BINDURA UNIVERSITY OF SCIENCE EDUCATION

FACULTY OF SCIENCE

DEPARTMENT OF CHEMISTRY

Development and Evaluation of a Tannin and Terpene Plant Based Termiticide for Termite Control

By

Chaparadza Jerryphanos

B221313B

Chemical Technology

June 2026

Supervisor: Prof P Dzomba

A Dissertation Submitted in Partial Fulfillment of the Requirements for The Bachelor of Science Honors Degree in Chemical Technology

APPROVAL FORM

The undersigned certify that they have supervised, read and recommend to the Bindura University of Science Education for the acceptance of a research dissertation entitled: **Development and Evaluation of a Tannin and Terpene rich plant based termiticide for termite control** submitted by Jerryphanos Chaparadza in the partial fulfilment of the requirements for the Bachelor of Science Honours Degree in Chemical Technology.

(Signature of student) … …… …….. Date …….

(Signature of Supervisor) ...…  …..…….. Date 22/08/2026

(Signature of Chairperson) ……  …… Date …….

DEDICATION

This work is dedicated first and foremost to God Almighty, my Creator, the source of my strength, wisdom, knowledge, and understanding. His guidance, grace, and unwavering presence have sustained me throughout this academic journey, and it is through Him that I have been able to persevere and succeed. I also dedicate this study to Prof. P. Dzomba, my supervisor, for his invaluable guidance, encouragement, patience, and continuous support throughout the entire research process. His insistence on excellence and completion greatly shaped the outcome of this work. Furthermore, I dedicate this work to my beloved parents, whose unconditional love, sacrifices, and constant support have been my foundation and motivation. Their belief in my abilities has been a driving force behind my academic achievements. Lastly, I dedicate this work to my sisters for their encouragement, understanding, and support during challenging moments of this journey.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to God Almighty for His grace, strength, and guidance throughout the course of this study. I am deeply grateful to Prof. P. Dzomba for his supervision, patience, and valuable guidance during this research. I also acknowledge the laboratory assistants in the Chemistry and Biology departments for granting access to the instruments and facilities required for the experimental work. My appreciation is extended to my colleagues for their encouragement and motivation during my studies. Special thanks to my best friend Panashe Zimhatye for all the support and encouragement. Finally, I am grateful to my late father, Mr Moses Chaparadza, whose emotional and financial support greatly contributed to my academic journey.

ABBREVIATIONS

E. Tirucalli – *Euphorbia Tirucalli*

GC-MS – Gas chromatography-mass spectrometry

UV-VIS – Ultraviolet-Visible Spectroscopy

GCA – Gallic Acid Standard

RF – Retention factor

VOCs – Volatile organic compounds

IPM – Integrated pest management

BA – Bio Assay

pH - Power of Hydrogen

ABSTRACT

Termites pose a major threat to agricultural productivity and infrastructure, causing economic losses and undermining food security. Conventional termite control relies largely on synthetic termiticides, which are associated with environmental contamination, toxicity to non-target organisms, and the development of resistance. This study developed and evaluated a plant-based termiticide rich in tannins and terpenes extracted from *Euphorbia tirucalli*. Tannins and terpenes were extracted by maceration using ethanol and an ethyl acetate-chloroform mixture, giving yields of 12.6% and 8.8%, respectively. Phytochemical screening confirmed the presence of both compound groups. Quantitative analysis showed a total phenolic content of 168 mg GAE/g, while GC-MS identified major terpenoid constituents including Ergost-5-en-3-ol, β-amyrin, neophytadiene, spathulenol, and caryophyllene. The extracts were formulated into an emulsifiable termiticide using Tween 90, ethanol, and water at different terpene: tannin ratios. Bioassays for contact toxicity, fumigant activity, repellence, antifeeding activity, and soil barrier effectiveness demonstrated strong biological activity. The 100:0 and 80:20 formulations achieved 98.9±0.2% and 97.8±1.0% mortality respectively within 24 hours, while the 0:100 formulation showed the highest antifeeding activity at 92.9±0.5% inhibition. The 80:20 formulation produced the highest soil barrier effectiveness at 92.9±2.2 %, whereas terpene-rich formulations showed the strongest repellence. The observed activity is attributed to synergistic interactions between tannins and terpenes through neurotoxicity, digestive inhibition, repellence, and disruption of feeding behaviour. Overall, the *Euphorbia tirucalli*-based formulation showed strong potential as a sustainable alternative to synthetic termiticides, although further optimisation and field validation are required.

Contents

APPROVAL FORM i

DEDICATION ii

ACKNOWLEDGEMENTS iii

ABBREVIATIONS iv

ABSTRACT v

LIST OF FIGURES ix

LIST OF TABLES xi

CHAPTER 1: INTRODUCTION 1

CHAPTER 2: LITERATURE REVIEW 5

2.1 Taxonomy of Termites 5

2.2 The Reclassification of termites: a molecular revolution 6

2.3 Changes to family and sub-family classification 6

2.4 Economic significance of termites 7

2.5 Effects on agriculture 7

2.6 Damage to constructed structures 7

2.7 Existing natural organic compounds available on the market 8

2.8 Disadvantages of conventional synthetic termiticides 8

2.9 Potential uses for natural organic product to control termites 8

2.10 *Euphorbia tirucalli* 9

2.10.1 Biopesticide Potential 9

2.11 Modes of Action of Tannins and Terpenes 10

2.12 Terpenes: Modes of action 10

2.13 Extraction Methods 12

CHAPTER 3: METHODOLOGY 18

3.1 Materials and equipment 18

a) Collection of Plant 18

b) Sample preparation 18
c) Maceration for Terpene Extraction 18
d) Maceration for Tannin Extraction 18
For the Terpene Extract 19
For the Tannin Extract 19
Salkowski test for terpenes and terpenoids 19
Liebermann-Burchard Test for terpenes and terpenoids 19
Lead Acetate test for tannins 19
Ferric Chloride Test for Tannins 19
3.2 Composition analysis of terpenes by GC-MC 20
3.3 Preparation of the calibration curve 20
Quantification 21
3.4 Ratios and Volume of Extracts Required 21
3.5 Evaluation of termite toxicity 22
3.6 Evaluation of the physiochemical parameters of the formulated termiticide (best overall performance.) 24
3.7 Conclusion 25
CHAPTER 4: RESULTS 26
4.1 Percentage Yield of Extracts 26
4.2 Qualitative Phytochemical Screening 26
4.3 Quantative and Compositional analysis 28
4.3.1 Total Tannin Content Quantification (UV-VIS) 28
4.3.2 GC–MS Analysis of Terpenes and Terpenoids 30
4.4 Bioassay Results 36
4.4.1 Contact Toxicity Assay 36
4.4.2 Fumigant Assay 37
4.4.3 Repellence Assay 38

4.4.4 Antifeeding Assay 40
4.4.5 Soil Barrier Assay 41
4.5 Integrated Assessment of Bioassay Performance 42
4.6 Physicochemical Properties of the Selected Formulation 44
4.7 Conclusion 45
CHAPTER 5: DISCUSSION 46
5.1 Phytochemical & Extractive Data interpretation 46
5.2 Tannin Quantification and GC-MS data interpretation 46
5.3 Bioassay performance explanation 46
5.4 Integrated performance of the 80:20 formulation 47
5.5 Limitations of the present study & further considerations for a field trial 47
5.6 Conclusion 47
CHAPTER 6: CONCLUSION AND RECOMMENDATIONS 49
6.1 Conclusion 49
6.2 Recommendations 49
1. Field trial and validation 49
2. Optimization of the formulation 50
3. Large scale production 50
4. Integration in IPM 50
5. Environmental and toxicological evaluation 50
6. Further phytochemical investigations 50
7. Study of storage stability 50
REFERENCES 52
APPENDIX 56

LIST OF FIGURES

Figure 2.1: Termite Taxonomy 5
Figure 2.2: *Euphorbia tirucalli* plant used as the source of tannin- and terpene-rich extracts in this study 9
Figure 2.3: Mechanisms of tannins and terpenes as potential insecticides 10
Figure 2.4 Classification of Terpenes Based on the Number of Isoprene Units 16
Figure 4.1: Ferric chloride test for the presence of tannins 16
Figure 4.2: Lead acetate test for tannins 17
Figure 4.3: Salkowski test for terpenes and terpenoids 27
Figure 4.4: Plant-based termiticide formulations prepared from different terpene: tannin ratios of *Euphorbia tirucalli* extracts. ..... **Error! Bookmark not defined.**
Figure 4.5: Gallic acid calibration curve used for UV-VIS quantification of total tannin content. 29
Figure 4.6: UV-VIS output used to estimate the tannin content of the *Euphorbia tirucalli* extract. 29
Figure 4.7 GC-MS chromatographic profile and report output for the terpene-rich extract of *Euphorbia tirucalli*. 30
Figure 4.8: Structural representations of selected dominant terpenoid compounds identified by GC-MS. 32
Figure 4.9 : Mass spectrum of Neophytadiene from GC-MS library 33
Figure 4.10: Mass Spectrum of β-Amyrin from GC-MS library 33
Figure 4.11: Mass Spectrum of Caryophyllene from GC-MS library 34
Figure 4.12: Mass Spectrum of Ergost-5-en- 1 from GC-MS library 34
Figure 4.13: Mass spectrum of spathulenol from GC-MS library 35
Figure 4.14: Contact toxicity responses of termites exposed to different terpene: tannin formulations after 24 hours. 37
Figure 4.15: Fumigant mortality responses of termites exposed to volatile components of different terpene: tannin formulations. 38
Figure 4.16 : Repellence of termites over 4 hours for different terpene: tannin formulations. 40
Figure 4.17: Antifeeding activity of different terpene: tannin formulations based on reduction in reed consumption 41
Figure 4.18: Soil barrier effectiveness of different terpene: tannin formulations against termite crossing. 42

Figure 4.19: Integrated comparison of formulation performance across contact toxicity, fumigant, repellence, antifeeding, and soil barrier assays. 43

LIST OF TABLES

Table 3.1 Formulation components for synthesis of the tannin- and terpene-rich plant-based termiticide 21
Table 3.2 Extract volumes used to prepare each terpene: tannin formulation ratio 21
Table 3.3 Evaluation of the physiochemical parameters the formulation 24
Table 4.1: Percentage yield of tannin and terpene extracts from *Euphorbia tirucalli* 26
Table 4.2: Summary of qualitative phytochemical screening results for *Euphorbia tirucalli* extracts 26
Table 4.3 Major terpenoid classes and relative composition identified by GC–MS 30
Table 4.4 Dominant terpenoid compounds and peak areas identified by GC–MS 31
Table 4.5: Contact toxicity assay results for different terpene: tannin formulations 36
Table 4.6: Fumigant assay results for different terpene: tannin formulations 37
Table 4.7 : Repellence assay results over 4 hours for different terpene: tannin formulations 39
Table 4.8: Antifeeding assay results for different terpene: tannin formulations 40
Table 4.9: Soil barrier assay results for different terpene: tannin formulations 41
Table 4.10: Overall comparison of bioassay performance across terpene: tannin formulations 43
Table 4.11: Physicochemical properties of the selected 80:20 formulation 44

CHAPTER 1: INTRODUCTION

1.1 Problems of termites in construction and fields

Though termites are important ecological decomposers, they are also known as one of the most devastating agricultural and construction pests. In agricultural systems termite infestation lead to destruction of plant roots, stems and other plant parts thereby disrupting their water and nutrient uptake and ultimately affecting their growth and yield (Jouquet *et al.*, 2011; Florence, Nyagumbo and Mafongoya, 2016). This causes more secondary fungal and bacterial infections on the damaged parts leading to weakening of stems and easy lodging of plants under high winds. Losses have been caused by termite damage to crops and other important cash crops like cereals, maize, sorghum, wheat, sugarcane etc resulting in food insecurity and posing a major challenge to smallholder farmers (Su and Scheffrahn, 2022). While some termites contribute positively to the cycling of nutrient in the ecosystem, large scale infestation and mound formation activity could also have negative impacts on the structure of the soil affecting the infiltration and on soil fertility in the long run (Jouquet *et al.*, 2011; Biswa, 2024). In construction termites are major structural pests causing damage to wooden posts of fence, furniture, floors, roofs and ceilings, utility poles etc thereby posing risks to the structure and maintenance costs (Rust and Su, 2012). Over a period, prolonged termite infestation will ultimately weaken crucial structural elements of the building thus making it vulnerable to damages from environmental conditions. In Zimbabwe most parts of the country have a hot climate hence favourable to termites causing damage to wooden materials and utility posts. This situation poses termite control as major hindrance to both agriculture and construction.

1.2 Current control chemicals and their limitations

Current termite control methods in agriculture and construction include cultural practices, treated timber, soil chemical barriers, baits and chemical sprays of synthetics termicides such as fipronil, pyrethroids, neonicotinoids, organophosphates and chitin-synthesis inhibitors (like hexaflumuron) among others (Logan, 1990; Su and Scheffrahn, 2022). Though such chemical formulations are useful in the immediate short term but application of these synthetics' products over a prolonged period of time raises environmental and health concerns. Termiticides applied either directly to the soil or the wood will have residuals which might leach to the water sources or contaminate the soil; affecting the soil chemistry and microorganisms; non-target organisms like pollinators, aquatic animals, beneficial insects, wild life and human beings are often

affected by application of such chemicals. Some synthetics like Chlorpyrifos, imidacloprid, clothianidin and thiamethoxam are associated with negative impact on nature and human beings. Moreover, the repeated application of chemicals has often resulted in the development of resistance by the pest population, making long term application inefficient. Such limitation has strengthened the drive toward application of environmentally friendly solutions such as botanicals and Integrated Pest Management practices.

1.3 The potential of plant-derived phytochemicals for termite control and their modes of action

Heightened environmental consciousness and deleterious effects of chemical termiticides has been the driving force for the greater interest and application of phytocompounds.

Phytocompounds are plant derived chemicals which affect insects. Previous studies have shown that phytocompounds are capable of exhibiting pest control through several mechanisms. For instance, application of plant extracts and terpene-based formulations was found to be active against various arthropod pests such as ticks and insects that act as vectors (Bekele, 2014; Coulibaly *et al.*, 2020; Kuvawoga *et al.*, 2025a). These results indicate that compounds derived from plants could act as a toxin, repellent or anti-feeding agent depending on its chemical composition. Phytocompounds exist in the form of terpenes, alkaloids, phenolics, flavonoids, tannins, saponins and essential oils which are normally synthesized by plants as part of their defensive system against pests(Ernst *et al.*, 2019; Kortbeek, Gragt and Bleeker, 2019). Unlike synthetics pesticides, they are normally biodegradable and do not normally pose a risk to non-target organisms, especially when combined with each other; this allows the compound to target different physiological sites hence it is harder to develop resistance. Some plants compounds like neem oil, lemongrass essential oil and products derived from plants containing rotenone along with mixtures of different plant compounds have been found to act as growth inhibitors, repellents, respiratory inhibitors and have synergism with other compounds(Verma *et al.*, 2021; Lin *et al.*, 2022; Xuan *et al.*, 2024). Less was reported on combination of tannins and terpenes as an anti-termite formulation, a gap filled by the present study.

1.4 Problem statement

Termites represent a severe threat to both agricultural production and infrastructure. Termites contribute significantly to crop yield losses and structural damage, which in turn influence food security, economic development and social well-being (Govorushko, 2019). Conventional termiticides are predominantly used for termite control, nevertheless, these can be ineffective

for long-term usages and pose health risk due to soil contamination and effects on non-target organisms (Furlong *et al.*, 2020). Despite the fact that phytochemicals derived from plants may be environmentally friendly, there is very little information available about the synergistic effect of tannins and terpenes derived from *Euphorbia tirucalli* on termiticidal activities. Therefore, there is a need to investigate the potential of phytochemicals extracted from *Euphorbia tirucalli* as active constituents in the development of sustainable and environmentally safe termiticidal formulations.

1.5 Justification

This study is important because it could contribute and lead to the development of sustainable termiticides for use in the agricultural and construction sectors, thereby reducing environmental damage, promoting food security, and improving people's health and well-being (Govorushko, 2019), hence improving health and well-being of the population (Furlong *et al.*, 2020). The reduced effectiveness of synthetic termiticides against termites and the development of resistance, has increased interest in the research and development of sustainable alternatives (Su and Scheffrahn, 2022). Several phytochemical studies on *Euphorbia tirucalli* revealed presence of terpenoids and phenolic compound which also includes tannin-related metabolites which make it as a viable source for bioactive insecticide components for environmentally friendly and potent termiticide. Annual termite infestation causes worldwide economic losses in agriculture, construction, housing and infrastructure of billions of dollars (Govorushko, 2019). Plant based pesticides can help mitigate this huge loss. In Africa, termites remain serious agricultural pests, causing substantial crop losses and contributing to food insecurity in rural communities (Logan, 1990).

1.6 Aim

- To develop a tannin and terpene rich plant based termiticide.

1.7 Objectives

- To extract tannins from *Euphorbia Tirucalli* using maceration method.
- To extract terpenes from *Euphorbia Tirucalli* using maceration method.
- To formulate a tannin and terpene rich termiticide.
- To evaluate effectiveness of the tannin and terpene rich termiticide through contact toxicity, antifeeding, fumigant, repellence and soil barrier.

1.8 Research Questions

1. Which terpenes and tannins in *Euphorbia Tirucalli* can be used as the principal actives against termites?

2. What is the level of effectiveness of tannins and terpenes isolated from *E. Tirucalli* on the contact toxicity, antifeeding, repellence, fumigant toxicity and soil barrier tests?

3. Which is the best formulation for producing a tannin and terpene based bioinsecticide?

1.9 Conclusion

Chapter 1 introduced the termiticides issues on crops and construction, highlighted on the demerits on use of synthetics chemicals, emphasized on plant-based alternative on pest control; especially on tannins and terpenes. It concluded with problem statement, justification, aim, objectives and research questions, which form the foundation for literature review of the second chapter.

CHAPTER 2: LITERATURE REVIEW

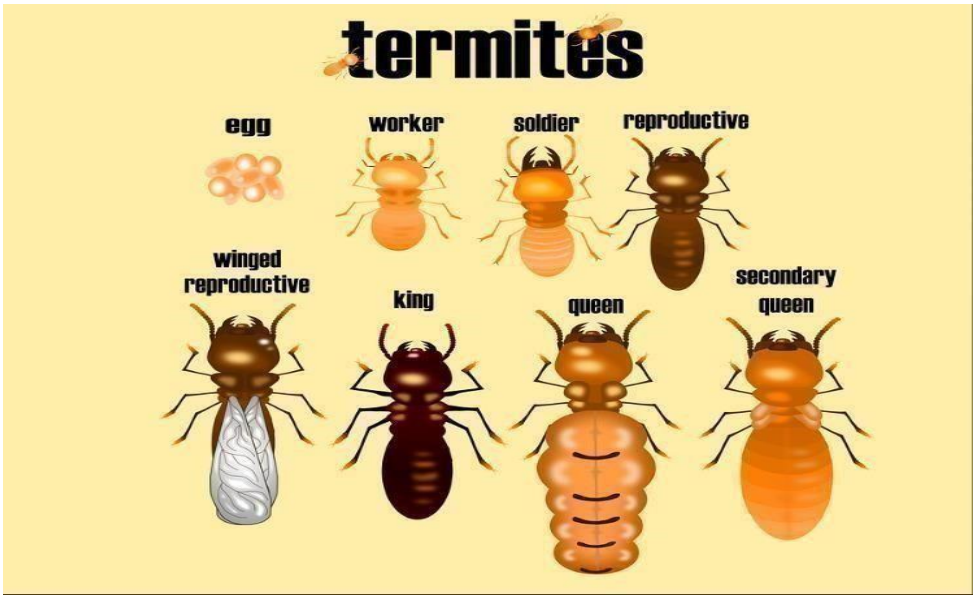


Figure 2.0.1: Termite Taxonomy

2.1 Taxonomy of Termites

Termites were once classified in order Isoptera but evidence from behaviour, morphology and DNA sequencing revealed that they should actually be placed in the same order as cockroaches. When the behaviour and morphology of termite and wood-eating cockroach species were examined, an apparent similarity between termites and the wood-eating cockroach genus Cryptocercus was found, both groups having the same gut symbionts and social behaviours (trophallaxis, allogrooming) (Korb, 2008). Later molecular studies of termite and cockroach genes, using 16S rRNA sequences proved that termites nested within Blattodea with termites being closely related to the Cryptocercus species. Termites now reside within the infraorder Isoptera in order Blattodea; 2,972 species of termites have so far been described, most in the family Termitidae. A general classification is shown below:

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Blattodea

Infraorder: Isoptera

Families: Termitidae, Rhinotermitidae, Kalotermitid, Rhinotermitid, Mastotermitidae, Serritermitidae, and Stylotermitidae.

The family Termitidae has over 85% of termite genera and is the largest of all the termite families. The most primitive is the family Mastotermitidae. It is believed that termites evolved from wood-eating cockroaches which include the genus Cryptocercus.

2.2 The Reclassification of termites: a molecular revolution

The classification of termites was recently re-written as a result of the introduction of molecular analyses. These proved that termites are not a separate order but nest within the Blattodea. Both the wood-eating cockroach genus Cryptocercus and termites are found to have many behavioral and morphological characteristics in common and termites are indeed eusocial cockroaches, rather than distinct from the Blattodea (Korb, 2008; Harrison *et al.*, 2018). The reclassification is significant because it impacts how termites evolve, are perceived and how we research into their social behavior and adaptive strategies; instead of observing eusociality as an evolutionary phenomenon in termites independently of other groups, we can view it as a progression along the evolutionary route from ancestor to descendant along cockroaches, and we are able to apply this to all the groups and consider how this change has affected our views of termite and cockroach biology. Recent genomic research on termites including sequences for a wide array of termites from multiple families together with cockroach outgroups revealed expanded gene and genomes within Termitidae, one of the largest termite families. These expanded genes and genomes are involved in dietary adaptation and diversification and were not observed in any cockroach species, therefore indicating an interesting evolutionary divergence within termites (Liu *et al.*, 2025) This shows the increasing emphasis on using not only molecular but also genomic techniques to understand termite taxonomy.

2.3 Changes to family and sub-family classification

Previously, traditional classifications for termites, which were largely based on morphology alone often resulted in classifications that were paraphyletic and polyphyletic, and revision of classifications has been necessary as a result of molecular research. Genome scale phylogenetic analysis using ultra conserved elements and multispecies coalescent models resulted in the proposal of a monophyletic framework for higher termites (Hellemans, 2024). It was also shown that within Neoisoptera, the most diverse termite group taxonomically, several

previously regarded sub-families were elevation in to families; Heterotermitidae, Psammotermitidae, and Termitogetonidae, to reflect the monophyletic status. Within the large Termitidae family several groups which were already identified as having monophyletic sub-family status were confirmed, and further monophyletic sub-families within termitidae were also recognized and established; these include; Crepititermitinae, Cylindrotermitinae, Forficulitermitinae, Neocapritermitinae, Protohamitermitinae, and Promirotermitinae and both Amitermitinae and Microcerotermitinae re-established (Hellemans, 2024).

2.4 Economic significance of termites

Although termites are ecologically beneficial through their roles as decomposers and recyclers, they have a detrimental effect as pests and can cause a great deal of damage to constructed structures, agriculture and the timber industry. Their feeding behaviour often leads to considerable damage because they can remain undetected inside structures for prolonged periods until catastrophic failure or a great deal of economic loss occurs; globally they are thought to cause billions of dollars worth of damage each year (Rust and Su, 2012).

2.5 Effects on agriculture

Termites have also proved to be significant agricultural pests and cause the loss of valuable production in numerous crops across the world. It was revealed that maize and haricot beans are most susceptible to termites, where small holder farmers in the Central Rift Valley of Ethiopia estimated a pre-harvest yield loss of maize and haricot beans of 18.02 2.67 kg ha-1 and 10.58 1.91 kg ha-1respectively (Debelo and Degaga, 2015). They can also cause considerable loss of profit by damaging farming structures, such as granaries and storage rooms thus exacerbating losses of stored crops (Rust and Su, 2012). Thus, a nation that depends heavily on agriculture such as Zimbabwe may suffer a large loss of economic benefit from these pests, for example, termite species cause catastrophic losses of sugarcane, cotton and wheat, of 20-45% at harvest time. Germination losses of sugarcane have been recorded as being as high as 90-100% in some areas. In addition to damaging crops, termites can also affect the agricultural economy through structural damage, but they are able to cause significant losses throughout agricultural systems dependent on the region, crop and intensity of infestation. Worldwide termites have been found to cause economic damage of billions of dollars each year (Rust and Su, 2012).

2.6 Damage to constructed structures

Structural damage caused by termites to buildings and wooden construction leads to significant loss in terms of property value, repair costs and inspections. It is estimated that in the USA, subterranean termites cause approximately $3 billion in damage per year to wooden structures (Rust and Su, 2012). This has serious implications on the longevity and value of buildings.

2.7 Existing natural organic compounds available on the market

The market for termites is steadily moving from synthetic chemicals towards botanical and naturally derived product as the dangers and potential damage of synthetics become increasingly apparent. An example of such compound that is already widely used for controlling termites is Neem oil (Azadirachta indica). Its principal compound azadirachtin is thought to work by inhibiting growth, feeding and reproduction (Pavela, 2016).

2.8 Disadvantages of conventional synthetic termiticides

Synthetic termiticides have traditionally been used to kill or prevent termites attacking structures via the construction of chemically treated soil and wood barriers. These may appear to work effectively in the short term but because termites can still find breaches in the barrier they may still be able to feed and thus attack wood components. Over time they can lose effectiveness due to degradation from the elements and chemical breakdown within the soil. Furthermore, the effect on non-target species, ecosystem and overall environment is a major concern with some chemicals contributing to ground and water contamination and depletion of beneficial insect species, and hence biodiversity. A problem that many chemical termiticides face is resistance, with termites adapting and evolving, and this results in ineffective termiticides that need to be replaced and further chemicals purchased. In the long-term repeated applications are costly and although they appear to be the most cost-effective option the environmental cost is significant and there are already several examples of long-term contamination from the use of some persistent chemicals in developing countries. This shows that more effective and safer chemical controls are necessary.

2.9 Potential uses for natural organic product to control termites

As we become increasingly concerned with the environment the number of natural organic products on the market for the control of termites is growing, mainly because they are biodegradable, nontoxic, and not damaging to the environment (Isman, 2006). Many plants produce chemicals such as tannins, flavonoids, terpenes and alkaloids that deter and kill insects, interfere with their diet, feeding and growth. There are several research studies that indicate

the effectiveness of these botanical compounds in protecting timbers and crops against termites. Synergistic interactions between extracts from different plants can also increase their effectiveness and potential use in controlling termites.

2.10 *Euphorbia tirucalli*

Euphorbia tirucalli (pencil cactus or milk bush) is a succulent belonging to family Euphorbiaceae and is distributed in tropical and sub-tropical regions. Traditionally this plant is used in folk medicine for various therapeutic effects and lately it has been the subject of interest in investigation of its phytochemical constituents and pharmacological properties. It has been revealed that various phytochemical constituents like alkaloid, flavonoids, tannin and terpenoids were isolated from *E. Tirucalli*(Salehi, B., 2019). Major constituents like euphol, tirucallol, phytosterols, and diterpenes which are well known to exhibit some antimicrobial, anti-inflammatory and pesticides properties were also determined.



Figure 2.0.2: Euphorbia tirucalli plant used as the source of tannin- and terpene-rich extracts in this study

2.10.1 Biopesticide Potential

Because of its diverse bioactivities the biopesticidal potential of *Euphorbia tirucalli* have received more and more attention. Experiments conducted in several laboratories indicate pesticidal effects of *Euphorbia tirucalli* extracts on numerous species of insects thereby proving its suitability for pest control strategy(Damme, 2011). *Euphorbia tirucalli* also has long been used in ethnomedicines and contains vital phytochemical elements like alkaloids,

terpenoids, tannins and phenolics that confer it biological effects (Upadhyay, 2010). This clearly shows that *Euphorbia tirucalli* can also be a good source for organic pest control compound and further studies are needed to optimize the formulation and its effect on non-target organism.

2.11 Modes of Action of Tannins and Terpenes

Terpenes and tannins are plant produced chemicals which show insecticidal and repellent activities on numerous insects. These products are considered as organic pesticides and are better alternatives to the synthetic chemicals due to their biodegradable nature and relatively low toxicity on non-target organisms (Isman, 2006).

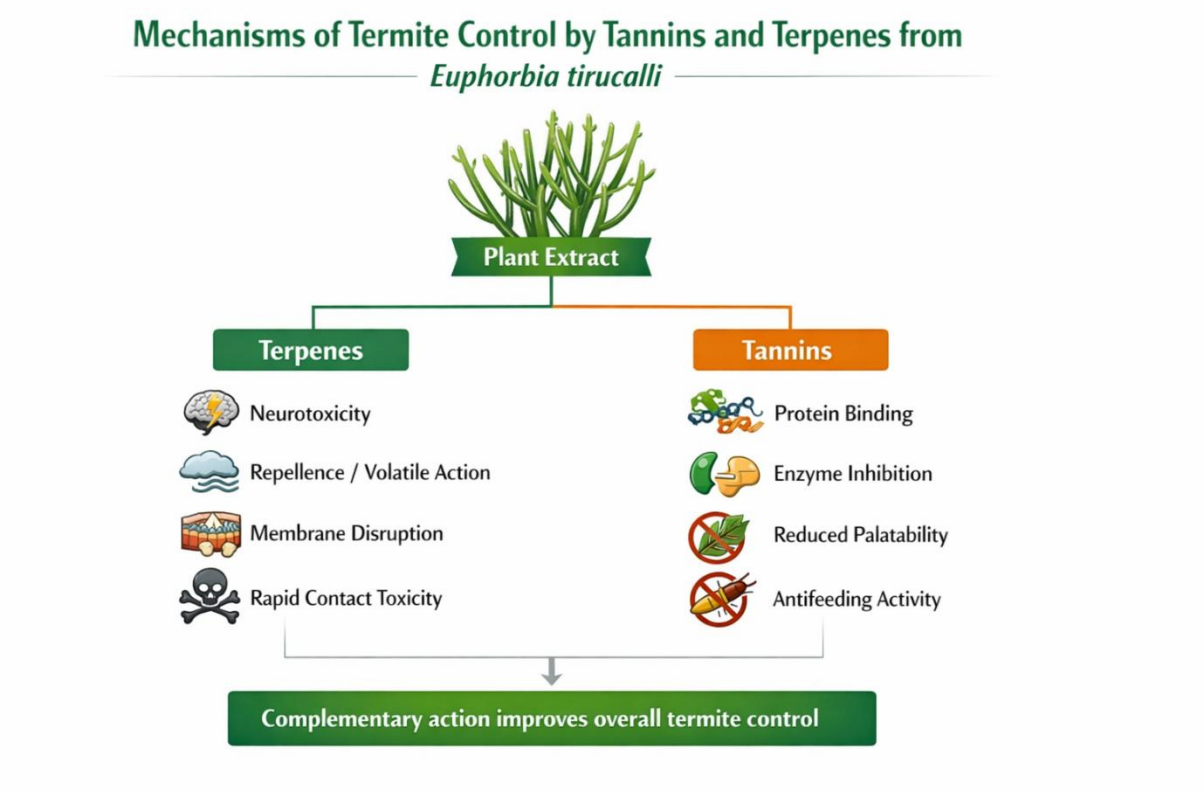


Figure 2.0.3: Mechanisms of tannins and terpenes as potential insecticides

2.12 Terpenes: Modes of action

There are different mechanisms of action which can be exploited in using terpenes to control insects

1. Neurotoxicity: The insecticidal activity of terpenes is due to its primary toxicity on nervous system. Most monoterpenes in their modes of action inhibition of acetylcholinesterase (enzyme which plays a vital role in functioning of nerve impulse in insects) leading to paralysis and finally death of the insect. Examples are limonene and pinene have shown remarkable toxic effects against different pests of insects via nervous system disturbance (Regnault-Roger, Vincent and Arnason, 2012).

2. Disruption of physiological process of insects: Some terpenoids affects hormone-based process like moulting and growth of insect. The insect growth regulating property of triterpenoid azadirachtin derived from seeds of neem plant is due to its interference in the hormonal mechanism in moulting. It also interrupts activities of ecdysone hormone in moulting.

3. Repellence: One of the important characteristics of terpene use in controlling insect pests is its repellency. The very volatile compounds of the plants which are capable of giving very strong odours can deter insects from feeding and oviposition the plants, in this category comes essential oils that produce repellency from strong olfactory and behavioural effects in insects, e.g citronellal found in citronella grass acts as a mosquito repellent (Pavela, 2016).

4. Feeding deterrence: The feeding deterrent properties of some terpenes are also responsible for its pesticidal activity. The feeding of plant compounds by the insects can also produce Starvation and ultimate death by blocking feeding stimuli. Examples are most bitter plants and oils have strong feeding deterrent activities. Bitter tasting properties of terpenes deter insect from feeding (Pavela, 2016).

5. Membrane integrity: Terpenes present in essential oils are reported to affect insect cell membranes permeability to various cellular components, thus destroying cell membrane integrity and leading to cell death. High terpene concentration in essential oils shows promising results against many pests due to the destruction of the cell membrane and thus physiology of the pest (Pavela, 2016).

2.12 Tannins: Modes of action

The role of tannins, which are abundant polyphenolic compounds in several plants in pest control is manifold:

1. Protein binding activity and anti-nutrition: Tannins act through many ways in inhibiting nutrition and feeding behaviour of insects. Tannins possess capability to bind protein of insect's

digestion system, like proteases, amylases which results in reduced efficiency in protein digestion in the alimentary canal. This in turn leads to starvations and consequently death of the insects(Barbehenn and Peter Constabel, 2011; Pavela, 2016).

2. Direct toxicity: Certain tannins, directly and indirectly contribute to insecticidal action due to their capability of interacting with cell metabolic pathways in order to interfere with physiological functioning of insects. Hydrolysable tannins when degraded lead to products like gallic acid that is toxic to insects(Barbehenn and Peter Constabel, 2011).

3. Repellency: Although it is not as widely known as it is with terpene- based repellents, some tannins can also produce the deterrence action through their astringent taste, this can be considered as an indirectly produced repellent action (Barbehenn and Peter Constabel, 2011; Pavela, 2016).

4. Inhibition of enzyme activity for detoxifying insects: Tannins along with some other secondary metabolites can inhibit the enzyme activity in insect detoxification system so that insect cannot detoxify toxic components in their physiology. In this way toxicity of other compounds also enhanced against insect (Barbehenn and Peter Constabel, 2011; Pavela, 2016).

2.13 Extraction Methods

Extraction is a very important stage in recovery of plant secondary metabolites since yield and composition is largely affected by the solvent polarity and extraction condition as well as the type of the phytochemicals present (Fonmboh *et al.*, 2020). There are various extraction techniques employed for the recovery of phytochemicals and they range from; maceration, Soxhlet extraction, steam distillation, hydro distillation, digestion and supercritical fluid extraction. The above mentioned methods differ significantly in costs, effectiveness, and heat sensitivity, scaling of extraction (Starmans and Nijhuis, 1996; Fonmboh *et al.*, 2020). However, for thermally unstable compounds and systems containing mixtures of phytochemicals, maceration is considered a favourable method since it is relatively inexpensive and can be used in laboratory extraction scale. Solvent type employed in extraction is also very important as phytochemicals vary in polarity and solubility so there is need to select suitable extraction method in order to get a representative and biologically active extract, when developing pesticide agents.

2.13.1 Maceration

Maceration is a widely used process of extraction for use in pharmacognosy and natural product research since the process is simple and straightforward to undertake. Maceration is the most basic technique to extract compounds present in a plant material, which serves as a method for extracting bioactive chemical constituents from plant material (Fonmboh *et al.*, 2020). In this technique, chopped or chunk material is immersed under a container by solvent during the process. The mixture is continually stirred using magnetic stirrer at room temperature throughout for hours or as long as it required. This allows the cell walls to be relaxed and ruptured; ease removal of solubilized compounds can also be carried out easily. Mixture obtained is then filter through Whatman no 1 filter paper to remove the solid plant material from the liquid extract. Solvent used plays very important part to determine particular phytochemicals present and extract them from the samples.

2.13.2 Soxhlet extraction

Soxhlet extraction involves placing the ground raw extract within the chamber of Soxhlet apparatus inside the thimble. Solvent within the flask at boiling point evaporates and is condensed, allowed to drip into the chamber containing crude extract. Crude extract is then leached by the solvent for a duration that leads to the removal of bioactive compounds of interest. The solvent is continuously re-evaporated until a drop from siphon tube, upon evaporation is observed to not yield any residue(Fonmboh *et al.*, 2020). Soxhlet extraction method has advantages over other methods since a larger number of constituents may be extracted using less amount of solvent which translates to energy saving as well as cost saving and time saving benefits. Although primarily used on a small scale for Batch extraction, this process can be modified to work on continuous medium or large-scale extraction (Fonmboh *et al.*, 2020).

2.13.3 Digestion

Digestion is a maceration technique that entails the application of gentle heat to the solvent. It is used when a temperature which is moderately above room temperature is required(Fonmboh *et al.*, 2020). This method helps improve the solvent power of the menstruum or solvent used for extraction.

2.13.4 Supercritical fluid extraction

Supercritical fluid extraction (SFE) is sample preparation technique used in attempts to reduce the use of organic solvents and speed up the analysis of the sample. Carbon dioxide (CO2) has

been used as the most favourable solvent owing to its various characteristics that make it more desirable as compared to organic solvents. However, CO2 does have a drawback in that it does not efficiently extract the polar analytes and those with strong matrix affinity, for these cases other solvents or even gases like Argon or organic solvents might need to be used with CO2. Increasing temperature or pressure enhances the extraction recovery of certain compounds using SFE. Some advantages include extraction at relatively lower temperatures thus less degradation, use of less organic solvents, SFE also find a number of applications like; pesticide extraction, oil extraction, polymer extraction, extraction of perfumes, and environmental samples. The primary drawback is the high investment costs (Fonmboh *et al.*, 2020).

2.14 Qualitative phytochemical screening

The qualitative phytochemical screening test is one of the commonly used method generally applied in showing whether bioactive compounds are present in the plant extract or not. Some commonly known bioactive phytochemicals which are reported to have varied pharmacological and biological activities that may be present in the plant extract are alkaloids, flavonoids, tannins, steroids, terpenoids, glycosides and saponins. Different solvents are used in the extraction of the phytochemical compounds from plant material, various qualitative tests have been developed so as to detect particular compound (e.g Salkowski test performed in order to detect Terpenoids and Mayer's test performed in order to detect Alkaloids). A chemical test usually shows its results through observation of colour change or precipitation of substance. The performed test would allow the determination of the chemical constituents of selected medicinal plants, this will lead to selecting of suitable medicinal plant species for pharmaceutical and drug substance production(Singh *et al.*, 2022).

2.16 Tannins Classification

Tannins are a class of polyphenol secondary metabolites which are widely distributed in the plant kingdom. They are known for precipitating proteins and bonding with alkaloid and metal ions and play an important role in their bioactivity (Mackov et al., 2017). There are broadly two main classes of tannins: hydrolysable tannins and condensed tannins. The basic unit of hydrolysable tannins is a carbohydrate molecule, commonly glucose, esterified with phenolic acid such as gallic and ellagic acid derivatives. They may be hydrolysed, using acid or enzymes to release phenolic compounds. Tannins are abundant in many fruits, legumes, cereals, and vegetables and have defensive role in plants against various abiotic and biotic stresses. Because of their protein-binding capacity and astringency they also have several industrial uses,

including in leather tanning, dyes, inks and adhesives. Furthermore, recent studies indicate their cardioprotective, antimicrobial, antioxidant and anticancer properties (Mackov et al., 2017). They can be hydrolysed, using acid or enzymes to release their phenolic compounds. Tannins occur naturally in fruits, nuts, cereals and vegetables providing protection against biotic and abiotic stresses (Smeriglio et al., 2017). Due to their binding action with proteins, they are widely used in leather tanning, adhesives, dyes and inks industry. Additionally, they possess therapeutic qualities of being cardioprotective, anti-cancerous, antioxidant and antimicrobial (Smeriglio et al., 2017).

2.16.1 Condensed Tannins (CTs)

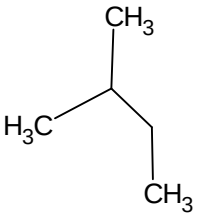
Condensed tannins (also called proanthocyanidins) are oligomeric or polymeric compounds composed of flavan-3-ol units, such as catechin, epicatechin, gallocatechin, and epigallocatechin, primarily linked through C-C bonds. Contrary to hydrolysable tannins, they do not possess a sugar moiety and their interflavan C-C bonds resist hydrolysis. Depending on the flavan-3-ol units they can be subcategorised into procyanidins, prodelphinidins and propelargonidins. These compounds are widely available in various plant sources and foods and drinks such as grapes, blueberries, cocoa, wine, nuts, seeds, flowers and bark and affect their taste, color and astringency. Condensed tannins are potent antioxidants with a broad range of biologic effects such as anti-cancerous, anti-diabetic, cardioprotective, anti-viral, neuroprotective and anti-inflammatory activities (Panzella and Napolitano, 2022). Hence, they are used widely in cosmetics, nutraceuticals, animal feed supplements, wood adhesives, fire resistant materials and improving wine quality. Due to their affinity for protein, condensed tannins are also widely used in leather processing for tanning.

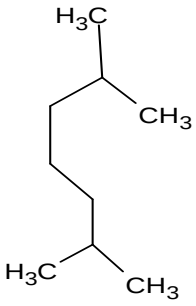
2.17 Classification of Terpenes

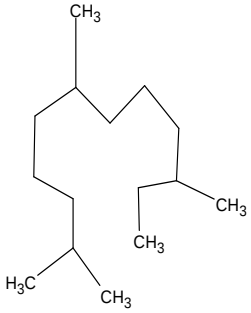
a) Based on the Number of Isoprene Units

The primary and simplest classification of terpenes is based on the number of isoprene (C) units found linked together by a head to tail combination. Terpenes are broadly divided into the following classes: Hemiterpenes(C5), Monoterpenes(C10), Sesquiterpenes(C15); Diterpenes(C20), Sesterterpenes(C25), Triterpenes(C30) and Tetraterpenes(C40)

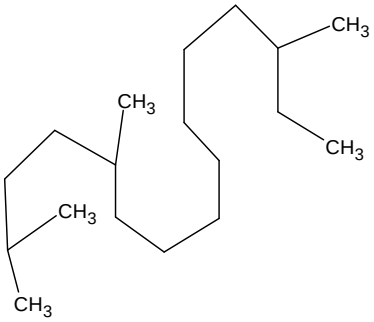
1. **Hemiterpenes (C5)** **2. Monoterpenes(C10)** **3. Sesquiterpenes (C15**



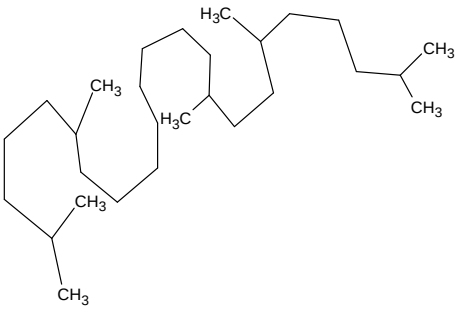




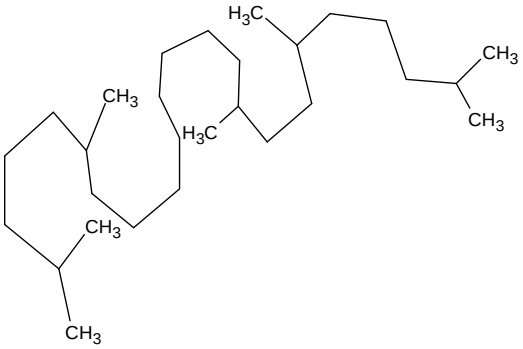
4**. Diterpenes(C20)**



5. Sesterterpenes(C25)



6. Triterpenes(C30)



7. Tetraterpenes(C40)

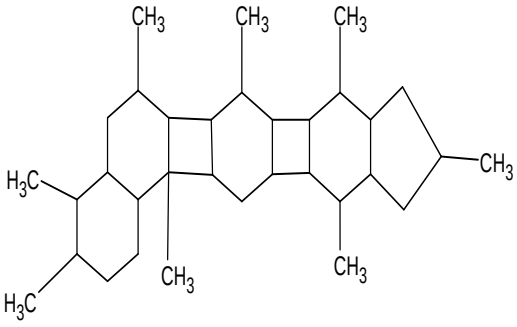


Figure 2.0.4 Classification of Terpenes Based on the Number of Isoprene Units

B) Based on the Cyclisation and Structure:

Terpenes have a remarkable structural diversity resulting from various cyclisation and molecular rearrangements that took place during biosynthesis(Davis and Croteau, 2000). Terpenes can be classified on basis of its structural diversity (non-cyclised, monocyclic, bicyclic, tricyclic etc.) (Zielińska-Błajet and Feder-Kubis, 2020). Limonene for instance, a monocyclic monoterpene, has only one ring in its structure whereas pinene, another monoterpene, possesses two rings within its structure.

Terpenes Vs Terpenoids:

Although term terpene is generally referred to as a hydrocarbon containing compound, the modified forms contain extra oxygen atom or additional functional groups and is called as terpenoids. But, in literature, these terms are often used synonymously. Terpenoids are abundant in plants and contribute to its fragrance and flavour, biological defence mechanism, and other health benefits. Examples of monoterpenoids are menthol while that of sesquiterpenoid is artemisinin.

2.18 Conclusion

In this chapter, the scientific background to termite control such as taxonomy and economic importance of termites, weakness of using chemicals for termite control, potentials of using organic materials as termite management approaches were reviewed. It examined the phytochemical and pesticidal significance of *Euphorbia tirucalli*, the mechanism of action of tannins and terpenes and the methods of its extraction. The literature shows the broad use of plant products for multi-faceted pest management and has revealed the need for further research on synergistic action of tannins and terpenes for control of termites which form the basis for present study's methodology.

CHAPTER 3: METHODOLOGY

3.1 Materials and equipment

Materials and equipment used in the study included chloroform, lead acetate, methanol, ferric chloride, distilled water, ethyl acetate, Tween 90, vanillin, sulphuric acid, a GC-MS system, a UV-VIS spectrophotometer, an incubator, a blender, a rotary evaporator, an analytical balance, a magnetic stirrer, sodium carbonate solution (Na_2CO_3), Folin-Ciocalteu reagent, and gallic acid standard.

a) Collection of Plant

Euphorbia tirucalli stems were collected from Mafodya Farm in Chiredzi, Zimbabwe, and transported to the laboratory for preparation and analysis.

b) Sample preparation

The stems were transported in clean plastic bags to the laboratory, washed under running tap water, rinsed with distilled water, and shade-dried. The dried stems were then ground into a fine powder and stored in an airtight glass container until extraction. Tannins and terpenes were extracted from *E. tirucalli* using the maceration method because it is simple, cost-effective, and suitable for preserving heat-sensitive phytochemicals.

c) Maceration for Terpene Extraction

For terpene extraction, 30 g of finely ground sample was weighed into a conical flask, and 500 mL of an ethyl acetate-chloroform solvent mixture was added. The flask was shaken to ensure uniform wetting of the plant material and covered with aluminium foil to minimise solvent loss. Maceration was carried out for 72 hours at room temperature with continuous stirring using a magnetic stirrer. After extraction, the mixture was filtered to separate the terpene-rich extract from the plant residue.

d) Maceration for Tannin Extraction

For tannin extraction, a separate 30 g portion of finely ground sample was weighed into a clean conical flask, and 500 mL of 99% ethanol was added. The flask was shaken to ensure even solvent distribution and covered with aluminium foil to reduce evaporation. The mixture was

macerated for 72 hours at room temperature with continuous stirring. After extraction, filtration was carried out to obtain the tannin-rich extract.

For the Terpene Extract

The terpene filtrate was concentrated by evaporating the ethyl acetate-chloroform solvent mixture in a water bath maintained at 60 °C. After complete solvent removal, the dry extract remaining in the pre-weighed flask was used to calculate percentage yield according to the expression $(W1_{terpenes}/W2) \times 100$, where $W1_{terpenes}$ is the mass of dry terpene extract and W2 is the initial mass of plant material.

For the Tannin Extract

The tannin filtrate was concentrated by evaporating ethanol in a water bath maintained at 60 °C. After complete solvent removal, the dry extract remaining in the pre-weighed flask was used to calculate percentage yield according to the expression $(W1_{tannins}/W2) \times 100$, where $W1_{tannins}$ is the mass of dry tannin extract and W2 is the initial mass of plant material.

Salkowski test for terpenes and terpenoids

To confirm the presence of terpenoids in the crude extract 3ml of concentrated sulphuric acid was added and a reddish-brown colour was noticed indicating the presence of terpenes in the sample.

Liebermann-Burchard Test for terpenes and terpenoids

A small amount of the concentrated terpene extract was taken and a few drops of acetic anhydride were then added to this solution, followed by the careful addition of a few drops of concentrated sulfuric acid (H_2SO_4). The observation of a colour change, starting from pink or red and gradually transitioning to blue and finally green, indicated the presence of terpenes.

Lead Acetate test for tannins

To confirm the presence of tannins in the crude extract a lead Acetate test was also employed and the formation of a yellow, buff-coloured, or whitish insoluble precipitate strongly suggested the presence of tannins.

Ferric Chloride Test for Tannins

A small amount of the concentrated tannin extract was taken and dissolved in a few millilitres of distilled water. Then, a few drops of a 5% ferric chloride ($FeCl_3$) solution were added. The

presence of tannins was indicated by the formation of a dark blue, bluish-black, green, or brownish-green colour, with the specific colour suggesting the type of tannins present (e.g., Hydrolysable tannins often yield a bluish-black colour, while condensed tannins may result in a brownish-green colour).

3.2 Composition analysis of terpenes by GC-MC

The terpenes were analysed and quantified using a gas chromatography mass spectrophotometer.

Quantification of tannins by UV-VIS

The tannins were quantified and analysed by UV-VIS by use of Folin-Ciocalteu Method.

Procedure

Step 1: 0.5mL aliquot of the sample was measured and put into a test tube.

Step 2: 1mL Folin-Ciocalteu reagent was added and thoroughly mixed.

Step 3: The reaction was allowed to stand for 5 min at room temperature to start the reduction of reagent by phenolic compounds.

Step 4: 1mL of 20% sodium carbonate solution was added to neutralize the reaction and cease colour development.

Step 5: After mixing the solution was incubated at room temperature for 30 min.

Step 6: Absorbance was determined at 765nm using a UV-VIS spectrophotometer.

3.3 Preparation of the calibration curve

Series of gallic acid standard solutions were prepared at the concentrations: 0, 0.0029, 0.0059, 0.0118, 0.0235 and 0.0353 mg/ mL.

The same procedure was followed for all the standard solutions, and their absorbance values were recorded.

A calibration curve of absorbance (y-axis) was plotted against concentration of gallic acid (x-axis).

Quantification

Using the absorbance measured from the sample, tannin content was determined by interpolating from the gallic acid calibration curve.

Table 3.1 Formulation components for synthesis of the tannin- and terpene-rich plant-based termiticide

Component	Volume (mL)	Function
Plant Extracts	5.00	Active ingredient (terpenes + tannins)
Tween 90	2.10	emulsifier
Ethanol	9.16	Co-solvent (part of 3:2 solvent mix)
Distilled Water	3.74	Solvent (part of 3:2 mix)
Total Volume	20.00	Final formulation

3.4 Ratios and Volume of Extracts Required

Table 3.2 Extract volumes used to prepare each terpene: tannin formulation ratio

Formulation ratio (Terpene: Tannin)	Terpene extract in 5.0 mL total extract (mL)	Tannin extract in 5.0 mL total extract (mL)
100:0	5.0	0.0
80:20	4.0	1.0
60:40	3.0	2.0
50:50	2.5	2.5
40:60	2.0	3.0
20:80	1.0	4.0
0:100	0.0	5.0

1. Preparation of the solvent base

9.16 ml of ethanol and 3.74 ml of distilled water were added into a clean beaker and stirred for homogeneity to create a 3:2 ethanol: water solvent carrier base.

2. Addition of emulsifier

2.1 ml of tween 90 were added to the solvent carrier base and stirred for 5-10 min to allow dispersal.

3. Preparation of extract mixture

5ml of the concentrated plant extract were measured for the 7 different ratios (100:0, 0:100, 20:80, 80:20, 40:60, 60:40 and 50:50). The extract was filtered and all residual solvent removed (ethyl acetate & chloroform for the terpenes and ethanol for the tannins).

4. Mixing of the extract and base

5ml of the plant extract were slowly added to the tween - ethanol: water mixture while it was stirred.

5. Homogenization

The mixtures were stirred for 15-30 minutes until they become homogeneous emulsions.

6. Bottling and storage

The emulsion was then poured into a dark (amber) coloured bottle and sealed. The storage conditions were at room temperature.

3.5 Evaluation of termite toxicity

1. Anti-feeding assay

The emulsifiable concentrate termiticide were applied. Dry reeds were sprayed with 7 terpenes: tannin ratio mixtures of 100:0, 0:100, 50:50, 80:20, 20:80, 60:40 and 40:60 at full strength and allowed to dry so that excess emulsifiable concentrate evaporated. Three replicate reeds per ratio combination were used. Each container contained 20 adult termites and treated reeds. All containers were kept at 25 degrees Celsius and 60% relative humidity. Consumption of the food (reeds) was evaluated on days 1, 3, 5 and 7 after weighing of the reeds before and after contact with the termites. The antifeeding activity (%) was calculated as follows:

$$\text{Antifeeding Activity (\%)} = \left(\frac{C - T}{C}\right) \times 100$$

where C represents the average mass loss of control reeds and T represents that of treated reeds.

2. Soil-Barrier Assay

Each of the seven formulations were treated into the soil. This soil was allowed to air dry. In petri dishes 28 termites were then introduced into one side of the soil barrier and after an exposure period number of termites entering the treated area was counted. Effectiveness of the soil barrier was then calculated using the following formula:

$$\text{Soil Barrier Effectiveness (\%)} = \left(\frac{C - T}{C}\right) \times 100$$

where C is the number of termites that crossed the untreated soil and T is the number that crossed the treated soil. The experiment was replicated thrice for each ratio to ensure robust statistical data.

3. Contact-Toxicity Assay

The Emulsifiable concentrate termiticide prepared using each of the seven terpenes: tannin ratios: 100:0, 0:100, 50:50, 80:20, 20:80, 60:40, and 40:60 were used. Each formulation was applied to sterile petri dishes and the solvent was evaporated to leave a fine film of termiticide on the bottom of the petri dish. 30 adult termites were added to each petri dish, the lids were tightly sealed and the number of dead termites recorded at the appropriate time points. Each treatment was performed in replicates of three, in order to generate robust statistical data. The negative control was an ethanol-treated petri dish.

4. Fumigant Assay

The Emulsifiable concentrate termiticide produced of the seven terpenes: tannin ratios: 100:0, 0:100, 50:50, 80:20, 20:80, 60:40, and 40:60, at a concentration of 5 mL per batch were used. Filter paper discs were impregnated with each formulation and allowed to air dry. These disks were placed in the lid of each 50 mL bottle (where there was no contact with the termiticide on the paper disk) with ten adult termites within the bottle. The lid was securely tightened to create an airtight seal. 10 adult termites were placed in each bottle, and the appropriate ratio filter papers were added to the lid of the bottle in each case (excluding the control). After 24 hours the number of dead termites were recorded for each filter paper disk. Each formulation was replicated three times to generate statistically robust data.

5. Repellence Assay

The Emulsifiable concentrate termiticides of terpene: tannin ratios: 100:0, 0:100, 50:50, 80:20, 20:80, 60:40, and 40:60, at a concentration of 5 mL per batch were used in this repellence assay. A filter paper disk cut in half, was treated on one half and left untreated on the other with each of the formulations listed above and then left to air dry. Ten termites were added to each petri dish on top of each treated disk, which was then placed in an airtight seal but with holes for aeration. The number of termites in the treated half of the filter paper disk (Nt) and in the untreated half of the filter paper disk (Nc) were recorded after 4 hours. The percentage of repellence is determined using the formula

$$\text{Repellence (\%)} = \left(\frac{N_c - N_t}{N_c + N_t}\right) \times 100$$

where Nc is the number of termites on the untreated side and Nt is the number on the treated side. The experiment was replicated 3 times for each ratio to ensure robust statistical data.

3.6 Evaluation of the physiochemical parameters of the formulated termiticide (best overall performance.)

Table 3.3 Evaluation of the physiochemical parameters the formulation

Parameter	**Method/Instrument**	**Condition**
Viscosity	Viscometer	50 rpm
Thermal stability	Incubator observation test	45 °C for 48 h
pH	Digital pH meter	Direct measurement
Emulsion stability	Centrifuge phase-separation test	Visual assessment after centrifugation
Organoleptic properties	Visual and odour assessment	Colour and odour

1. **Viscosity**

The viscosity of the prepared ointment was measured using a viscometer at 50 revolutions per minute

2. **Thermal stability test**

The best overall termiticide was placed in a plastic bottle and stored in the incubator at 45 ℃ for 48 hours after which the product was observed for any physical change.

3. **pH of the formulated termiticide**

The pH of the formulation was measured using a digital pH meter.

4. **Emulsion Stability**

The emulsion stability was done through phase separation using a centrifuge.

5. **Organoleptic evaluation**

Colour and odour of the formulation were evaluated

3.7 Conclusion

This chapter described the materials, extraction procedures, phytochemical screening methods, quantitative analytical methods, formulation approach, and bioassay procedures used to develop and evaluate the plant-based termiticide. The methodology was designed to isolate tannins and terpenes from *Euphorbia tirucalli*, formulate them into different ratios, and assess their biological activity and physicochemical properties under controlled laboratory conditions. These procedures provided the framework for generating the results presented in the next chapter.

CHAPTER 4: RESULTS

4.1 Percentage Yield of Extracts

The table 4.1 illustrates the % yield of plant extract obtained by the maceration procedure.

The maceration process was used to extract tannins and terpenes from the prepared *Euphorbia tirucalli* plant material. The percentage yield for each extract was calculated based on the initial dry weight of the plant material.

Table 4.1: The maceration extraction process yielded 12.6 % for the tannin extract and 8.8 % for the terpene extract, calculated from an initial dry weight of 30.0 g for each.

4.2 Qualitative Phytochemical Screening

The phytochemical screening of the *Euphorbia tirucalli* extract detected the presence of Tannins and Terpenes.

Table 4.1: Percentage yield of tannin and terpene extracts from Euphorbia tirucalli

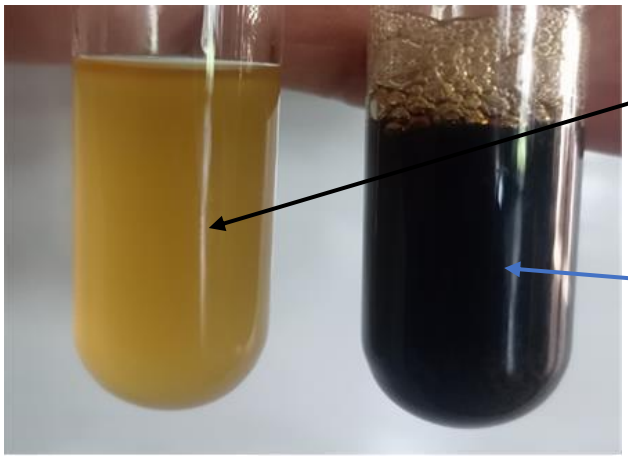
Extract Type	Initial Dry Weight (g)	final Extract Weight (g)	Percentage Yield (%)
Terpene Extract	30.0	2.64	8.8 %
Tannin Extract	30.0	3.78	12.6%

Table 4.2: Summary of qualitative phytochemical screening results for Euphorbia tirucalli extracts

Phytochemical group	**Test used**	**Observation**	**Result**
Tannins	Ferric chloride test	Blue-black colour developed	Positive
Terpenes/Terpenoids	Salkowski test	Reddish-brown colour observed	Positive
Terpenes/Terpenoids	Liebermann-Burchard test	Red to blue to green colour change	Positive

Tannins	Lead acetate test	white coloured precipitate	positive

After adding 10% Ferric chloride solution a blue-black colour was observed indicating the presents of tannins.

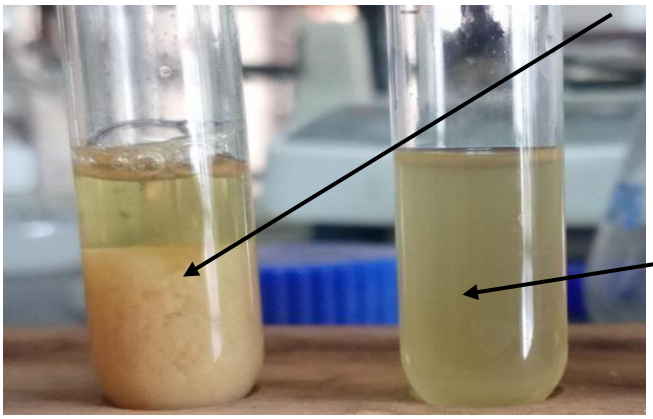


A negative control (plant extract without adding 10% ferric chloride for tannins determination)

Tested positive for the presence of tannins. The extract exhibited the presence of tannins, a group of polyphenolic compounds.

Figure 4.1: Ferric chloride test for the presence of tannins

d positive for the presence of tannins.



A negative control (Plant extract without adding lead acetate)

Figure 4.2: Lead acetate test for tannins

Terpenoids

Terpenes were identified in the extract.

Salkowski test for terpenes and Terpenoids tested positive with reddish-brownish colour

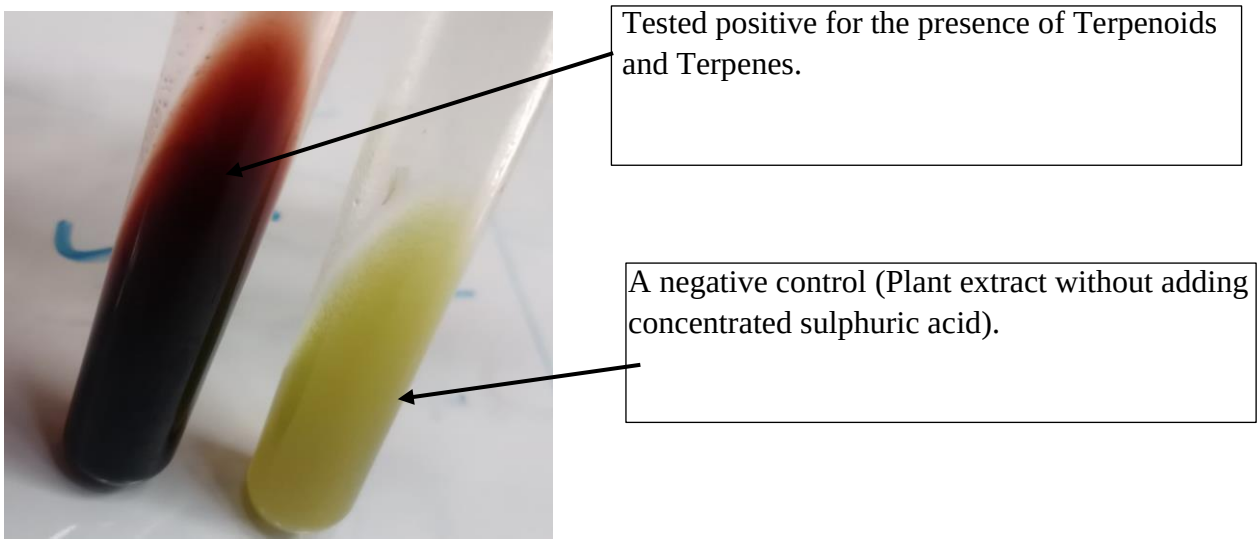


Figure 4.3: Salkowski test for terpenes and terpenoids

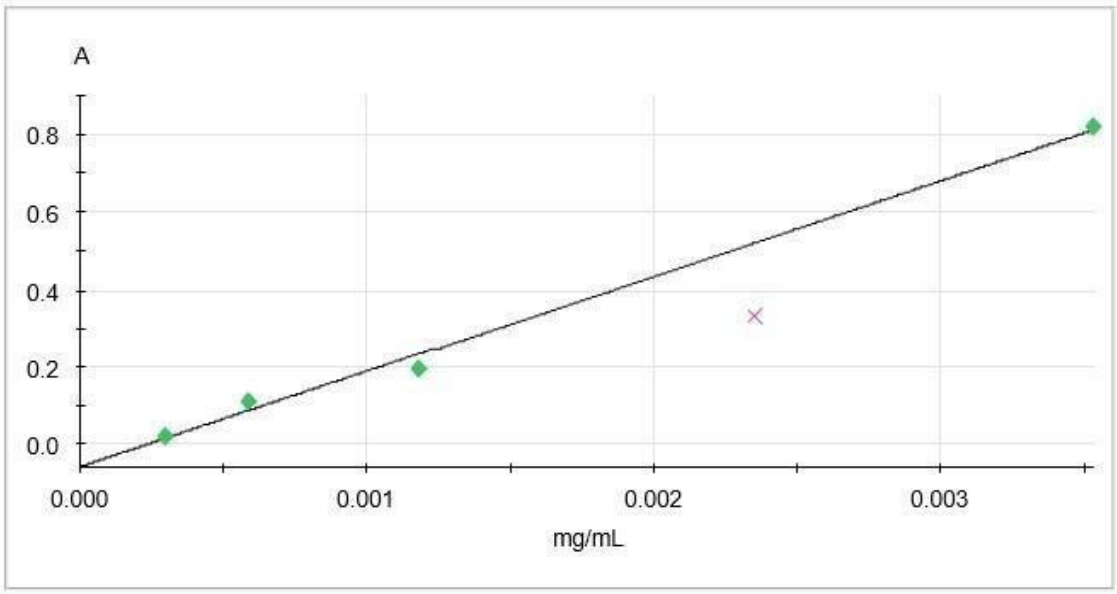
Liebermann-Burchard Test for terpenes and Terpenoids

Tested positive for terpenes and Terpenoids. A colour change, starting from red and gradually transitioning to blue and finally green, indicated the presence of terpenes.

4.3 Quantitative and Compositional analysis

4.3.1 Total Tannin Content Quantification (UV-VIS)

Total phenolic content was quantified in terms of total tannin content using the method of Folin-Ciocalteu. A standard calibration curve of known concentration of gallic acid was plotted. Figure 4.5 shows the plot of Absorbance at 765 nm vs Gallic Acid concentration (mg/mL). The total tannin content of the tannin extract sample based on the measured absorbance was calculated using the calibration curve and was found to be 168 mg GAE/g.



Curve parameters: y = 24.65466 x -5.917217E-03
Residual error: 0.0030 Correlation coefficient: 0.99768
Date: 06-Dec-25 9:02:55 AM

Figure 4.5 Gallic acid calibration curve used for UV-VIS quantification of total tannin content.

Standards

No.	Concentration [mg/mL]	Ordinate [A]	Error [A]	Used
1	0.0029	0.02	0.001	Yes
2	0.0059	0.11	0.002	Yes
3	0.0118	0.20	-0.004	Yes
4	0.0235	0.33	-0.019	No
5	0.0353	0.82	0.001	Yes

Sample	Dilution Factor	Ordinate [A]	Concentration [mg/mL]
ethanol 1	300	1.305	0.53167
ethanol 2	300	1.184	0.48251
ethanol 3	300	1.378	0.56112

Figure 4.6: UV-VIS output used to estimate the tannin content of the Euphorbia tirucalli extract.

4.3.2 GC–MS Analysis of Terpenes and Terpenoids

Terpenoid Composition (GC–MS, EMA Report)

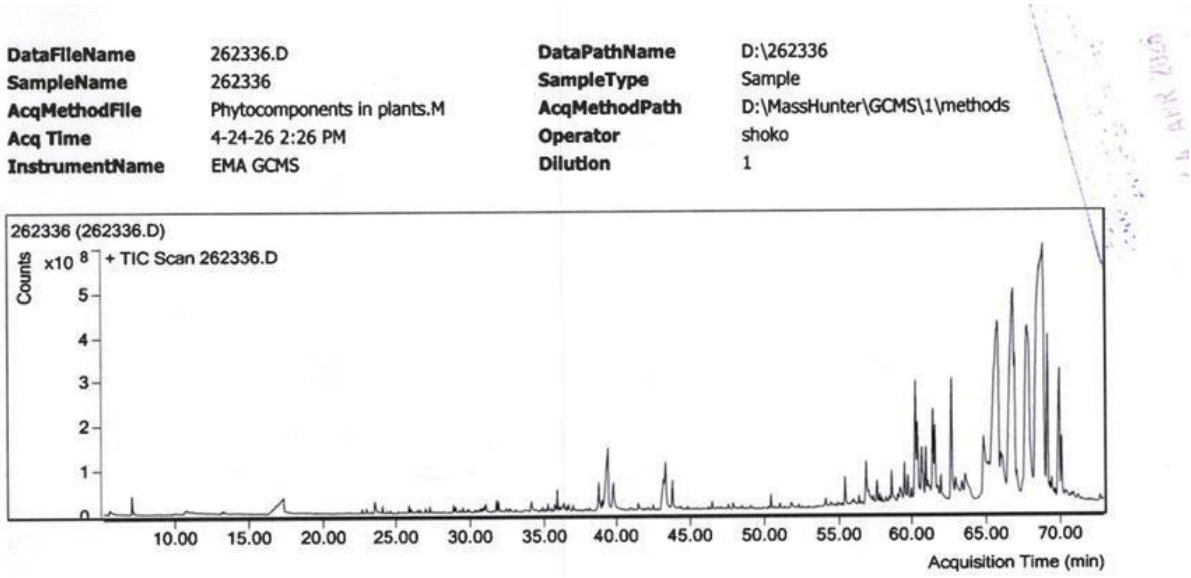


Figure 4.7 GC-MS chromatographic profile and report output for the terpene-rich extract of Euphorbia tirucalli

Table 4.3 Major terpenoid classes and relative composition identified by GC–MS

Class	**Major components**	**Relative %**
1. Monoterpenes	Limonene	4.1%
2. Sesquiterpenes	Caryophyllene, γ-Muurolene, α-Copaene, Spathulenol, Ledene oxide, α-Cadinol, δ-Cadinene, α-Humulene, β-Selinene α-Muurolene	41.2%
3. Diterpenes	Neophytadiene, Phytol, Phytol acetate.	7.9%
4. Triterpenes	β-Amyrin, Squalene, A-Neooleana-3(5),12diene	14.7%

5. Sterols / Derivatives	Ergost-5-en-3-ol, Cholest-5-en-3-ol, Cholest-5-en-3-ol acetate, γ-Tocopheryl methyl ether	32.1%

GC- MS of the *E. Tirucalli* extract detected a total of 25 terpenoids. All major terpenoid classes were present in the chromatographic profile and represented as monoterpenoids, sesquiterpenoids, diterpenoids, triterpenoids and sterols. Monoterpenoids only appeared in minor quantities in the GC profile and Limonene eluted at the retention time of 10.777 and represented 4.1% of the total profile. The biggest fraction detected was sesquiterpenoids at 41.2% with caryophyllene, muurolene, copaene, spathulenol, ledene oxide derivatives, cadinol, cadinene, humulene, selinene and muurolene represented in this fraction. The presence of 3 diterpenoids was noted in the fraction with Neophytadiene, Phytol, Phytol Acetate contributing 7.9%, whilst the 3 triterpenoids and related compounds were detected at 14.7% and were A Neoleana-3(5),12-diene, Squalene and β-Amyrin. Sterols and steroids were the second largest fraction at 32.1%, the compounds represented were ergost-5-en-3-ol, cholest-5-en-3-ol, cholest-5-en-3-ol Acetate, and Tocopheryl Methyl Ether. From all of these it was possible to assign the structure ergost-5-en-3-ol as being the main peak and eluting with a peak area of 369,036,223 therefore this would be considered the most abundant compound in the *E. Tirucalli* extract.

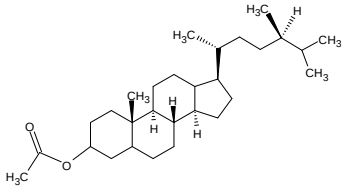
Major Peaks: Dominant Terpenoids.

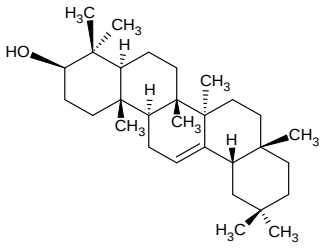
From the GC–MS, five major or dominant terpenoid components were identified. They are ergost-5-en-3-ol (steroid, peak area 369,036,223), β-Amyrin (triterpenoid, 215,972,857), Neophytadiene (diterpenoid, 148,942,309 + 43,735,066), Spathulenol (sesquiterpenoidoxide,61,364,586) and Caryophyllene (sesquiterpenoid, 49,388,333). Among the components, ergost-5-en-3-ol followed by β-Amyrin and Neophytadiene are abundant. The molecular structures of the top three components are given below.

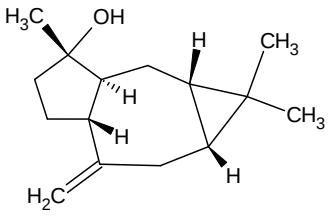
Table 4.4 Dominant terpenoid compounds and peak areas identified by GC–MS

Compound	Class	Peak Area
Ergost-5-en-3ol	Sterol	369,036,223
β-Amyrin	Triterpene	215,972,857
Spathulenol	Sesquiterpene oxide	61,364,586
Caryophyllene	Sesquiterpene	49,388,333
Neophytadiene	Diterpene	148,942,309 + 43,735,066

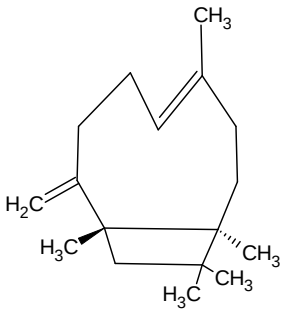
1. Ergost-5-en-3ol 2. β-Amyrin 3. Spathulenol







4. Caryophyllene 5. Neophytadiene



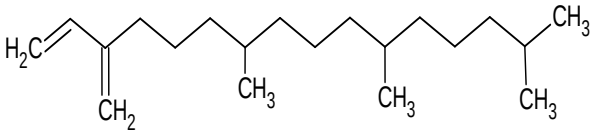


Figure 4.8: Structural representations of selected dominant terpenoid compounds identified by GC-MS.

Mass Spectra's for major Terpenes

1. **Neophytadiene**

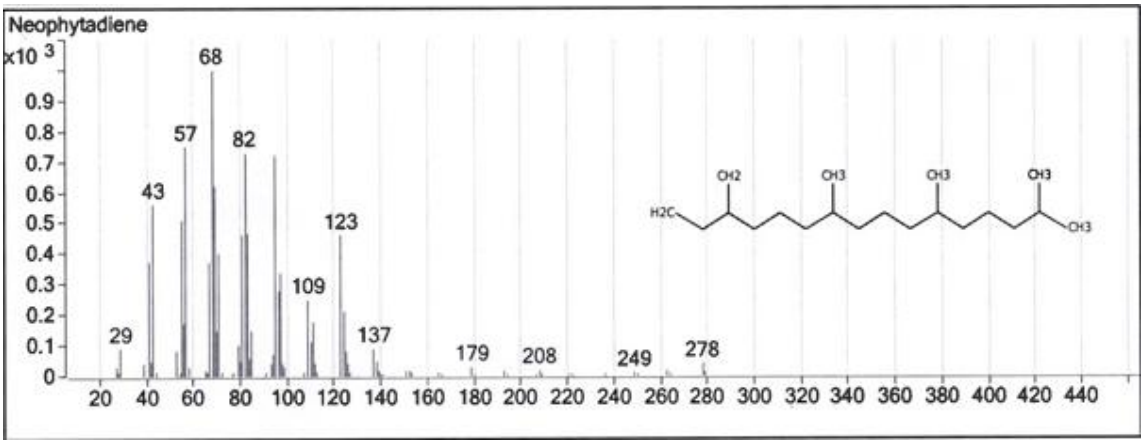


Figure 4.9: Mass spectrum of Neophytadiene from GC-MS library

Mass spectrum of Neophytadiene that was found in the terpene abundant extract of *Euphorbia tirucalli*. Key fragmentation peaks with base peak at m/z 68 showed similarity with mass spectra of reference spectra in GC-MS library and confirming the identity of the compound.

2. β-Amyrin

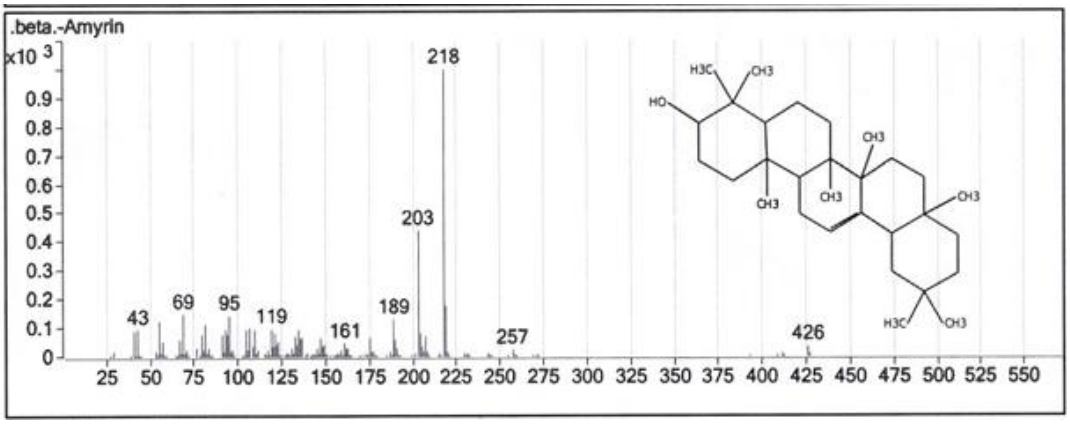


Figure 4.10: Mass Spectrum of β-Amyrin from GC-MS library

Mass spectrum of β-Amyrin found in the terpene rich extract of *Euphorbia tirucalli*. Fragment patterns and largest ions peaks obtained were in good match with GC-MS reference library

spectra and confirms the presence of compound β-Amyrin is a biologically active terpene which gives termiticidal effects to the formulation.

3. Caryophyllene

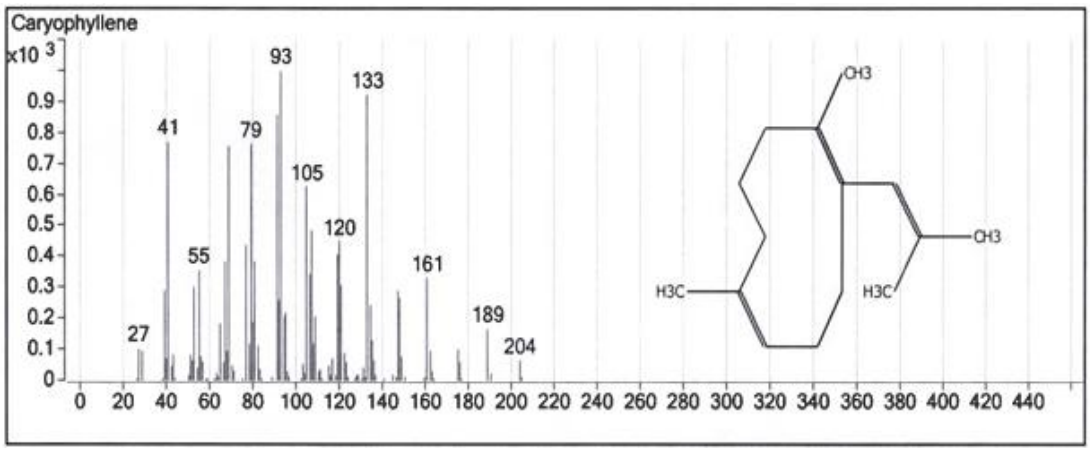


Figure 4.11: Mass Spectrum of Caryophyllene from GC-MS library

Mass spectrum for Caryophyllene identified within the terpene rich extract from *Euphorbia tirucalli*. The presence of fragmentation patterns typical of this compound and major ion peaks which match those found within a GC-MS reference library were utilized to determine compound identity. Caryophyllene is an active terpene which contributes to both the insecticidal and termiticidal properties of the formulation**.**

4. Ergost-5-en-3-ol

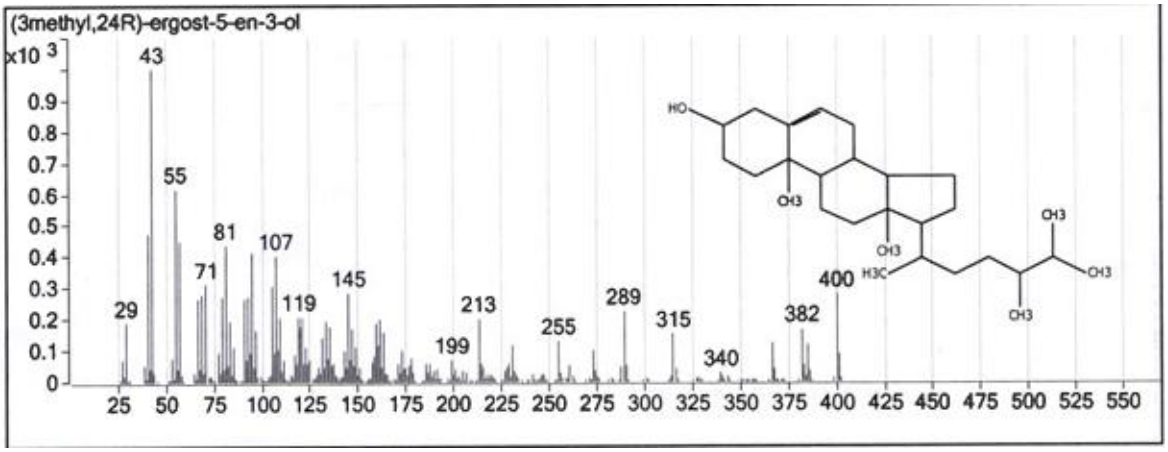


Figure 4.12: Mass Spectrum of Ergost-5-en- 1 from GC-MS library

Mass spectrum of Ergost-5-en-3-ol in terpene rich extract of *E. tirucalli*, whose Fragmentation and main ions were identified with the GC-MS reference library spectra indicating the presence of the compound. Ergost-5-en-3-ol accounts for the biological and termiticidal activities of the formula.

5. **Spathulenol**

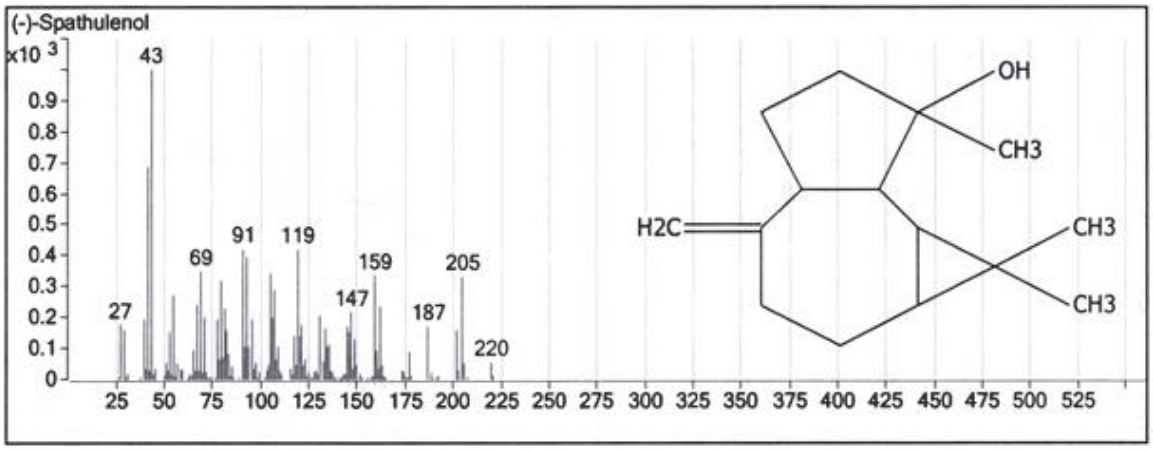


Figure 4.13: Mass spectrum of spathulenol from GC-MS library

Mass spectrum of Spathulenol isolated from the terpene-rich fraction of *Euphorbia tirucalli*. Mass spectrum was similar to GC-MS library spectra and showed the fragmentation pattern, key ion peaks indicated Spathulenol. Spathulenol provides termiticidal activity to the formulation.

Different Terpene: Tannin termiticide formulations

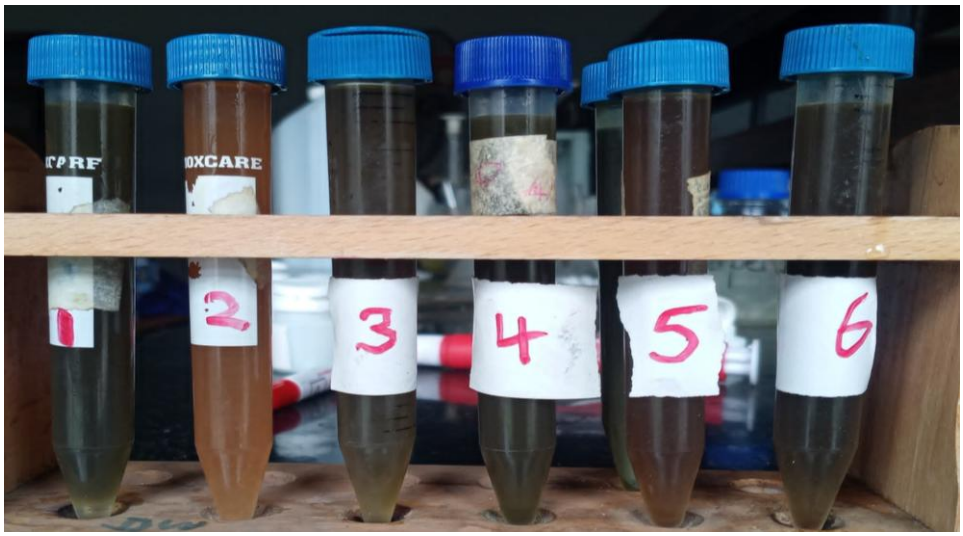


Figure 4.14: Plant-based termiticide formulations prepared from different terpene: tannin ratios of Euphorbia tirucalli extracts.

Plant-based termiticides of different ratios of terpene and tannin from *Euphorbia tirucalli*. The ratios terpene to tannin used in formulation were 100:0, 0:100, 80:20, 20:80, 50:50, 60:40 and 40:60 of terpene: tannin ratio respectively

4.4 Bioassay Results

The results of the contact toxicity, fumigant, repellence, antifeeding, and soil barrier assays are presented in the following subsections.

4.4.1 Contact Toxicity Assay

30 soldier termites were exposed directly to a film of each formulation. Mortality was recorded after 24 hours. Mortality results were presented as Mean ± SD (n = 3).

Table 4.5: Contact toxicity assay results for different terpene: tannin formulations

Formulation (Terpene: Tannin)	Mean Mortality % after 24hr
control	1.1±0.6
100:0	98.9±0.2
0:100	46.7±1.8
50:50	81.1±1.5
80:20	97.8±1.0
20:80	60.0±1.7
60:40	88.9±1.3
40:60	82.2±1.4

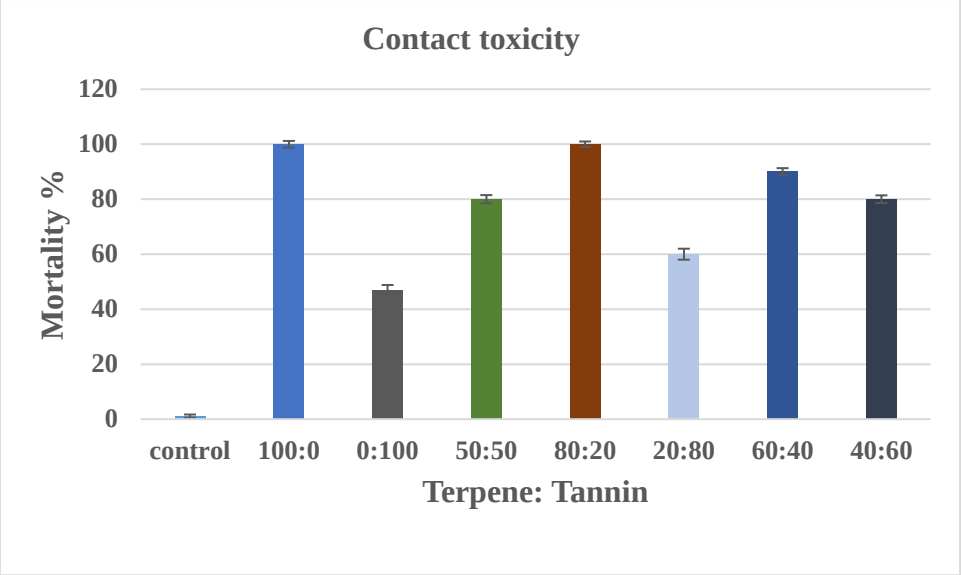


Figure 4.14: Contact toxicity responses of termites exposed to different terpene: tannin formulations after 24 hours.

Figure 4.10 shows the contact toxicity of terpene: tannin formulation against termites after 24hr exposure. Only (1.1±0.6 %) mortality was observed in the non-treated control, suggesting that termite death resulted from the treatment application. Higher mortalities were obtained for formulation 100:0 (98.9±0.2%)and 80:20 (97.8±1.0%). The 0:100 formulation showed the lowest mortality among the applied treatments (46.7±1.8%). The remaining formulations exhibited from moderate to high effectiveness. Mortality ranged from (60.0±1.7%)in the formulation 20:80 to (88.9±1.3%) in the formulation 60:40. Higher proportions of terpene in the formulation resulted in higher termite mortality, thus, the contact toxicity was mainly contributed by the terpene and perhaps through interaction with tannins resulting in some positive interaction.

4.4.2 Fumigant Assay

10 adult termites were exposed to the volatile components of each formulation without direct contact. Mortality was recorded after 24 hours and expressed as a percentage of individuals dead out of the total exposed. Fumigant mortality values were expressed as Mean ± SD (n = 3)

Table 4.6: Fumigant assay results for different terpene: tannin formulations

Formulation (Terpene :Tannin)	**Mean Mortality % after 24hr**

Control	0.0±0.0
100:0	70. 0 ± 2.1
0:100	0.0±0.0
50:50	40.0±1.8
80:20	70. 0 ± 1.2
20:80	20.0±2.4
60:40	60.0±2.7
40:60	40.0±1.5

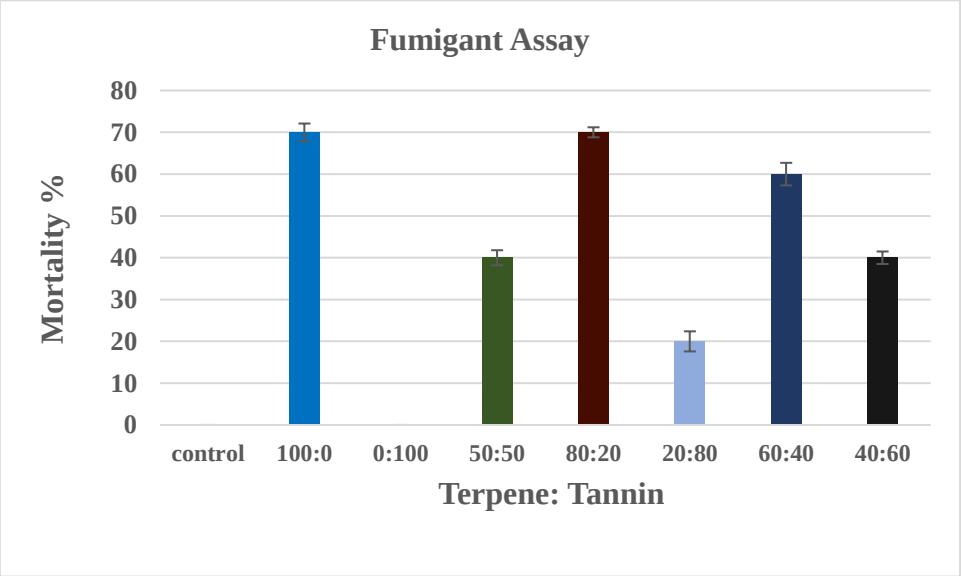


Figure 4.15: Fumigant mortality responses of termites exposed to volatile components of different terpene: tannin formulations.

All treatments of fumigant toxicity assay were performed in replicates of three, with readings taken 24h after treatment, soldier termites were directly exposed to the vapor created by the formulations. There was a high mortality of (70. 0 ± 2.1%) and (70. 0 ± 1.2%) both when terpene alone was used (100:0) and when the mix of terpene and tannin was used (80:20) confirming terpene as the primary fumigant agent, no mortality was recorded when (0:100) was used. Mixtures that were balanced (50:50, 40:60 and 60:40) resulted in medium mortality (40±1.5% to 60±2.7%), demonstrating a partial synergism, while, at (20:80) tannin alone produced weak mortality of (20±2.4%) showing that tannin dominated formulations have low to no fumigant activity.

4.4.3 Repellence Assay

10 adult termites were introduced into each petri dish which contained the Emulsifiable concentrate with 7different terpene and tannin ratios, which was then sealed promptly with a perforated lid to allow for ventilation. % repellence was recorded for each and every hour for 4 hours. Repellence percentages were reported as Mean ± SD (n = 3).

Table 4.7 : Repellence assay results over 4 hours for different terpene: tannin formulations

Formulation (Terpene: Tannin)	**%Repellence 1hr**	**%Repellence 2hr**	**%Repellence 3hr**	**%Repellence 4hr**
Control	1.0± 1.0	0.3 ±0.5	0.0±0.0	0.0±0.0
100:0	98.0 ± 2.1	97.0 ± 3.0	79.7 ± 3.5	78.3±4.0
0:100	19.3 ± 2.5	17.0 ± 2.6	1.0±1.0	0.3±0.5
50:50	79.0 ±4.0	59.0±4.6	19.3 ± 3.6	17.7±4.0
80:20	98.3±1.5	98.3 ± 1.5	95.0 ± 2.0	76.7±4.6
20:80	37.7 ± 4.5	19.3 ±2.5	1.7±1.5	0.3. ±0.5
60:40	80. 0 ± 3.5	58.3 ±4.5	39.3±3.6	38.3±3.5
40:60	60.7 ±3.5	40. 0 ± 3.0	39.0 ± 3.0	19.3±3.5

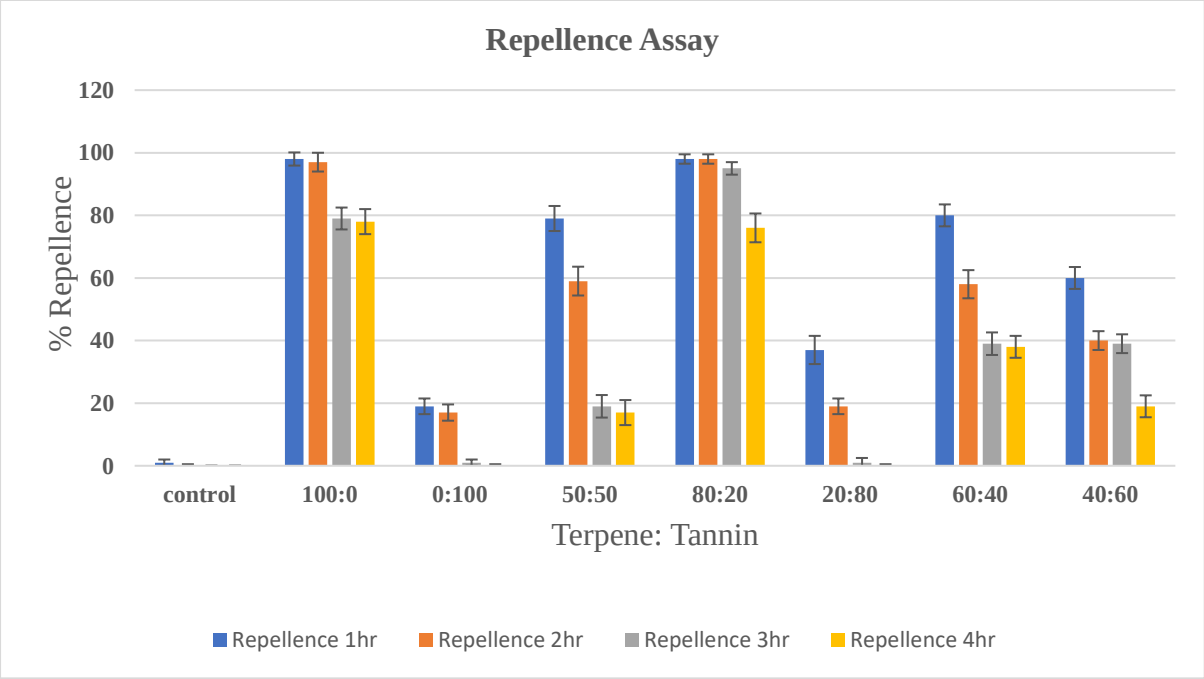


Figure 4.16: Repellence of termites over 4 hours for different terpene: tannin formulations.

Terpene dominated mixtures (100:0 and 80:20) are the only to exhibit good and long-lasting repellence, achieving78.3±4.0 % and 76.7±4.6% avoidance respectively after 4 hours of exposure. Balanced mixtures (50:50, 60:40 and 40:60) demonstrate moderate repellence that diminishes with time (17.7±4.0% to 38.3.0±3.5% by 4 hours), whereas tannins alone (0:100) and the tannin dominated mixtures (20:80) displayed poor and transient repellence, diminishing to 0% and 1.7±1.5% respectively by 3 hours. Thus, terpene dominance is key to producing sustained repellence and the impact of tannins appears minimal in the vapor-phase.

4.4.4 Antifeeding Assay

The reduction in food consumption by termites was measured after 7 days of exposure to treated reeds. Antifeeding activity values were expressed as Mean ± SD (n = 3). Twenty termites were used for each replicate.

Table 4.8: Antifeeding assay results for different terpene: tannin formulations

Formulation (Terpene: Tannin)	Mean Reed Mass Loss (Mg)- control	Mean Reed Mass Loss (mg)-treated	Antifeeding Activity (%)
control	45.2	38.2±0.3	15.5±0.7
100:0	45.2	18.1±0.4	60.0±1.3
0:100	45.2	3.2±0.2	92.9±0.5
50:50	45.2	5.0±0.3	88.9±0.9
80:20	45.2	6.3±0.3	86.1±0.8
20:80	45.2	3.9±0.4	91.4±0.9
60:40	45.2	5.4±0.5	88.1±1.0

40:60	45.2	4.6±0.4	89.8±0.9

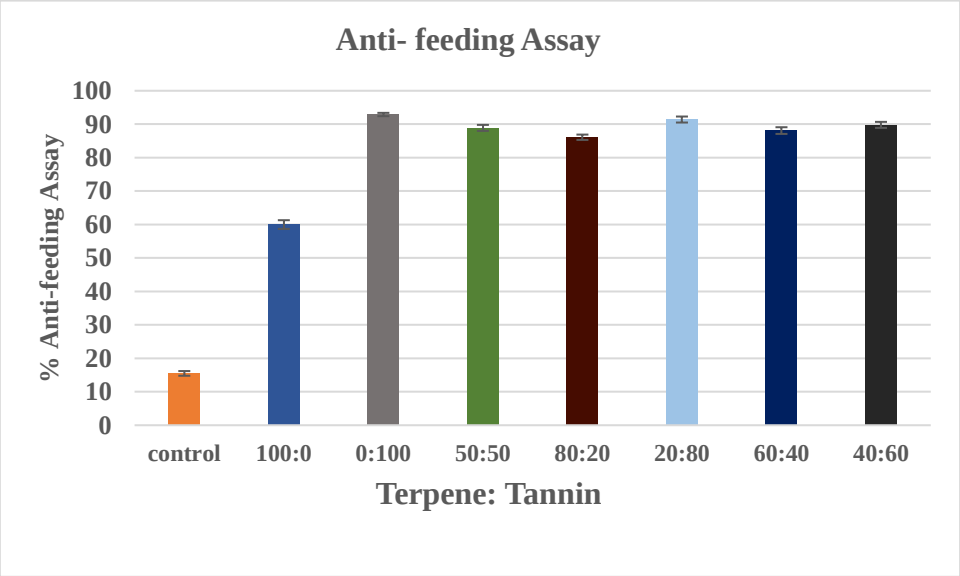


Figure 4.17: Antifeeding activity of different terpene: tannin formulations based on reduction in reed consumption

The antifeeding assay was conducted using reed as substrates and each treatment had three replicates. In each of the replicates, the reed mass loss by control sample was 45.2 mg and it was reduced as a function of terpene: tannin ratio in treatment samples. The samples that were high in tannin concentration (0:100, 20:80, 40:60) had most potent antifeeding activity (>89.8±0.9%), whereas terpene-alone (100:0) had weakest antifeeding activity (60.0±1.3%). Mixtures with balanced components (50:50, 60:40, 80:20) displayed sustained high activity (86.1±0.8% to 89.8±0.9%), implying synergism. Majority of samples with dominance of tannins showed greater antifeeding activity whereas terpene gave protection with a certain extent showing that tannins has more anti feeding properties compared to terpenoids.

4.4.5 Soil Barrier Assay

The effectiveness of treated soil in preventing termites from crossing was evaluated. Soil barrier effectiveness results were presented as Mean ± SD (n = 3).

Table 4.9: Soil barrier assay results for different terpene: tannin formulations

Formulation (Terpene: Tannin)	Termites crossed (control)	Termites Crossed (treated)	Soil Barrier effectiveness (%)

Control	28	27,28,27	2.4±0.7
100:0	28	3,4,2	89.3±3.1
0:100	28	11,10,12	60.7±2.5
50:50	28	6,6,5	79.8±2.8
80:20	28	2,1,3	92.9±2.2
20:80	28	9,10,8	67.9±2.7
60:40	28	5,4,6	82.1±2.4
40:60	28	8,8,9	70.2±2.6

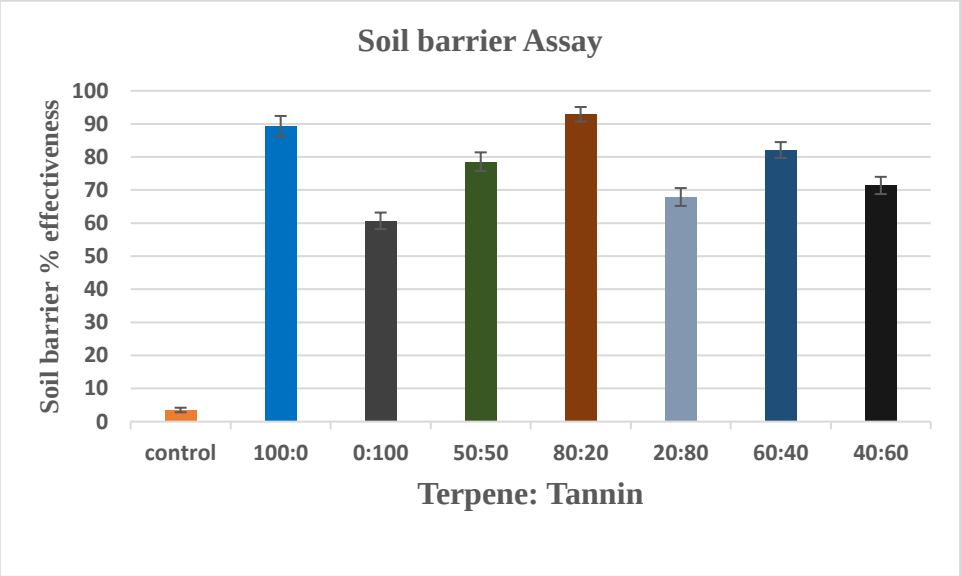


Figure 4.18: Soil barrier effectiveness of different terpene: tannin formulations against termite crossing.

The soil barrier assay consisted of 28 termites per replicate, with each treatment being run 3 times to provide accurate results. Different ratios of terpene and tannin in the soil barrier performed differently in blocking termites. Soil treatments containing high proportions of terpene were most effective, the soil mixture consisting of 80:20 terpene: tannin produced the best barrier effect (92.9±2.2%). Tannin alone gave a medium protection effect and mixtures of the two were synergistic.

4.5 Integrated Assessment of Bioassay Performance

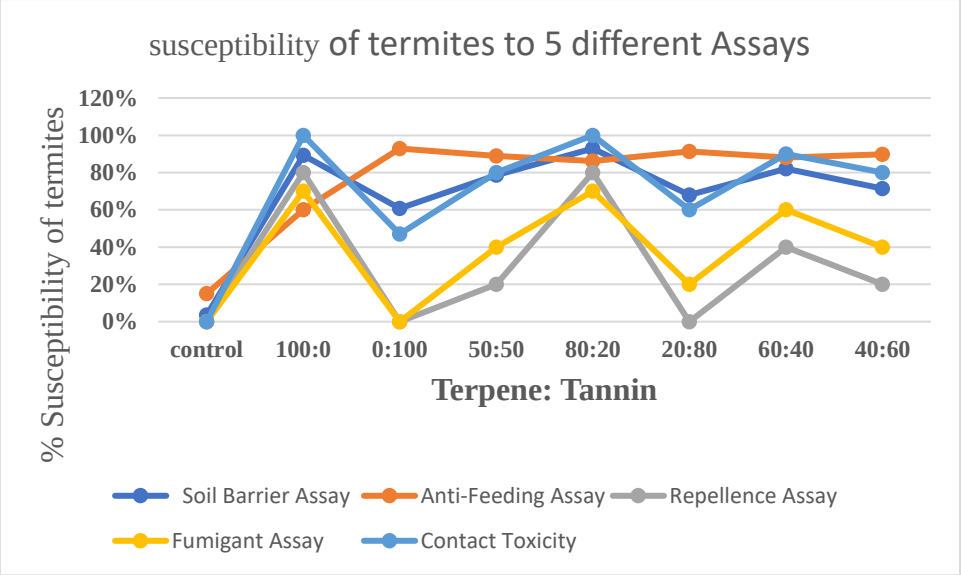


Figure 4.19: Integrated comparison of formulation performance across contact toxicity, fumigant, repellence, antifeeding, and soil barrier assays.

Table 4.10: Overall comparison of bioassay performance across terpene: tannin formulations

Formulation	**Contact toxicity**	**Fumigant activity**	**Repellence**	**Antifeeding**	**Soil barrier**	**Overall interpretation**
100:0	Very high	High	Very high	Moderate	High	Strong terpene-driven activity
0:100	Low	Very low	Low	Very high	Moderate	Best for feeding inhibition
80:20	Very high	High	Very high	High	Very high	Best overall formulation
50:50, 60:40, 40:60	High	Moderate	Moderate	High	Moderate to high	Balanced synergistic performance
20:80	Moderate	Low	Low	Very high	Moderate	Tannin-dominant with

						limited volatile action

When the results of the five bioassays were integrated, the 80:20 terpene: tannin mixture had the highest all-around performance, having effective contact toxicity, repellence, and effective soil barrier properties. Tannin-rich mixtures had the strongest effects in antifeeding tests, but did less well in fumigant and repellence bioassays. Terpenes therefore appeared to mediate the high acute toxicity and volitant deterrence observed, while the tannins appeared to mediate feeding inhibition more effectively. The performance of mixtures suggests that the two types of compounds in combination yielded better overall results than either would in isolation supporting the hypothesis of complementary or synergistic action in termite control.

4.6 Physicochemical Properties of the Selected Formulation

Based on the superior performance in the contact toxicity and soil-barrier assays and across all other assays, the 80:20 (Terpene: Tannin) formulation was selected for physicochemical evaluation.

Table 4.11: Physicochemical properties of the selected 80:20 formulation

Parameter	**Result**
Colour	Milky, pale-yellow emulsion
Odour	Strong, sharp herbal and citrus-like scent
pH	6.8
Thermal Stability (45°C, 48h)	No phase separation, slight deepening of colour.
Emulsion Stability (Centrifuge)	No phase separation

4.7 Conclusion

This chapter showed the yield obtained for tannin and terpenoid extraction, phytochemical screening carried out, tannin content determined, composition determined by GC-MS analysis, performance on bioassays, and physical and chemical parameters for the selected plant-based termiticide. It has also showed that *Euphorbia tirucalli* has the presence of both tannins and terpenes. Tannin extraction resulted in a higher yield in percentage as compared to extraction of terpenes. From the GC-MS analysis it was revealed that the extraction yielded several groups of terpenoids and on testing through the various bioassays it was observed that generally those that were enriched in terpenes and mixed formulations fared well when evaluated for contact toxicity, fumigant activity, repellence and soil barrier action but on the other hand tannins played a more significant role in antifeeding actions of the formulation. From these formulations it was also established that a ratio of 80:20 of terpene to tannin had an optimum performance and it was tested for its physical and chemical parameters. From these results it was possible to derive the conclusions that were made in the following chapter.

CHAPTER 5: DISCUSSION

5.1 Phytochemical & Extractive Data interpretation

The phytochemical screening conducted as part of this investigation confirmed that *Euphorbia tirucalli* possesses phytochemicals with termicidal potential. Larger yield for tannin rich fraction than terpene rich fraction suggested that the more polar and semi polar constituents were more efficiently extracted under the selected parameters. This observation is consistent with previous studies reporting efficient recovery of tannins during ethanol extraction of phenolic compounds.(Fonmboh *et al.*, 2020; Singh *et al.*, 2022). Qualitative phytochemical screening further indicated the presence of tannins and terpenes in the plant, rendering it suitable for Multi-component Botanical Formulations (Lengai, Muthomi and Mbega, 2020).

5.2 Tannin Quantification and GC-MS data interpretation

The tannin content of the extract was measured to be 168mg GAE/g extract, thereby indicating a considerable number of phenolic compounds. Tannins have already shown to possess properties that hinder termite digestion, reduce palatability and exert antifeeding action through interaction with protein components in the insect gut(*Barbehenn* and *Peter Constabel*, 2011) .The results from the GC-MS analysis of the terpene-rich fraction revealed that many compounds found in the terpene fraction belonged to sesquiterpenes, diterpenes, triterpenes and sterols class of compounds. These compounds included ergost-5-en-3-ol, β-amyrin, neophytadiene, spathulenol and caryophyllene which have shown insecticidal, repulsive and membrane-disruptive properties (Acheuk *et al.*, 2022; Demouche *et al.*, 2023; Kuvawoga *et al.*, 2025b). Taken together, these findings provide strong evidence that the phytochemicals identified in both fractions contribute to the observed termiticidal activity.

5.3 Bioassay performance explanation

From the various bioassays conducted it was clear that the two formulations differed in their mechanism of action against the termites. The percentage mortality in contact toxicity assay increased steadily until the 80:20 formulation and then it reduced down considerably as the quantity of tannins increased further in the formulation. Thus, terpenes were predominantly responsible for the quick death of the termites via the toxic and membrane disruptive mechanism characteristic of volatile and lipophilic compounds (Acheuk *et al.*, 2022). The same

phenomenon was observed in both the fumigant toxicity and repellent bioassay as the terpene fraction performed much better than the tannin fraction. These were the bioassays dependent on the volatile nature of the compounds and it reaffirmed the well-known behavior modifying properties of the terpene fraction against insects (Kuvawoga *et al.*, 2025b). Conversely, higher feeding deterrence activity was observed in tannin-dominated fractions, which is consistent with the findings of Barbehenn and Constabel (2011), who reported that tannins reduce insect feeding by decreasing protein digestibility and palatability. The terpene dominated formulations had higher barrier action in soil barrier assay particularly the 80:20 formulation. This was due to combined toxicity, repellence and feeding deterrence properties against termites. It is therefore clear that the two classes of compounds act differently on the termites.

5.4 Integrated performance of the 80:20 formulation

The best overall formulation was found to be the 80:20 formulation. The 100:0 terpene formulation showed excellent contact and repellent activity but 80:20 formulation performed well for both contact and repellent assay, as well as fumigant assay, barrier activity and also for feeding deterrence. It clearly indicated that terpenes and tannins work synergistically and also exhibit complementary activities to each other(Lengai, Muthomi and Mbega, 2020). Inclusions of even smaller amounts of tannins with the terpene fraction enhance the overall performance while not drastically lowering the activity provided by the terpene fraction. These findings suggest that the 80:20 formulation is the most promising candidate for the development of an integrated termite management product.

5.5 Limitations of the present study & further considerations for a field trial

A limitation of the present study is that the entire experimental study was carried out under controlled laboratory conditions and the action against the termites under field conditions might be different due to factors like variation of soil pH, soil moisture content, soil composition, temperature etc. Further detailed analysis like more statistical treatment is warranted for certain bioassays and also further studies for field performance should be done against more species of termites to develop a reliable and effective formulation for use. The formulated product also needs to be tested for its formulation stability and shelf life before it can be adopted for the commercial market.

5.6 Conclusion

The findings of this study were consistent with the known biological activities of tannins and terpenes. The results support the hypothesis that these phytochemicals contribute to termite control through complementary mechanisms. Terpene-rich formulations were associated with rapid mortality, fumigant activity and repellence, whereas tannin-rich formulations were more effective as antifeedants. The 80:20 terpene: tannin formulation combined these desirable properties and therefore demonstrated the most balanced and effective overall performance.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

A termiticidal formulation composed of tannin and terpene fractions extracted from *Euphorbia tirucalli* was successfully formulated and characterized. The presence of tannins (12.6%) and terpenes (8.8%) from extracts of *Euphorbia tirucalli* indicated that the plant is rich in bioactive secondary metabolites. From chemical screening and quantitative studies, it has been confirmed that these extracts were rich in both phenols and terpenoids. Gas Chromatography-Mass Spectrometry (GC-MS) revealed that the main terpene constituents in the extracts were ergost-5-en-3-ol, β-Amyrin, neophytadiene, spathulenol and caryophyllene. These comprise the major chemical groups (sterols, triterpenes, diterpenes and sesquiterpenes) shown to exhibit insecticidal, repellent and antifeedant properties against insects (Bekele, 2014; Kuvawoga *et al.,* 2025b)and likely contribute to the plant's natural defence mechanisms . In the bioassays conducted, it was found that the tannin-rich and terpene-rich formulations produced effective termiticidal results. Terpenoids exhibited insecticidal, repellent and fumigant activities whereas tannins primarily exhibited antifeedant activity. Broad-spectrum activity including toxicity, repellence, fumigant action, soil barrier protection and antifeedant activity was achieved from a ratio of 80:20 of terpene: tannin. The terpene-rich emulsions were highly effective in the soil barrier assay with no infestation recorded ($92.9 \pm 2.2\%$). The observed termiticidal effects can be explained by the known biological activities of these phytochemicals(Acheuk *et al.*, 2022). Terpenoids cause damage by interfering with respiration and neurological functions or are simply through neurotoxic, membrane-disruptive and respiratory effects(Acheuk *et al.,* 2022; Kuvawoga *et al.,* 2025a). Tannins primarily affect the insect's digestive system by inhibiting digestive processes, and reducing nutrient absorption and feeding efficiency(*Barbehenn* and Peter Constabel, 2011).

6.2 Recommendations

1. Field trial and validation

There is need for field trials and validation of *Euphorbia tirucalli* derived termiticide using several termite species, particularly *Macrotermes* spp, in different ecological settings in Zimbabwe. Termite control efficacy under various soil and termite infested scenarios needs

long term testing and effectiveness/impact of formulation to the environment should also be investigated.

2. Optimization of the formulation

Future studies may focus on the optimal ratio of tannins and terpenes that would offer maximum insecticidal, repellent and antifeedant activities. Use of appropriate emulsifying and stabilizing agents in enhancing the stability and shelf life of the formulation could also be tested.

3. Large scale production

The extraction and formulation processes should be optimized to facilitate the large-scale production of the termiticide. Economical manufacturing techniques and best formulation approaches need to be evaluated.

4. Integration in IPM

Future studies should evaluate the integration of *Euphorbia tirucalli*-based termiticides into Integrated Pest Management (IPM) programmes and assess their compatibility with existing pest management strategies in order to reduce reliance on synthetic pesticides(Lengai, Muthomi and Mbega, 2020).

5. Environmental and toxicological evaluation

The effects on the environment should be assessed comprehensively by evaluating the environmental and toxicological effects of the formulation on non-target organisms including soil microbes, wildlife, aquatic organisms, livestock and humans. Information gained regarding non-target species should be compared with effects associated with synthetic pesticides used.

6. Further phytochemical investigations

More in-depth studies aimed at the isolation, purification and identification of specific bioactive termiticidal compounds are recommended. Once purified and characterized, such compounds should be evaluated individually and in combination to determine their contributions to termiticidal activity and to elucidate their mechanisms of action.

7. Study of storage stability

Tests to determine the storage stability of the termiticide formulation under different storage conditions including temperature, humidity and light need to be conducted.

REFERENCES

Acheuk, F. *et al.* (2022) 'Status and Prospects of Botanical Biopesticides in Europe and Mediterranean Countries', *Biomolecules*, 12(2), p. 311. Available at: https://doi.org/10.3390/BIOM12020311/S1.

Barbehenn, R. V. and Peter Constabel, C. (2011) 'Tannins in plant-herbivore interactions', *Phytochemistry*, 72(13), pp. 1551–1565. Available at: https://doi.org/10.1016/J.PHYTOCHEM.2011.01.040.

Bekele (2014) 'Larvicidal and Adulticidal Effects of Extracts from Some Indigenous Plants against the Malaria Vector, Anopheles arabiensis (Diptera: Culicidae) in Ethiopia', *Journal of Biofertilizers & Biopesticides*, 05(02). Available at: https://doi.org/10.4172/2155-6202.1000144.

Biswa, R. (2024) 'Termites as Ecosystem Engineers : Their Role in Soil Health and Biodiversity', 14(694), pp. 165–168.

Coulibaly, A. *et al.* (2020) 'Acaricidal activity of three plants extracts from the central region of Burkina Faso on adult Rhipicephalus (Boophilus) microplus cattle ticks', 14(June), pp. 1511–1519.

Damme, S.P. Van (2011) 'Evaluation of pesticidal properties of *Euphorbia tirucalli L* . (Euphorbiaceae) against selected pests Tedsonjulius Mwine', pp. 119–121.

Davis, E.M. and Croteau, R. (2000) 'Cyclization Enzymes in the Biosynthesis of Monoterpenes , Sesquiterpenes , and Diterpenes', 209.

Debelo, D.G. and Degaga, E.G. (2015) 'Farmers ' knowledge , perceptions and management practices of termites in the central rift valley of Ethiopia', 10(36), pp. 3625–3635. Available at: https://doi.org/10.5897/AJAR2014.9283.

Demouche, L. *et al.* (2023) 'Insecticidal activity and physiopathological effects of Cotula cinerea crude extract against Culex pipiens', *Tropical Biomedicine*, 40(2), pp. 241–249. Available at: https://doi.org/10.47665/tb.40.2.016.

Ernst, M. *et al.* (2019) 'Assessing Specialized Metabolite Diversity in the Cosmopolitan Plant Genus Euphorbia L', *Frontiers in plant science*, 10. Available at: https://doi.org/10.3389/FPLS.2019.00846.

Florence, E., Nyagumbo, I. and Mafongoya, P. (2016) 'Termite prevalence and crop lodging under conservation agriculture in sub-humid Zimbabwe', *Crop Protection*, 82, pp. 60–64. Available at: https://doi.org/10.1016/j.cropro.2016.01.004.

Fonmboh, D.J. *et al.* (2020) 'An Overview of Methods of Extraction , Isolation and Characterization of Natural Medicinal Plant Products in Improved Traditional Medicine Research', 9(August), pp. 31–57. Available at: https://doi.org/10.9734/AJRIMPS/2020/v9i230152.

Furlong, M.A. *et al.* (2020) 'International Journal of Hygiene and An epigenome-wide association study of ambient pyrethroid pesticide exposures in California ' s central valley', *International Journal of Hygiene and Environmental Health*, 229(November 2019), p. 113569. Available at: https://doi.org/10.1016/j.ijheh.2020.113569.

Govorushko, S. (2019) 'Economic and ecological importance of termites: A global review', *Entomological Science*, 22(1), pp. 21–35. Available at: https://doi.org/10.1111/ENS.12328.

Harrison, M.C. *et al.* (2018) 'of termite eusociality', *Nature Ecology & Evolution*, 2(March). Available at: https://doi.org/10.1038/s41559-017-0459-1.

Hellemans, S. (2024) 'Genomic data provide insights into the classi fi cation of extant termites', (October 2023). Available at: https://doi.org/10.1038/s41467-024-51028-y.

Isman, M.B. (2006) 'Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world', *Annual Review of Entomology*, 51, pp. 45–66. Available at: https://doi.org/10.1146/ANNUREV.ENTO.51.110104.151146.

Jouquet, P. *et al.* (2011) 'European Journal of Soil Biology In fl uence of termites on ecosystem functioning . Ecosystem services provided by termites', *European Journal of Soil Biology*, 47(4), pp. 215–222. Available at: https://doi.org/10.1016/j.ejsobi.2011.05.005.

Korb, J. (2008) 'Termites , hemimetabolous diploid white ants ?', 9, pp. 1–9. Available at: https://doi.org/10.1186/1742-9994-5-15.

Kortbeek, R.W.J., Gragt, M. Van Der and Bleeker, P.M. (2019) 'Endogenous plant metabolites against insects', pp. 67–90.

Kuvawoga, E.R. *et al.* (2025a) 'Development and evaluation of a herbal anti-tick grease using Cissus quadrangularis terpenes and terpenoids as active compounds for controlling ticks on live stock', *Tropical animal health and production*, 57(3). Available at: https://doi.org/10.1007/S11250-025-04401-Y.

Kuvawoga, E.R. *et al.* (2025b) 'Development and evaluation of a herbal anti-tick grease using Cissus quadrangularis terpenes and terpenoids as active compounds for controlling ticks on live stock', *Tropical Animal Health and Production*, 57(3). Available at: https://doi.org/10.1007/S11250-025-04401-Y.

Lengai, G.M.W., Muthomi, J.W. and Mbega, E.R. (2020) 'Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production', *Scientific African*, 7. Available at: https://doi.org/10.1016/J.SCIAF.2019.E00239.

Lin, S. *et al.* (2022) 'Low Concentration of Rotenone Impairs Membrane Function of Spodoptera litura Cells by Promoting Their Aggregation'.

Liu, C. *et al.* (2025) 'Unravelling the evolution of wood-feeding in termites with 47 high-resolution genome assemblies', pp. 1–14.

Logan (1990) 'Loganetal1990.pdf'.

Panzella, L. and Napolitano, A. (2022) 'Condensed Tannins, a Viable Solution To Meet the Need for Sustainable and E ff ective Multifunctionality in Food Packaging: Structure, Sources, and Properties'. Available at: https://doi.org/10.1021/acs.jafc.1c07229.

Pavela, R. (2016) 'History, presence and perspective of using plant extracts as commercial botanical insecticides and farm products for protection against insects - A review', *Plant Protection Science*, 52(4), pp. 229–241. Available at: https://doi.org/10.17221/31/2016-PPS.

Regnault-Roger, C., Vincent, C. and Arnason, J.T. (2012) 'Essential oils in insect control: Low-risk products in a high-stakes world', *Annual Review of Entomology*, 57, pp. 405–424. Available at: https://doi.org/10.1146/ANNUREV-ENTO-120710-100554.

Rust, M.K. and Su, N.Y. (2012) 'Managing social insects of urban importance', *Annual Review of Entomology*, 57, pp. 355–375. Available at: https://doi.org/10.1146/ANNUREV-ENTO-120710-100634.

Salehi, B., et al. (2019) *Antioxidant and Anti-Inflammatory Properties of Natural Compounds in Neurological Disorders. Molecules, 24, 3611. - References - Scientific Research Publishing*. Available at: https://www.scirp.org/reference/referencespapers?referenceid=4139371 (Accessed: 11 May 2026).

Singh, P.K. *et al.* (2022) 'Phytochemical Screening, Quantification, FT-IR Analysis, and In Silico Characterization of Potential Bio-active Compounds Identified in HR-LC/MS Analysis of the Polyherbal Formulation from Northeast India', *ACS Omega*, 7(37), pp. 33067–33078. Available at: https://doi.org/10.1021/ACSOMEGA.2C03117.

Starmans, I.D.A.J. and Nijhuis, H.H. (1996) 'metabolites from plant material : A review', 71(June).

Su, N.-Y. and Scheffrahn, R.H. (2022) 'Termites as Pests of Buildings', *Termites: Evolution, Sociality, Symbioses, Ecology*, pp. 437–453. Available at: https://doi.org/10.1007/978-94-017-3223-9_20.

Upadhyay (2010) 'E -m , p a s e l.', 2(4), pp. 65–77.

Verma, S. *et al.* (2021) 'A Review on Response of Neem Seed and Leaf Extract on Crop Protection and Production', 33(6), pp. 22–27. Available at: https://doi.org/10.9734/IJPSS/2021/v33i630440.

Xuan, T.T. *et al.* (2024) 'Biocontrol Potential of Different Entomopathogenic Fungi and Neem Oil for Control of Sucking Insects and Two-Spotted Spider Mites in Vegetables', *AgBioForum*, 26(3), pp. 148–155.

Zielińska-Błajet, M. and Feder-Kubis, J. (2020) 'Monoterpenes and Their Derivatives-Recent Development in Biological and Medical Applications', *International journal of molecular sciences*, 21(19), pp. 1–38. Available at: https://doi.org/10.3390/IJMS21197078.

APPENDIX



sample preparation



finely grounded sample



30g of the sample

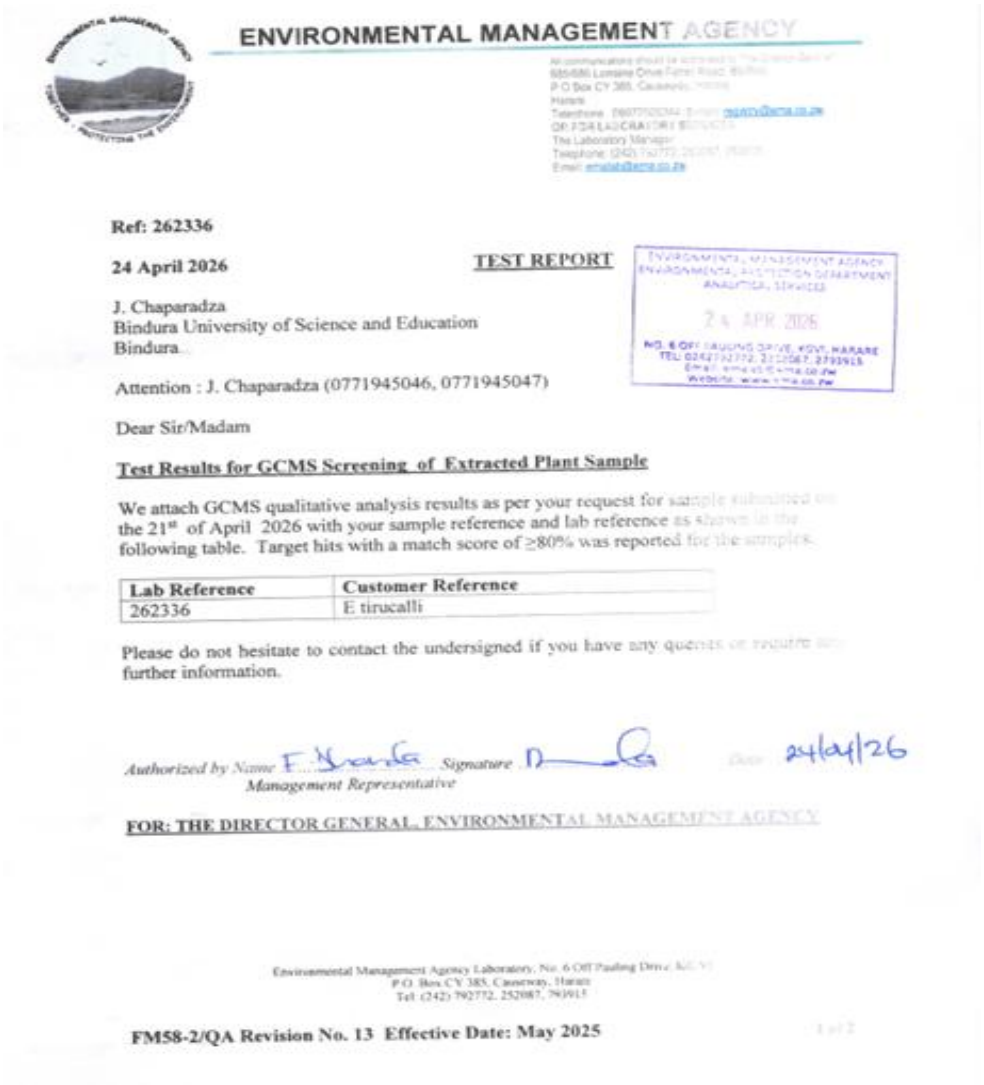


ethanol and ethyl acetate-chloroform





GC-MS ANALYSIS REPORT



ENVIRONMENTAL MANAGEMENT AGENCY

Ref: 262336

24 April 2026

TEST REPORT

J. Chaparadza
Bindura University of Science and Education
Bindura

Attention : J. Chaparadza (0771945046, 0771945047)

Dear Sir/Madam

Test Results for GCMS Screening of Extracted Plant Sample

We attach GCMS qualitative analysis results as per your request for sample submitted on the 21st of April 2026 with your sample reference and lab reference as shown in the following table. Target hits with a match score of ≥80% was reported for the samples.

Lab Reference	Customer Reference
262336	E tirucalli

Please do not hesitate to contact the undersigned if you have any queries or require any further information.

Authorized by Name F. Nyanga *Signature* *Date* 24/04/26
Management Representative

FOR: THE DIRECTOR GENERAL, ENVIRONMENTAL MANAGEMENT AGENCY

Environmental Management Agency Laboratory, No. 6 Off Pauling Drive, Kelvin
P.O. Box CY 385, Causeway, Harare
Tel: (242) 792772, 252087, 793915

FM58-2/QA Revision No. 13 Effective Date: May 2025