ASSESSING THE EFFICACY OF *MYROTHAMNUS FLABELLIFOLIUS* (MUFANDICHIMUKA) IN CONTROLLING COCCIDIOSIS IN INDIGENOUS CHICKENS.

A dissertation submitted in partial fulfilment of the requirements for the Master of Science Degree in Food Security and Sustainable Agriculture
(Production)

Bindura University of Science Education

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DECLARATION

I hereby declare that the research project entitled **"Assessing the efficacy of Myrothamnus Flabellifolius (Mufandichimuka/ Resurrection tree) in controlling coccidiosis in indigenous chickens"** submitted to Bindura University of Science Education, Department of Agriculture Economics, Education and Extension is a record of an original work done by me under the guidance and supervision of **Dr Renias Chivheya** and this work is submitted in partial fulfilment of the requirements for the award of a Master of Science Degree in Food Security and Sustainable Agriculture. The results embodied in this thesis have not been submitted to any University or Institute for the award of any degree or diploma.

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DEDICATION

This dissertation is dedicated to:

My beloved spouse, whose consistent backing and motivation facilitated the completion of this endeavour. My cherished offsprings, Anopaishe, Akanakaishe, and Zane, who motivate me each day to strive for more. Furthermore, I extend my gratitude to my mum, whose sacrifices and unwavering affection have served as a continual wellspring of fortitude. May this undertaking pay tribute to the profound influence you all have had on my existence and stand as a testimony to the resilience of our familial connections.

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To God be the glory.

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ABSTRACT

Eimeria, a codiosis causing bacteria leads to huge economic losses to the poultry farming industry. To mitigate the challenge there has been conventional formulations as an endeavour to boost chicken growth rate and health in general. To date there are several challenges being faced by poultry farmers because of the conventional codiostat available, which can face bacterial resistance from the virulent *Eimeria*. The study assessed the qualitative and quantitative bioactive chemicals in *Myrothamnus flabellifolius (Mufandichimuka)* aqueous and acetone extract. It also investigated the effectiveness of the plant as a codiostat. Lastly, there *was M. flabellifolius* codiostat formulation and packagingand packagingdevelopment and packaging and packaging. For the qualitative analysis, phytochemical compounds were identified using standard screening tests, including tests for phenols, tannins, saponins, glycosides, alkaloids, flavonoids, and terpenoids. The data was analysed using descriptive statistics to provide a summary of the characteristics of the coccidial compounds. This included measures such as mean and standard deviation. Also, the results were subjected to an independent samples t-test. The qualitative results revealed that, phenols, tannins, saponins, glycosides, alkaloids, flavonoids, and terpenoids were present in the plant extracts. Furthermore, quantitative analysis, quantified phenols, flavonoids, tannins, saponins and alkaloids. Overall, the results indicate that the acetone solvent was more effective in extracting these phytochemical compounds from the plant material compared to the hot water solvent. A multi-faceted approach using ethanol, methanol, and hydro distillation, combined with advanced processing techniques, can maximize the extraction

efficiency of *M. flabellifolius*, ensuring the retrieval of its diverse bioactive compounds for various therapeutic applications. To address the issue of level of effectiveness of the plant's extracts, phytochemicals were quantified, and birds' growth rates, invitro assays, and mortality rates were studied. To analyze the growth rate, all the birds used in the study were weighed every 7 days. The reduction of oocyst counts, focused on the faecal oocyst concentration reduction rate. Birds were observed for mortality, counting total deaths post inoculation. The data for weight gain was analyzed using one way ANOVA. Also, the weight gain was subjected to Bonferroni post-hoc test for pairwise comparisons. The results for oocyst reduction rate were expressed as percentages. The mortality rates were expressed in percentages. The results indicate that the *M. flabellifolius* treatment group had the highest mean weight gain at day 42. Group which received the *M. flabellifolius* treatment, also exhibited lower mortality rates compared to the untreated group. Lastly, the data suggests that the two treatment interventions, particularly the Coccidiostat and ESB3 combination, had a significant positive impact on the oocyst count reduction, resulting in a much more substantial and sustained increase compared to the control group. Overall, this study demonstrated that *M. flabellifolius* can reduce coccidiosis infected poultry mortality, reduce oocyst count and improve the growth weight. The development and packaging and packaging of the plant codiostat, involved, labelling, packaging material, packaging design, packaging, quality control and stability testing.

Keywords: Coccidiosis; Phytochemical; Qualitative; Quantitative; *Myrothamnus flabellifolius*; Aqueous extract; Solvent extract; Level of effectiveness; Natural remedies; Traditional medicine; Veterinary medicine.

LIST OF ACRONYMS AND ABBREVIATIONS

OPG	Oocyst per gram
NRs	Natural Regions
Ha	Hectares
HIT	Harare Institute of Technology
DCS	Distributed Control System

ICH	International Council for Harmonisation of Technical Requirements of Pharmaceuticals for Human Use
HIV	Human Immune Virus
HPLC	High-performance liquid chromatography
GC-MS	Gas chromatography-mass spectrometry
TPC	Total phenolic content
TFC	Total flavonoid content
DPPH	DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay
(GAE)/g	Gallic acid equivalent (GAE)/g
NADES	Natural Deep Eutectic Solvents
DKL	Dry kinkeliba leaves
PVPP	Polyvinylpolypyrrolidone method
DR	Dragendorff's reagent
FCR	Feed conversion ratio
TNBC	Triple negative breast cancer
HPLC	High Performance Liquid Chromatography
TLC	Thin-layer chromatography
GC	Gas chromatography
FTIR	Fourier Transform Infrared Spectroscopy
NMR	Nuclear magnetic resonance
MS	Mass spectrometry

MFEO	Essential oil from *M. flabellifolius* leaves
MLTE	*M. flabellifolius* leaf tea extract
SAD	Solid amorphous dispersions
CSP	Concentration-sustaining polymers
MLTE	*M. flabellifolius* leaf tea extract

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ABSTRACT

Eimeria, a coccidiosis causing bacteria leads to huge economic losses to the chicken farming industry. To mitigate the challenge there has been conventional formulations as an endeavour to boost chicken growth rate and health in general. To date there are several challenges being faced by chicken farmers because of the conventional coccidiostat available, which can face bacterial resistance from the virulent *Eimeria*. The study assessed the qualitative and quantitative bioactive chemicals in *Myrothamnus flabellifolius (Mufandichimuka)* aqueous and acetone extract. It also investigated the effectiveness of the plant as a coccidiostat. Lastly, there *was M. flabellifolius* coccidiostat formulation and packagingand packagingdevelopment and packaging and packaging. For the qualitative analysis, phytochemical compounds were identified using standard screening tests, including tests for phenols, tannins, saponins, glycosides, alkaloids, flavonoids, and terpenoids. The data was analysed using descriptive statistics to provide a summary of the characteristics of the coccidial compounds. This included measures such as mean and standard deviation. Also, the results were subjected to an independent samples t-test. The qualitative results revealed that, phenols, tannins, saponins, glycosides, alkaloids,

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CSP	Concentration-sustaining polymers

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

The global prevalence of chicken coccidiosis is significant, with rates varying among different chicken species. Research indicates that coccidiosis is detrimental in chicken. It's prevalence in

chicken's ranges from 36.3% to 43.3%. Affected are both local and exotic breeds(Chatterjee et al., 2023; Usman et al., 2022). In chicken coccidiosis presents short-term and long-term effects on general health. Short term, coccidiosis shows symptoms like anorexia, lethargy, and diarrhea. Additionally there are lesions such as hemorrhages in the ceca and small intestines(Tompkins et al., 2023). Moreover, coccidiosis causes a decline in various hematological parameters in commercial layer chickens, indicating significant health implications(Alsayeqh & Abbas, 2023). In the long term, coccidiosis alters bone homeostasis in broilers, resulting in suppressed bone growth rate, increased bone resorption, and lower bone mineral density and content under Eimeria infection.

The prevalence of chicken coccidiosis in various African regions has been extensively studied. Ethiopia and Somalia have presented prevalence and associated risk factors of coccidiosis in chickens. Prevalence rates ranged from 17.7% to 43.3%, with differences noted based on age, breed, sex, and management systems(Jeilani Busuri Mio et al., 2022; Tanga & Abdu, 2015, 2015; Wondimu et al., 2019). The chicken industry faces significant economic losses due to coccidiosis, with costs exceeding billions of Euros globally(Chapman, 2017). Chicken coccidiosis in Zimbabwe is of concern to various parties. Smallholder farmers in Zimbabwe rely on traditional remedies like Aloe vera and Aloe spicata for managing chicken health due to the high cost of conventional drugs(Makaya et al., 2012a). Additionally, an ethno veterinary survey identified 36 plant species used in chicken ethno medicine, with potential for developing phytogenic feed additives, highlighting the importance of traditional knowledge in bird health care(Mwale et al., 2005).

Smallholder farmers, researchers, and veterinary authorities are concerned about chicken coccidiosis in Zimbabwe. Coccidial infection in chicken has been a prevalent issue in various regions, including Zimbabwe. Studies from Nigeria, Ethiopia, and Serbia highlight the widespread occurrence of Eimeria species causing coccidiosis in chickens. The findings indicates that different *Eimeria* species, such as *E. tenella, E. acervulina, E. necatrix*, and *E. maxima*, have been identified in infected chicken samples. This has resulted to economic losses and health challenges for the birds(Dikwa et al., 2023; Molla et al., 2015; Musa et al., 2010; Shahraki et al., 2018). Several Risk factors for chicken coccidial infection have been noted. Included are

practices such as increasing stock in smallholder farms, self-mixing of concentrate, usage of stream water, pen odour, non-adherence to recommended chicken vaccination, and lack of biosecurity measures(Maayan et al., 2023). In rural communities of Zimbabwe, chicken farming plays a crucial role. Smallholder farmers hold over 90% of the country's livestock (Gobvu et al., 2022). Farmers raise chicken to meet household food demands and generate additional income, especially in areas where access to modern veterinary services is limited (Kanyama et al., 2022). By elucidating the potential benefits of ethno veterinary practices, This research aims to evaluate the effectiveness of Myrothamnus flabellifolius (Mufandichimuka) in controlling coccidiosis in indigenous chickens.

1.2 Statement of the Problem

Coccidiosis is a significant concern in chicken production due to its association with high mortality rates. Both chickens and turkeys, are vulnerable to coccidiosis. Caeca coccidiosis is a severe threat (Jaiswal et al., 2023). Chicken coccidiosis is a significant issue affecting avian species, particularly chickens and turkeys, with high mortality rates due to caeca coccidiosis(Saeed & Alkheraije, 2023). The disease is caused by *Eimeria* species, leading to economic losses in broiler production globally(Atif et al., 2023). Traditionally coccidiostats to combat coccidiosis. Risen concerns with regards to resistance and public health led to exploration of alternative methods. These include vaccines and botanicals(Martins et al., 2022). The use of *M. flabellifolius* in the management of chicken coccidiosis presents a notable gap in current research and practical application(Peek & Landman, 2011; Ziam et al., 2020). While various strategies such as live vaccines, drug combinations, and herbal additives have been explored for coccidiosis control(Chapman & Rathinam, 2022; Mansoor et al., 2017; Williams, 2005), the specific utilization of *M. flabellifolius* remains limited. Research predominantly focuses on other herbal plants and their derivatives, showcasing their efficacy against coccidiosis.

The potential benefits of Myrothamnus flabellifolius, known for its stress resistance properties, in combating coccidiosis warrant further investigation to assess its effectiveness, safety, and

practicality in chicken production. The paucity of data suggests a need for more studies exploring the use of *M. flabellifolius* as a potential alternative in the management of chicken coccidiosis. Chicken coccidiosis in Zimbabwe has been associated with various observations. Studies have shown that coccidiosis outbreaks commonly affect chickens over 3 weeks old(Makaya et al., 2012b). Additionally, coccidiosis is caused by Eimeria species, with seven known to infect chickens(Musa et al., 2010). Furthermore, the prevalence of coccidial infection in chicken farms has been documented, with significant associations found between infection risk and factors such as chicken age, breed, study site, and management systems(Matekaire & Bwakura, 2004). The study aims to evaluate the potential of *M. flabellifolius* in treating coccidiosis in chicken. *M. flabellifolius* crude extracts have been used for the control of coccidiosis based on estimates in communal areas by folks to date. There are no measured effective dosages as its use is based on the assumptions that the concoction is ready for use. There is a need for the determination of the effective dosage as well as the appropriate methods of application as a plant based coccidiostat.

1.3 Objectives of the study

1.3.1 The main objective

To assess the efficacy of *M. flabellifolius* in controlling coccidiosis in indigenous chicken.

1.3.2 Specific objectives

The specific objectives of the study seeks to:

1. Characterization and Quantification of the coccidiostat compounds in *M. flabellifolius* plant
2. Determine the level of effectiveness of plant extracts from *M. flabellifolius* on treating coccidiosis in indigenous chicken.
3. Formulate and package a *M. flabellifolius* plant based coccidiostat.

1.4 Research Questions

The aforementioned objectives will result in a series of research inquiries concerning phytochemical identification, quantification, effectiveness of *M. flabellifolius* against chicken coccidiosis and formulation and packagingand packagingand packaging of the chicken coccidiostat, which, in pursuing its main goal, this study aims to address. The following are these research questions: -

1. Which coccidiostat compounds are found in *M. flabellifolius*?
2. What is the efficacy of *M. flabellifolius* in controlling coccidiosis in Indigenous Chickens?
3. What is the impact of *M. flabellifolius* on the growth performance, feed efficiency, and mortality rates of infected with coccidia at Nhema farm?

1.5 Hypotheses

Null Hypothesis (H_0):

The use of "Myrothamnus flabellifolius" has no significant effect on controlling coccidiosis in indigenous chickens.

Alternate Hypothesis (H_1):

The use of "Myrothamnus flabellifolius" has a significant effect on controlling coccidiosis in indigenous chickens.

1.6 Significance of the study/Justification

1.6.1 Prevalence of Coccidiosis

The prevalence of chicken coccidiosis in Zimbabwe varies depending on the study area and farming practices. Studies have shown that coccidial infections are common in both intensive and free-range chicken farming systems(Gebeyeh & Yizengaw, 2017; Makaya et al., 2012b). In Zimbabwe, the prevalence of coccidiosis was found to be 65.10% in intensive chicken farms and

smallholder farms(Matekaire & Bwakura, 2004). Also, there has been a study reporting a prevalence rate of 19.5% in free-ranging, intensively managed chickens, infected by *Eimeria tenella* and *Eimeria acervulina* (Garbi et al., 2015). These findings highlight the significant presence of coccidiosis in chicken populations in Zimbabwe, emphasizing the importance of implementing effective control measures to minimize the impact of this parasitic disease on chicken health and production(Gebeyeh & Yizengaw, 2017).

1.6.2 Limited Access to Chemical Treatments

Limited access to chemical treatments in chicken coccidiosis treatment in Zimbabwe has led to a reliance on herbal medicines(Eftekhari Hasan Abad & Ghaniei, 2023). The use of medicinal plants, such as those from the Fabaceae and Solanaceae families, is prevalent among non-commercial chicken producers in Zimbabwe(Gobvu et al., 2022). Additionally, the high botanical and veterinary consistency of herbal remedies for various chicken ailments underscores their importance as alternative treatments(Matekaire & Bwakura, 2004). The interest in natural sources for coccidiosis control has increased, with a focus on products derived from plants and other natural sources to produce drug-free birds(Greif et al., 2001).

1.6.3 Traditional Medicinal Knowledge

Traditional medicinal knowledge in Zimbabwe for chicken coccidiosis treatment is rich, with various plants being utilized for their potential benefits(Eftekhari Hasan Abad & Ghaniei, 2023; Gobvu et al., 2022; Jambwa et al., 2022). The ethno veterinary survey in Zimbabwe documented 36 plant species used for treating chicken ailments, with Fabaceae being the dominant family. Plants like Rumex nervosus leaves and Cinnamomum verum bark have shown moderate anticoccidial activity, comparable to synthetic agents like salinomycin. Aloe species were widely used among non-commercial chicken producers, highlighting the significance of traditional plant-based treatments. The potential of herbal medicines as alternative treatments for chicken coccidiosis is emphasized, indicating the importance of further research to explore their efficacy and active ingredients.

1.6.4 Organic Farming and Consumer Preferences

Organic farming practices are gaining popularity due to consumer preferences for antibiotic-free chicken products. In Zimbabwe, coccidiosis treatment in chicken is traditionally managed using botanical remedies with high consistency(Matekaire & Bwakura, 2004). However, the use of modern anticoccidial drugs has led to resistance issues, prompting the exploration of alternative strategies like phytotherapy(Uddin et al., 2016). The incorporation of synthetic coccidiostats in feed is a common practice but is being challenged due to concerns about residual antimicrobials and drug-resistant microorganisms(Barbour et al., 2015). Organic chicken farming, which prohibits antibiotic use, has shown promise in reducing antibiotic-resistant bacteria, highlighting the potential benefits of organic practices in addressing antibiotic resistance concerns(Holtcamp, 2011). As the search for effective and safe coccidiosis control methods continues, natural products are being explored as potential solutions, aligning with the trend towards holistic and natural approaches in chicken health management(Cobaxin-Cárdenas, 2018).

1.6.5 Promotion of Sustainable Agriculture

Sustainable agriculture practices in chicken coccidiosis treatment can be promoted through various strategies. One approach is the utilization of alternative methods such as probiotics, phytotherapy, and vaccines to control coccidiosis without relying heavily on antimicrobials(Aida et al., 2022; Alsayeqh & Abbas, 2023; Tewari & Maharana, 2011; Uddin et al., 2016). These alternatives help in reducing the emergence of drug-resistant strains and promote environmentally safe practices in chicken farming. Additionally, incorporating nutritional supplements like organic acids, minerals, vitamins, and probiotics as feed additives can enhance productivity, improve immunity, and prevent inflammation in chicken affected by coccidiosis(Tanyanyiwa et al., 2022). By adopting these sustainable and effective methods, the chicken industry in Zimbabwe can address coccidiosis challenges while ensuring long-term viability and reduced environmental impact.

1.6.6 Empowering Rural Communities

Empowering rural communities in Zimbabwe involves integrating ethno veterinary knowledge into orthodox veterinary practices for chicken diseases like coccidiosis. Traditional remedies for chicken ailments show high botanical and veterinary consistency, emphasizing the importance of standardization and validation(Gobvu et al., 2022). Community-based projects in Zimbabwe aim to empower rural chicken producers by utilizing indigenous medicinal plants for treating chicken diseases, with Aloe species being the most commonly used(Matekaire & Bwakura, 2004). To enhance empowerment, there is a need for further research to document more plants used in different regions and validate their therapeutic effectiveness under defined experimental conditions(Malinga et al., 2017).

1.6.7 Research Gap

A research gap in chicken coccidiosis treatment in Zimbabwe lies in the need for further investigation into the therapeutic effectiveness of medicinal plants used traditionally(Matekaire & Bwakura, 2004). While indigenous medicines are relied upon due to limited access to modern veterinary services, there is a lack of comprehensive studies evaluating the efficacy of these plant-based treatments under controlled conditions(Eftekhari Hasan Abad & Ghaniei, 2023). Additionally, the review of herbal medicines for chicken coccidiosis emphasizes the importance of conducting more research on standardized doses and clinical trials to validate their efficacy as alternative treatments(Adeyemi et al., 2023). Integrating traditional knowledge with orthodox veterinary medicine requires standardization and validation to enhance the effectiveness of ethno veterinary practices(Gobvu et al., 2022). Therefore, there is a significant research gap in Zimbabwe regarding the scientific validation and isolation of potentially beneficial compounds from medicinal plants used in chicken coccidiosis treatment.

1.7 Delimitations of the study

1.7.1 Scope of Mufandichimuka

- The study focuses specifically on the efficacy of Mufandichimuka in controlling coccidiosis in indigenous chicken. It does not explore other potential uses or applications of Mufandichimuka in veterinary medicine or other areas.

1.7.2 Specific Parasite Strains

- The study may focus on the effects of Mufandichimuka on specific strains or species of coccidial parasites. Variations strains show differences in virulence and susceptibility to treatments. Therefore, results from this study, may not be applicable to all coccidial strains.

1.7.3 Method of Administration

- The study may delimit the investigation to a specific method of administering Mufandichimuka extracts, such as oral administration. Other routes of administration or formulations of Mufandichimuka may not be explored in this particular study.

1.7.4 Evaluation Parameters.

- The study may focus on specific outcome measures, such as oocyst counts, weight gain, and mortality rates. This study will be limited not to not extensively analyse parameters such as immune response, histopathological changes, or economic analysis.

1.8 Limitation of the study

1.8.1 Sample Size

- The study may be limited by the availability of Nhema farm chicken populations and the feasibility of obtaining a sufficiently large sample size. Statistical power and generalizability of the findings can be affected by the limited sample size.

1.8.2 Duration of the Study

- The time constraints of the study may limit the duration of the experimental period. Coccidiosis is a complex disease with various stages, and a short study duration may not capture the long-term effects of Mufandichimuka treatment.

1.8.3 Controlled Environment

- Conducting the study in a controlled environment, such as separating the study models from the other brood of chickens, may not fully reflect the real-life conditions and challenges faced by chicken farmers. The results obtained in controlled settings may differ from the outcomes in practical or field conditions.

1.8.4 Generalizability

- The study's findings may be specific to the particular breed of chicken under investigation. Careful considerations should be taken in the extrapolation of the results to other chicken populations. These variations are, environmental factors, management practices, and genetic diversity.

1.9 Outline of Thesis

The remainder of the thesis is as follows:

Chapter two presents a review of the literature on other studies done by other scholars on an overview of chicken coccidiosis, previous studies unveiled: an overview of findings, relevance of findings to the study: implications and significance, critical evaluation of reviewed literature: strengths and weaknesses, addressing literature gaps and weaknesses: proposed methodology and research approach, differences between previous research, current study: comparative analysis, research focus and objectives: defining the thrust of the study and conceptual framework. This chapter is significant because it establishes the foundation for comparison with research findings.

Chapter three describes the study area. Additionally, the chapter outlines the study's methodology. It focuses on study design, sampling, data collection, and analysis, as well as ethical aspects. Chapters four, five, and six include details on the analysis for each distinct target.

The fourth chapter identifies phytoconstituents in *M. flabellifolius* using Million's test, Benedict's test, NaOH test,FeCl3 test, Froth test, Keller-kilani test, Wagner's test, Lead acetate test and Chloroform+H2SO4 test. Additionally, the chapter quantifies phytoconstituents in *M. flabellifolius* using The Folin-Ciocalteu assay, Aluminium chloride colorimetric, Polyvinylpolypyrrolidone (PVPP), Vanillin-sulphuric acid, and Dragendorff's methods at Harare Institute of Technology.

Chapter five, evaluated the effectiveness of *M. flabellifolius* as a coccidiostat using flotation method, regular weighing and monitoring of bird's body weight over a specified period and recording the number of bird's deaths over a specified period at at Nhema Chickens farm.

Chapter six, presents the formulation and packaging ofa *M. flabellifolius* plant based coccidiostat at Harare Institute of Technology. The herbal formulation and packagingand packagingwas encapsulated and packaged. The label for the coccidiostat was designed and affixed the container. Quality control for the formulation and packagingand packagingwas carefully considered.

Chapter seven brings together the findings, interpretations, and general suggestions from the preceding chapters. The chapter clarifies the qualitative and qualitative bioactive compounds found in *M. flabellifolius*. It also elaborated on the effectiveness of the plant based coccidiostat on chicken coccidiosis. Lastly it showed the formulation and packaging of*M. flabellifolius*, a potential coccidiostat.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter provides a comprehensive overview of empirical literature analysis on characterizing the coccidiostat compounds in *Myrothamnus flabellifolius* plant through qualitative methods, determination the level of effectiveness of plant extracts from *M. flabellifolius* on treating coccidiosis in indigenous chicken. It furthermore, reviews the formulation and packagingand packaginga *M. flabellifolius* plant based coccidiostat. Additionally, the chapter provides the conceptual framework utilized in the thesis, followed by a synopsis of the thesis's literature review.

Characterization of *M. flabellifolius* plant bioactive compounds through qualitative and quantitative methods.

Qualitative methods: *M. flabellifolius*, a medicinal plant traditionally used in southern Africa, has been extensively studied for its bioactive compounds and their potential health benefits. A qualitative analysis of its extracts reveals a rich phytochemical profile which includes Alkaloids, reducing sugars, terpenoids, triterpenes, cardiac glycosides, anthocyanins, flavonoids, saponins, phlobatanins, tannins, polyphenols, and steroids (Cheikhyoussef, Summers and Kahaka, 2015). Hydroethanolic extract has shown significant antioxidant and antidiabetic activities. This is owed to the *M. flabellifolius* ability to inhibit α-glucosidase and α-amylase enzymes(Ajao and Ashafa, 2017). Phytochemical compounds such as gallic acid, caffeic acid, ferulic acid, methyl gallate, and epicatechin supports its antioxidant properties (Chukwuma *et al.*, 2019). Arbutin and lupeol has been isolated from butanol and ethyl acetate extracts, respectively, and heterogeneous pectic polysaccharides composed of arabinose, rhamnose, xylose, mannose, galactose, and glucose(Nako, 2014). These findings underscore the plant's diverse bioactive profile, which includes secondary metabolites essential for cell function and regulation, making it a promising candidate for various therapeutic applications, including diabetes management, cancer treatment, and as a natural antioxidant source(Kwape *et al.*, 2020; Nantapo and Marume, 2022).

Quantitative methods: a quantitative analysis of the essential oil from *M. flabellifolius* leaves, analyzed by gas chromatography-flame thermionic detector (GC-FTD) and GC-mass spectrometry (GC-MS) showed results of bioactive quantities. The results showed presence of twenty compounds, namely monoterpenes and non-terpenoid derivatives, with β-myrcene, mentha-1,5,8-triene, and α-pinene been dominant(Ajao *et al.*, 2023). Analysis of bioactive compounds by high-performance liquid chromatography (HPLC) identified gallic acid, caffeic acid, ferulic acid, methyl gallate, and epicatechin in the leaf tea extract, which also showed significant antioxidant properties(Chukwuma *et al.*, 2019). Additionally, an untargeted liquid chromatography-tandem-mass spectrometry (LC-MS/MS) approach detected forty-one phenolic compounds, including nine anthocyanins, highlighting regional phenolic variability(Bentley, Moore and Farrant, 2019). The ethanol: water (70:30) extract of *M. flabellifolius* demonstrated anti-diabetic potential in type 2 diabetes mellitus (T2DM) rats, lowering glucose levels and improving various biochemical markers, with further analysis needed to explore other mechanisms of action(Kwape *et al.*, 2020). In the context of cancer research, fractionation of the plant extract using HPLC identified a galloyl glucose hexahydroxy diphenic acid derivative as a potent anti-triple negative breast cancer (TNBC) compound(Brar *et al.*, 2018). Advanced analytical methodologies, HPLC, TLC, and GC, detection techniques such as FTIR, NMR, and MS, are important in the isolation and characterization of *M. flabellifolius* bioactive compounds(Thakur *et al.*, 2022). The root-associated microbiome of *M. flabellifolius*, characterized by high-throughput amplicon sequencing, further suggests that microbial diversity may aid in the plant's drought tolerance and overall function(Tebele, Marks and Farrant, 2023).

2.1.1 *M. flabellifolius* plant extracts antimicrobial effectiveness.

***M. flabellifolius* plant extracts** several secondary metabolites, including polyphenols, flavonoids, and terpenoids, attributed to its antimicrobial efficacy(Cheikhyoussef, Summers and Kahaka, 2015; Nantapo and Marume, 2022). Previous research elaborated M. *flabellifolius* extracts, inhibits the adhesion of *Porphyromonas gingivalis*. *P. gingivalis,* a pathogen causing disease, inhibition *M. flabellifolius* is by interacting with bacterial outer membrane proteins and reducing gingipain activity. thus demonstrating its potential in preventing bacterial

infections(Löhr *et al.*, 2011). Additionally, the essential oil from M. flabellifolius leaves possess potent antioxidant and antimicrobial activities. The essential oils inhibit carbohydrate-metabolizing enzymes, further supporting its use in managing microbial infections(Ajao *et al.*, 2023). The plant's extracts also exhibit anti-inflammatory and cytoprotective effects, which can enhance its antimicrobial action by reducing inflammation and protecting host cells from microbial damage(Löhr *et al.*, 2011). Phytochemicals such as galloyl and quinic from in *M. flabellifolius*, contributes to its antimicrobial and anticancer properties. The plant's extract, selectively targets cancer cells without harming normal cells. This underscores *M. flabellifolius* potential as a complementary therapy in cancer treatment(Dhillon *et al.*, 2014; Cheikhyoussef, Summers and Kahaka, 2015). In the triple-negative breast cancer (TNBC), a derivative of galloyl glucose hexahydroxy diphenic acid identified as a potential anti-TNBC compound(Brar *et al.*, 2018; Fultang *et al.*, 2018).

M. flabellifolius has a rich antioxidant profile, including high total phenolic and flavonoid content. These antioxidants play a crucial role in its antimicrobial effectiveness. This is achieved through the neutralization of free radicals and enhancing the immune response against pathogens(Cheikhyoussef, Summers and Kahaka, 2015; Chukwuma *et al.*, 2019).

2.1.2 *M. flabellifolius* extract formulations

M. flabellifolius extract has been formulated into various products due to its diverse medicinal properties. The essential oil from *M. flabellifolius* leaves (MFEO) exhibits potent antioxidant and antidiabetic activities, inhibiting carbohydrate-metabolizing enzymes and containing compounds like β-myrcene, mentha-1,5,8-triene, and α-pinene(Ajao *et al.*, 2023). *M. flabellifolius* is also explored as a phytogenic feed supplement in the diet of animals, enhancing growth performance and health due to its antimicrobial and antioxidant properties(Nantapo and Marume, 2022). In diabetes research, *M. flabellifolius* extract has shown significant potential in lowering blood glucose levels and improving lipid profiles in type 2 diabetes mellitus (T2DM) rats, while preserving liver and pancreatic structures(Kwape *et al.*, 2020). Additionally, *M. flabellifolius* leaf tea extract (MLTE) is rich in polyphenols and vitamins, providing nutritional benefits and protecting against oxidative hepatic cell injury(Chukwuma *et al.*, 2019). In skincare, *M. flabellifolius* extract is incorporated into products like moisturizing masks and anti-aging

compositions, enhancing skin hydration, elasticity, and reducing wrinkles by protecting lipid membranes and improving cell membrane flexibility.

2.2 Conceptual framework

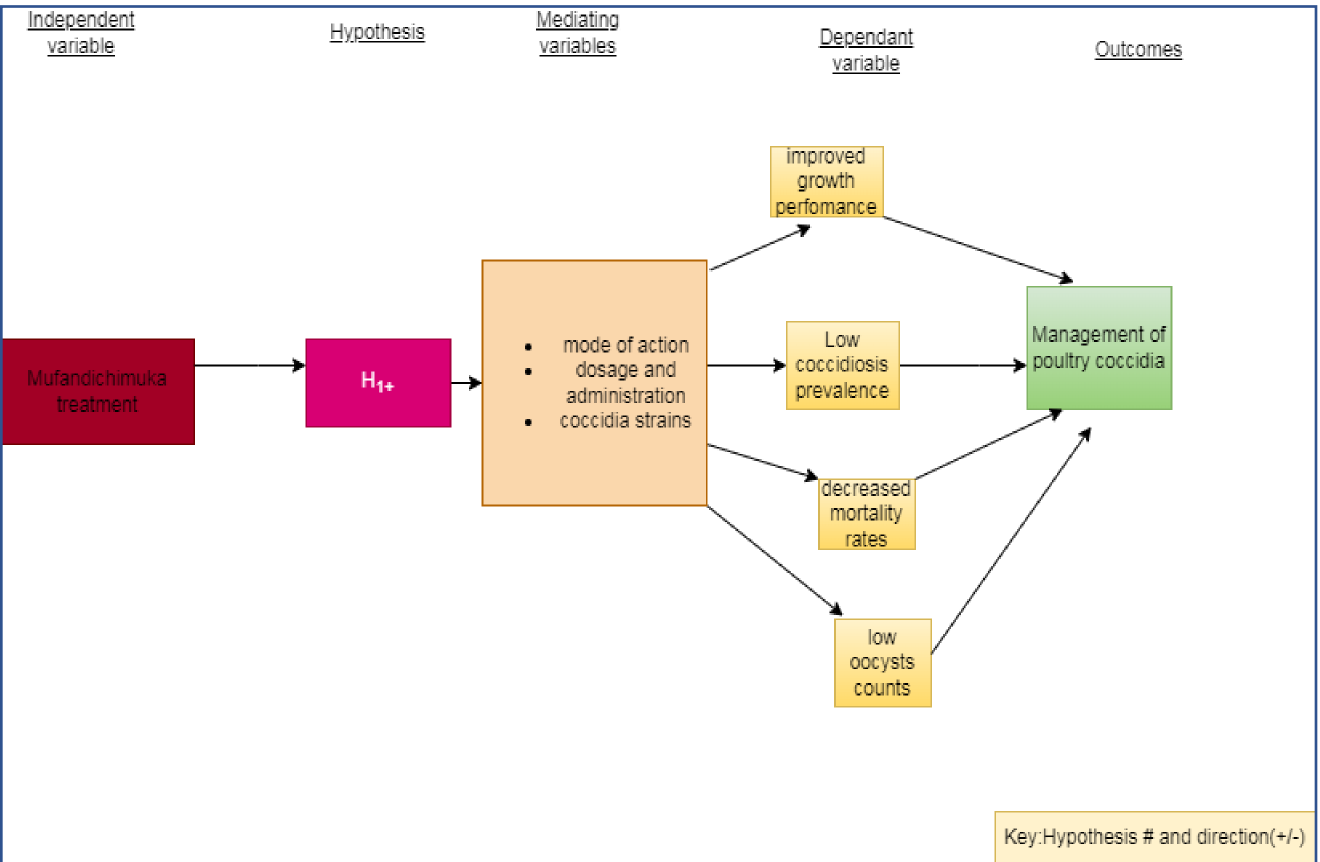

Figure 1.8.4-1: A conceptual framework showing the management of chicken Coccidia

The above conceptual framework is showing effects of *M. flabellifolius* treatment on chicken coccidia. The concept, takes into consideration the assumption of the alternative hypothesis, stating, that the use of "Myrothamnus flabellifolius" has a significant effect on controlling coccidiosis in indigenous chickens. Moving on, the pathway towards management of coccidiosis considers mediating factors such treatment mode of action, formulation and packagingand packagingdosage and administration as well as different coccidia strains. Eventually, this will lead to improved growth performance, low coccidiosis prevalence, decreased mortality rates and low oocysts counts. The subsequent results show management of management of chicken coccidian.

2.3 Summary of literature Review

An empirical literature review on research investigations opened the chapter assessing the efficacy of *M. flabellifolius* in controlling coccidiosis in indigenous chickens. The scholars revealed that *M. flabellifolius* has bioactive compounds with potential health benefits. A qualitative analysis of its extracts reveals a rich phytochemical profile which includes flavonoids, anthocyanins, alkaloids, steroids, terpenoids, triterpenes, cardiac glycosides, saponins, phlobatanins, tannins, polyphenols, and reducing sugars. The scholars revealed the presence of different phytochemical quantities with the use of advanced analytical methodologies. The results showed presence of twenty compounds, namely monoterpenes and non-terpenoid derivatives, with β-myrcene, mentha-1, 5, 8-triene, and α-pinene been dominant. Aqueous and acetone extract of *M. flabellifolius* exhibits potent phytochemicals, thus demonstrating antimicrobial and anticancer activities. This nominates the herb as a potential natural therapeutic agent therefore validating its traditional use.

Scholarly evidence, reveals that *M. flabellifolius* plant extracts several secondary metabolites, including polyphenols, flavonoids, and terpenoids, attributed to its antimicrobial efficacy. Literature reiterates that the plant's extract, selectively targets cancer cells without harming normal cells. *M. flabellifolius* plant extracts have diverse pharmacological activities which underscores its effectiveness as natural therapeutic agent with minimum side effects.

Finally, the chapter reviewed literature on research studies done regarding the formulation and packaging ofa *M. flabellifolius* extract formulation. Literature reveals that, *M. flabellifolius* extract has been formulated into various products due to its diverse medicinal properties. *M. flabellifolius* is also explored as a phytogenic feed additive in animal nutrition, enhancing growth performance and health due to its antimicrobial and antioxidant properties. Although *M. flabellifolius* has been used to manage human ailments, there is still paucity of data on the herb to improve chicken growth performance, feed quality and overall health. The conceptual

framework is offered, along with topics that need to be addressed in order to enhance chicken coccidia management. The study methodology is described in the following chapter.

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CHAPTER 3

METHODOLOGY

3.1. Introduction

A summary of the research methodologies, indicating whether the study is quantitative or qualitative, is provided in this chapter. In addition, the chapter presents the research design, the target population, the study unit, and the study region in precise terms. In terms of sample size, procedures, and technique, it also consists of sampling methods. Techniques for data analysis and their structure. The ethical considerations and a summary of the research methods round out the chapter.

3.2 Description of study area/sites

3.2.1 Serima, Gutu

The study area (Serima CA) is located in the northern part of Zimbabwe's Gutu district in Masvingo province. Serima Gutu has geographic coordinates of 19° 27' 30"S and 30° 58' 17"E. The district is located 202 kilometres southwest of Harare, the capital of Zimbabwe. Climatically, the area falls under Natural Region III. Natural Regions (NRs) in Zimbabwe's context are areas delineated on the basis of soil type, rainfall and other climatic factors. Soils in Serima communal area are predominantly coarse-grained sandy loams ranging in depth from shallow to deep with.

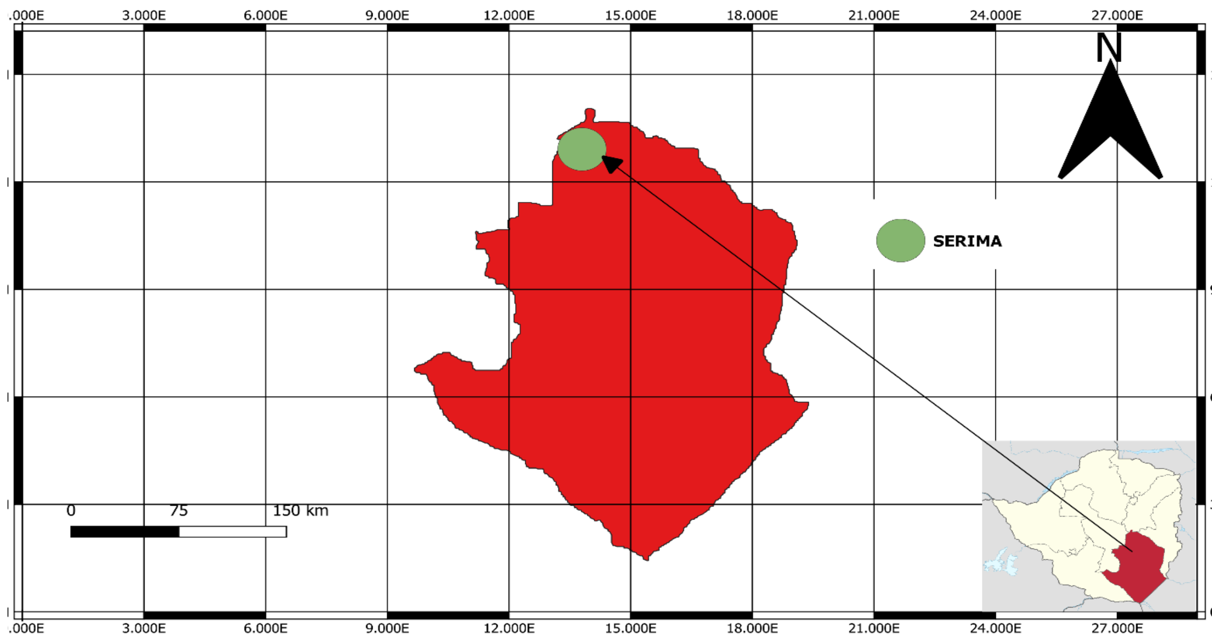

Figure 3.2.1-1: A map showing Serima in Gutu, Masvingo

3.2.2 Nhema Chicken farm

The experiment was done at Nhema Chickens farm covering a total of 10 Ha, which is located in Norton. Nhema Chickens farm has geographic coordinates of 17.8765° S, 30.6742°. The commuter town is located 40 kilometres west of Harare, the capital of Zimbabwe. The farm lands surrounding the town produce a range of crops including tobacco, maize and wheat, and cattle rearing for the beef and dairy industries is also an important element of the local economy. The farm has a capacity of 10 000 birds of different pure breeds which include Boschveld, Sasso, Light Sussex, Koekoek, Plymouth Rock, Mottled oppington, Brahma, Light Sussex and Black Austrolope.

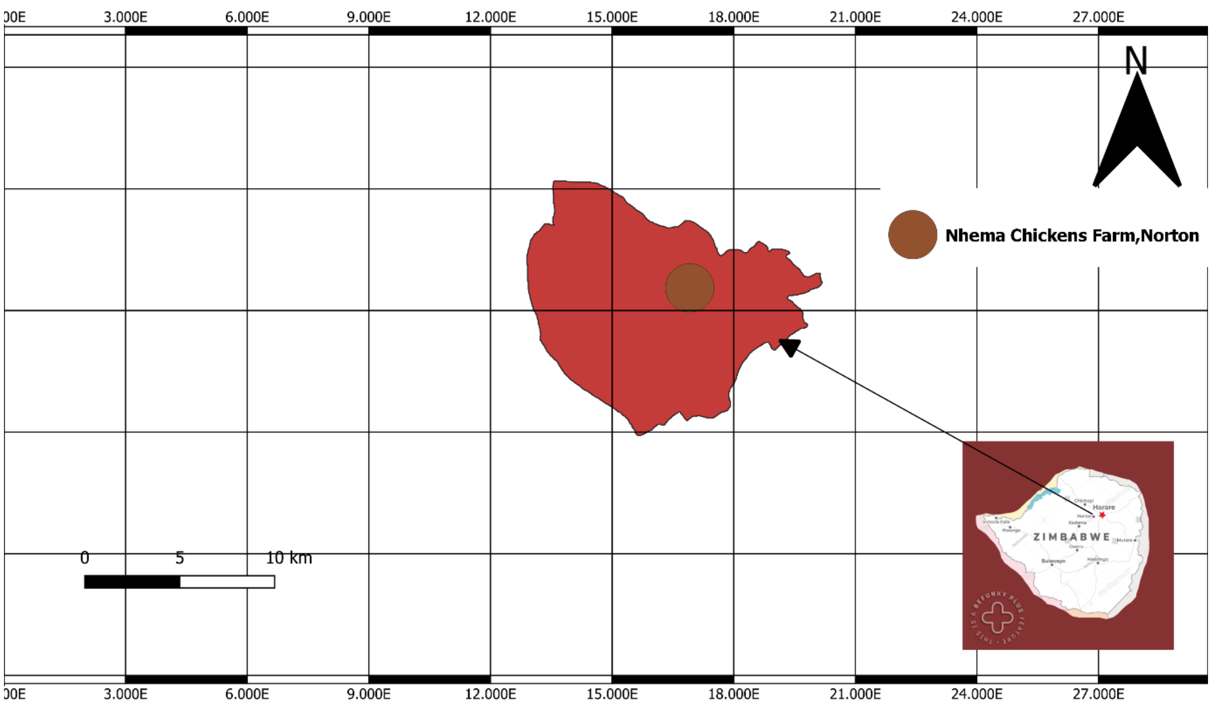

Figure 3.2.2-1: A map showing Nhema Chicken Farm in Norton

3.2.3 Harare Institute of Technology

The formulation and packagingand packagingwas designed at Harare Institute of Technology (HIT), a leading state-owned university located in Harare, the capital city of Zimbabwe. HIT is a Zimbabwean university offering courses mainly in technology and has geographic coordinates of 17.838°S 31.008°E. The district is located in Harare, the capital of Zimbabwe.

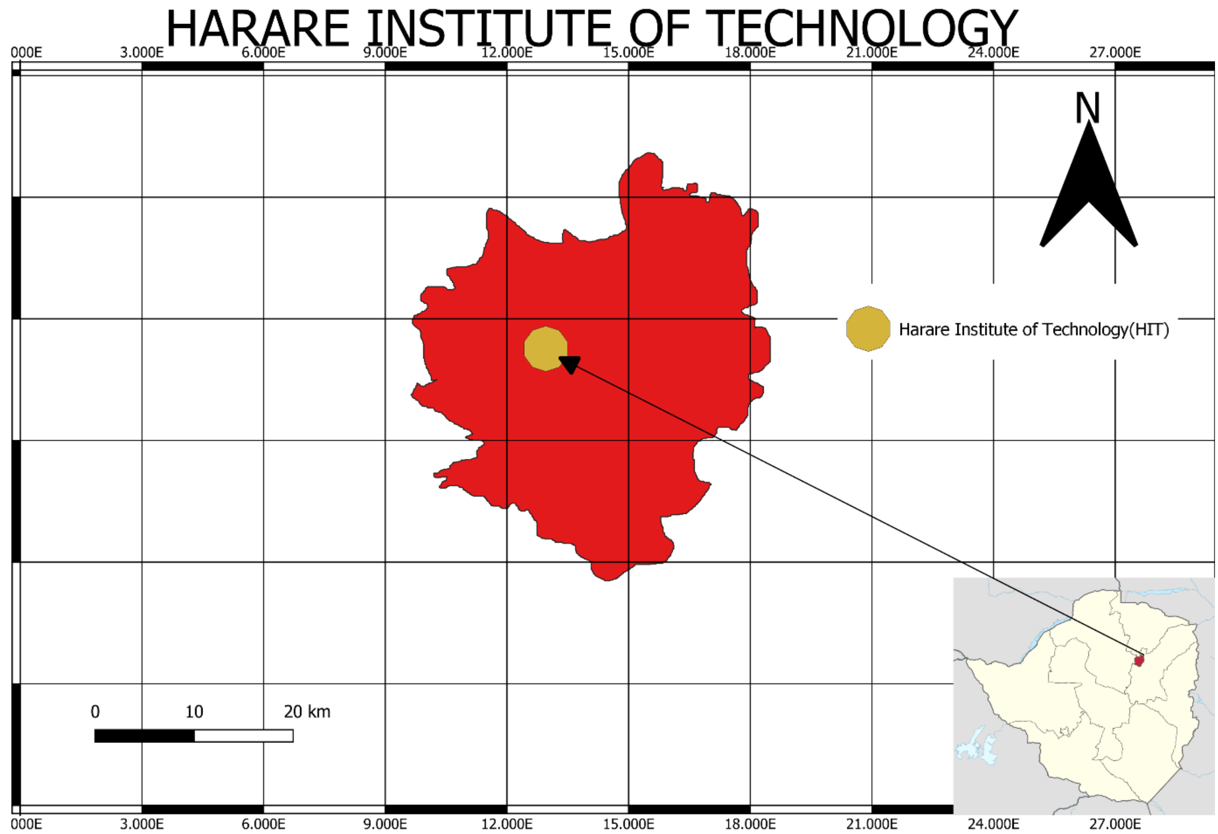

Figure 3.2.3-1: A map showing Harare Institute of Technology

3.3 Research Design

3.3.1 Experimental design

In this experiment, pure breeds, Boschveld, Sasso and light Sussex were used. The chickens were divided into 3 groups and housed in different adjacent fowl runs. Each group had 90 chickens, 30 from the aforementioned breeds. Litter of wood shaving was put as bedding and drinkers and feeders used were made of plastic. The birds were fed with road runner starter mash and growers mash made by the same company, Nhema Chickens. The groups were named as follows:

AA- No prophylaxis control group.

AB- Coccidiostat (Amprolium and ESB_3) control group.

AC- *M. flabellifolius* experimental group, which was further divided into three subgroups which were given *M. flabellifolius* at different concentrations which are: AC_1, at 5%, AC_2 at 10%, and AC3 at 15%.

3.3.1.1 Preparation of *M. flabellifolius*

The plant was collected, washed, dried and was grinded and then slaved. Afterwards, the powder was then mixed with water at a rate of 100g: 100ml of water, this was considered as the 100% concentration solution. Concentration of 5, 10 and 15% of the extract was then mixed with 800, 900 and 950ml of water respectively.

All chickens from the 3 groups were vaccinated against New Castle, infectious Bursal disease and fowl pox at day 1, day 9 and day 18 respectively. The experiment was carried over 40 days. Coccidiosis infested dropping was collected from a fowl run where coccidiosis infested chickens were housed. The dropping were then introduced to drinking water of all the 3 groups at day 21. Birds in AA were given feed and water without any additives Birds in AB (coccidiostat prophylaxis were given 1,2g of Amprolium 200 per 25 liters of water from day 1 to day 21, form day 22 to day 40, they were given ESB_3 at a rate of 2g liters using a 3-4-3 rules (3 days treatment, 4 day rest, 3 day treatment). Birds in AA were given the *M. flabellifolius* solution from day 1 to day 40, at different concentration rates of 5 % (AC_1), 10 % (AC_2) and 15 %(AC_3).

3.3.1.2 Oocytes per gram (OPG)

To determine the anticoccidial effects of *M. flabellifolius*, the OPG in all groups were measured from each group after 10 days post infection based on the methods by (Gadzirayi and Mupangwa, 2005). The faecal oocyst concentration reduction rate was determined by using the following formula as per (Tchankugni *et al.*, 2019).

$$\text{Faecal oocyte concentration reduction rate} = \frac{\text{initial mean OPG final mean OPG}}{\text{Initial means OPG}} \times 100$$

3.3.1.3 Growth rate.

All the birds used in the study were weighed every 7th day using a scale, in kilograms. The weights were then recorded so as to know the growth rate against the normal indigenous chicken growth curve.

3.3.1.4 Mortality rate.

This was determined by the number birds that died post infection.

3.3.1.5 Sampling procedures

Analyses of the phytochemical compounds in *M. flabellifolius* plant through hot water extraction and solvent extract

The fresh plant samples of *M. flabellifolius* were collected from Serima, Gutu District. The extraction of the plant involved the use of hot water and acetone to separate a diverse array of phytochemicals, such as flavonoids, tannins, and various phenolic compounds. A qualitative and quantitative analysis of the plant was carried out.

3.3.1.6 Determination of the level of effectiveness of plant extracts of *M. flabellifolius* on treating coccidiosis

To determine the anticoccidial effects of *M. flabellifolius*, the OPG in all groups were measured from each group after 10 days post infection. For growth rate determination, all the birds used in the study were weighed every 7th day using a scale, in kilograms. Mortality was determined by the number birds rat died post infection.

3.3.1.7 Formulation and packaging ofa *M. flabellifolius* plant based coccidiostat

The sampling plan was to formulate a *M. flabellifolius* plant based coccidiostat. Gutu, Masvingo was the sampling location.

3.4 Data collection methods

3.4.1.1 Collection of plant materials

Fresh stems of *M. flabellifolius* were collected from Serima in Gutu district, Masvingo province.

3.4.1.2 Identification and quantification of *M. flabellifolius* bioactive compounds

There were standard qualitative and quantitative tests that were performed to identify the presence of various classes of the metabolites present. The results were recorded as negative or positive for the presence of each phytochemical class. Total phenolic compounds, total flavonoid compounds, total saponin compounds, and total alkaloid compounds were quantified through the process of quantitative analysis.

3.4.1.3 *M. flabellifolius* coccidiostat effectiveness

There was measurement of infection intensity, growth performance trials and mortality rate assessments performed to determine the effectiveness of *M. flabellifolius* as a coccidiostat. The results were Oocytes per gram (OPG), % weight gain, % mortality rate respectively.

3.4.1.4 Formulation and packaging of*M. flabellifolius* based coccidiostat

The formulation and packagingand packagingdevelopment and packaging and packaging of a coccidiostat can be described as; labelling, packaging material, and packing design, packaging sizes, quality control, stability testing, chemical stability and microbiological stability.

3.4.1.5 Formulation and packaging of*M. flabellifolius* based coccidiostat.

The Freeze-dried dried ethanol extract of *M. flabellifolius* was prepared. Hard Gelatin Capsules (Size 00), Colloidal Silicon Dioxide (Aerosol), Magnesium Stearate, Microcrystalline Cellulose MCC, Hydrochloric Acid (0.1NHCl), Buffer pH6.8 Solution, Citric Acid, and Ethanol were obtained from HIT. All chemicals used were all of analytical grade and other materials.

3.5 Formulation and packagingand packagingand Evaluation of *M. flabellifolius* Extract

3.5.1 Determination of the Organoleptic Properties of Extract

The following organoleptic qualities of the extract were evaluated: physical appearance, odor, and taste. Extracts of M. flabellifolius were examined and evaluated using the natural senses (e.g., eyes, nose, and mouth). As illustrated in Figure 2.5.1 1.

Figure 3.5.1-1: Semisolid and Powder of M. flabellifolius Bush Extract

3.5.2 Determination of the Solubility of Extract

The solubility of a substance fundamentally depends on the solvent used as well as on temperature and pressure. The extent of solubility of a substance in a specific solvent is measured as the saturation concentration where adding more solute does not increase its concentration in the solution. Oral ingestion is the most convenient and commonly employed route of drug delivery due to its ease of administration, high patient compliance, cost effectiveness, least sterility constraints, and flexibility in the design of dosage form. As a result, many of the generic drug companies are inclined more to produce bioequivalent oral drug products. So, the solubility application according to standard parameters of solubility as shown in Table 3.5.2-1.

Table 3.5.2-1: Standard Parameters of Solubility.

Description	**Part of The Solvent Required Per Part of Solute**
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100

Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Practically insoluble	More than 10,000

3.5.3 Determination of the Density of Extract

Reformulation and packagingand packagingparameters like bulk density, tap density and Carr's index, were obtained for the powders. A known quantity of powder was poured into the measuring cylinder carefully level the powder without compacting, if necessary and read the unsettled apparent volume, Vo, to the nearest graduated unit as shown in Table 3.5.3-1.

Calculate the bulk density, in gm per ml, by the formula.

Bulk density = Bulk Mass/ Bulk Volume Carr's compressibility index: Carr's index (%) = (Tapped density – Poured density) / Tapped density

Table 3.5.3-1: Carr's Index Parameters of Powder Flowability.

Carr's Index%	Type of Flow
5-15	Excellent
12-16	Good
18-21	Fair to Passable
23-35	Poor
33-38	Very Poor
>40	Extremely Poor

3.5.4 Determination of the Flowability of Extract

The angle of repose (θ) is an important metric for describing the flowability of powders. In this investigation, a specialized apparatus was used for testing. The device had a glass cylinder in the center of the plate, a plate with a scale, and a ruler for measuring the height of the powder mound. To establish the angle of repose, the glass cylinder was filled with 10g of plant extract (passed through a 180 screen), then smoothly lifted, allowing the powder to pour out the bottom

into the plate, leaving a conical mound as illustrated in Table 3. The height and radius of the mound were measured, and the angle of repose was computed using the equation below:

$$\tan \theta = h / r$$

Θ: Angle of repose.

h: height of the conical mound.

r: radius of the conical mound.

Table 3.5.4-1: Flow property and Angle of Repose

Flow Property	Angle of Repose (Degrees)
Excellent	<20
Good	20-30
Passable	30-34
Very poor	.40

3.5.5 Formulation and packaging of*M. flabellifolius* Extract Capsules

A uniform powder is obtained by mixing the semisolid of *M. flabellifolius* extract with the appropriate adsorbent microcrystalline cellulose. lubricant as mg stearate, and glidant as colloidal silicon dioxide (Aerosol), the materials filled into the capsules as shown in Table 4.

Table 3.5.5-1: The Formulation and packaging ofM. flabellifolius Extract Capsules.

Ingredients	Amount (mg)
M. flabellifolius	250
Microcrystalline Cellulose	240
Mg Stearate	5

Colloidal Silicon Dioxide (Aerosil)	5

3.6 Evaluation of *M. flabellifolius* Extract Capsules

3.6.1 Determination of Uniformity of Weight and The Amount of *M. flabellifolius* Capsules

For the determination of the uniformity of weight, the British Pharmacopoeia method was used. Weight homogeneity was determined using the British Pharmacopoeia technique. The contents of twenty M. flabellifolius capsules were individually weighed, and the average weight (mass) of the contents was obtained. Only two of the individual weights (masses) could differ from the average weight (mass) by more than 7.5%, and none could deviate by more than double that proportion. The actual amount of powder placed into the capsules was compared to the desired quantity, and the difference was determined. The formulation and packagingand packagingcalled for 250mg of M. flabellifolius extract in a single capsule. Twenty capsules were selected at random, their contents weighed, and the percentage difference between this and to assess the accuracy of the filling process, the intended weight was computed and averaged over 20 capsules.

3.6.2 Determination of Moisture Content of *M. flabellifolius* Extract Capsules

Firstly, the capsule contents were removed using a suction element to avoid contamination and to ensure an accurate measurement of the capsule shell itself. Subsequently, the capsule shell was cut open and any residual substances were wiped off with a solvent. For the actual moisture determination, headspace gas chromatography was utilized. It involved heating the sample in a headspace vial and measuring the water vapor using gas chromatography signals, providing high sensitivity and automation.

3.6.3 Determination of The In-Vitro Dissolution of *M. flabellifolius* Extract Capsules

The dissolving test examines the rate at which a drug is released into solution from a dosage form and is used to assess a pharmaceutical product's bioavailability and quality. To make testing methods simpler, In this study the paddle method was used. The quantitation of the amount of

extract dissolved was measured based on UV absorbance measured at 330nm, the wavelengths for maximum UV absorbance of solutions of the *M. flabellifolius* extract determined by using a UV- Vis Spectrophotometer. For the dissolution study the following requirements and Procedure were used:

Apparatus: Paddle.

Medium: 6.8 citric acid buffer. Volume of medium: 900ml.

Temperature: 37±0.5°C. Rotation speed: 100 rpm.

Dissolution time: 10, 20, 30, 40, 50 and 60 minutes.

To prepare the equipment, 900 ml of pH 6.8 citric acid buffer was degassed and warmed to 37±0.5°C in a water bath. One capsule was deposited in each vessel, the paddle was lowered into position, and the apparatus was turned on immediately at 100 rpm. At various time intervals, namely 10, 20, 30, 40, 50, and 60 minutes after start, 3ml samples of the medium were extracted from a location halfway between the surface of the dissolving medium and the top of the revolving paddle and no less than 10 mm from the vessel's wallEach time the withdrawn medium was immediately replaced by 3 ml of pH 6.8 citric acid buffer introduced into the vessel. The UV absorbance of the solution was determined at the wavelengths mentioned earlier and using the solution of one of the empty capsule shells dissolved in the 900 ml volume of dissolution medium as a blank reference solution.

3.7 Stability testing

a) Storage conditions

i. Temperature and Humidity

In capsule storage conditions determination, a Distributed Control System (DCS) was used. The DCS collected temperature and humidity parameters through an environment module, transmitting these to a data storage module for processing and comparison against set standards.

b) Physical appearance

Color changes for the *M. flabellifolius* extract capsules was monitored over 14 days.

c) Chemical Stability

The *M. flabellifolius* extract capsules were exposed to various stress conditions such as light, oxidation, and dry heat, acidic, basic, and hydrolytic environments, as recommended by the International Council for Harmonization of Technical Requirements of Pharmaceuticals for Human Use (ICH) guidelines. It was analyzed after 14 days on the previously mentioned conditions, using High performance liquid chromatography.

d) Labelling

— label includes:

1) product name logo
2) Active ingredients and concentration
3) Instructions for use
4) Warning
5) Storage conditions
6) Shelf life.

e) Packaging material

- food grade and safe for animal consumption
- resistant to moisture, light and temperature fluctuations
- easy to handle and store.
- compatible with the product formulation

f) Packing design

- attractive and visually appealing
- easy to open and close
- tamper resistant/temper evident

g) Packaging sizes

- different sizes for different customer needs and affordability i.e. small, medium and large

h) Quality Control

- Accurate labelling and packaging
- correct product formulation and packagingand packagingand concentration
- clean and sanitized packaging material and equipment

3.8 Data analysis methods

This section describes the analytic tools to be used in the research project on each objective. As for objective one, the results we expressed as mean +/- standard deviation to estimate the variability within the data sets, followed by an independent samples t-test which was done to show no violation for the assumption of equal variances between the hot water and acetone extracts for the total alkaloids parameter.

On objective two, mortality was observed by counting dead birds after infection. An analysis of variance (One way ANOVA) was performed on the bird's weight gain. The bird's weight gain was analysed using the Bonferroni post-hoc test for pairwise comparisons. The results for oocyst reduction rate were expressed as percentages. The mortality rates were expressed in percentages.

3.9 Chapter Summary

The chapter first described the study regions of Gutu, Nhema Chicken Farm, and Harare Institute of Technology. The chapter covered research design, sample procedures, data collection, and data analysis for all three objectives. In addition, the chapter thoroughly evaluated the data analysis methods and analytic presentation for each study purpose. The chapter also covered data coding, entry, cleaning, and processing, as well as statistical tools used for data analysis.

The next chapter will show and examine the findings from Objective One. This chapter focuses on the quantitative and qualitative analysis. The results were expressed as mean +/- standard

deviation, followed by an independent samples t-test descriptive statistics and tables were used to aid the analysis of the data.

3.10 References

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CHAPTER 4

ANALYSING THE PHYTOCHEMICAL COMPOUNDS IN *MYROTHAMNUS FLABELLIFOLIUS* PLANT THROUGH HOT WATER EXTRACTION AND SOLVENT EXTRACT

ABSTRACT

The variability in phenolic content across different geographical regions necessitates careful consideration in sourcing and standardizing the *Myrothamnus flabellifolius* plant for consistent efficacy. To mitigate the challenge, phytochemical screening on *M. flabellifolius* plant was conducted on both hot water and acetone extracts. The objective was to investigate the presence of various primary and secondary metabolites. For the qualitative analysis, phytochemical compounds were identified using standard screening tests, including tests for phenols, tannins, saponins, glycosides, alkaloids, flavonoids, and terpenoids. The data was analysed using descriptive statistics to provide a summary of the characteristics of the coccidial compounds. This included measures such as mean and standard deviation. Also, the results were subjected to an independent samples t-test. The qualitative results revealed that, phenols, tannins, saponins, glycosides, alkaloids, flavonoids, and terpenoids were present in the plant extracts. Furthermore, quantitative analysis, quantified phenols, flavonoids, tannins, saponins and alkaloids. Overall, the results indicate that the acetone solvent was more effective in extracting these phytochemical compounds from the plant material compared to the hot water solvent. A multi-faceted approach using ethanol, methanol, and hydro distillation, combined with advanced processing techniques, can maximize the extraction efficiency of *M. flabellifolius*, ensuring the retrieval of its diverse bioactive compounds for various therapeutic applications.

Keywords: Coccidiosis; Phytochemical; Qualitative; Quantitative; *Myrothamnus flabellifolius*; Aqueous extract; Solvent extract

4.1 Introduction

Coccidiosis, induced by the Apicomplexan protozoan parasite *Eimeria*, is a disease with a worldwide presence that impacts the gastrointestinal tract of diverse animal species, such as chicken and wild birds, resulting in substantial financial setbacks in both extensive and intensive farming operations (Noguera Z. et al., 2022).

In the African continent, the prevalence of this ailment is notable, especially in areas characterized by substandard sanitation conditions and insufficient access to veterinary services, factors that contribute to the escalation and consequences of the disease. Zimbabwe, facing numerous healthcare challenges such as HIV, tuberculosis, and malaria, also contends with fungal infections and other parasitic diseases like coccidiosis, which receive less attention despite their significant impact on public health and agriculture (Dermauw *et al.*, 2018).

Water sources in peri-urban settlements around Harare were contaminated with various pathogens, including protozoan parasites like Cryptosporidium, which shares similar transmission routes with coccidia, highlighting the risk of waterborne coccidiosis in these areas(Manyenyeka *et al.*, 2022). Addressing the issue of coccidiosis in Zimbabwe necessitates a comprehensive strategy that encompasses the amalgamation of traditional veterinary expertise with contemporary methodologies, enhancement of water purity, and reinforcement of monitoring and management protocols to minimize the dissemination of coccidiosis as well as other concurrently infecting agents.

Addressing coccidiosis in Zimbabwe has been suboptimal due to several factors. The reliance on traditional remedies, while showing high botanical and veterinary consistency, lacks standardization and validation, which hinders their integration into orthodox veterinary medicine (Martin *et al.*, 2023). Addressing the issue of coccidiosis in Zimbabwe necessitates a comprehensive strategy that combines conventional ethno veterinary methods with contemporary veterinary science, enhanced hygiene practices, and health education initiatives rooted in the community. Traditional remedies for coccidiosis in chicken have shown high botanical and veterinary consistency, indicating their potential effectiveness and the need for standardization and validation to integrate them fully into orthodox veterinary practices (Matekaire and Bwakura, 2004).

Research efforts to address coccidiosis in Zimbabwe, a significant intestinal disease affecting chicken, have been multifaceted, focusing on both traditional and innovative approaches. Historically, control has relied heavily on anticoccidial drugs and live vaccines, but issues such as drug resistance and public health concerns have necessitated the development and packaging and packaging of new strategies(Pfavayi *et al.*, 2021). Coccidiosis, a significant disease affecting chicken, is a concern in Zimbabwe, but there is limited comprehensive knowledge and standardized practices to address it effectively. The aim of this research is to discover and measure the phytoconstituents present in M. flabellifolius, a promising coccidiostat.

4.2 Materials and methods

Details about the study area and technique, which include the characterization of M. flabellifolius' coccidial chemicals. The objective of this chapter is to provide a synopsis.

4.2.1 Description of study area

The study was conducted at Harare Institute of Technology (HIT), a leading state-owned university located in Harare, the capital city of Zimbabwe.

4.2.2 Research Design

The study employed an analytical research design comprising hot water extraction, solvent extraction and the phytochemical analysis (quantitative and qualitative) using different chemicals for each compound Initially, conducting preliminary phytochemical screening is crucial for the identification of various classes of compounds like flavonoids, alkaloids, tannins, and phenolics, which have been documented in *M. flabellifolius*. The utilization of solvent extraction methods employing ethanol, methanol, and water is imperative to enhance the extraction of these compounds from diverse plant components, including leaves and twigs. High-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) are highly recommended for the thorough analysis and quantification of specific phytochemicals, such as polyphenols and essential oils. The incorporation of standards like gallic acid and quercetin is essential for the determination of total phenolic content (TPC) and total flavonoid content (TFC). Furthermore, evaluating the antioxidant potential of the extracts through various assays like DPPH, superoxide anion, and hydroxyl radical scavenging assays is necessary to establish a correlation between phytochemical content and biological activity. Moreover, the isolation and

characterization of individual compounds such as arbutin and lupeol using methodologies like NMR spectroscopy for structural elucidation is a critical step in the process. Details regarding the research design is given in Section 3.3 of Chapter 3.

4.2.3 **Sampling procedure**

The fresh plant samples of *M. flabellifolius* were collected from Serima, Gutu District. The extraction of the plant involved the use of hot water and acetone to separate a diverse array of phytochemicals, such as flavonoids, tannins, and various phenolic compounds. A qualitative and quantitative analysis of the plant was carried out.

4.2.4 **Data collection procedure**

Based on the information provided in section 3.4 of Chapter 3, the data collection of phytochemical analysis of *M. flabellifolius* can be described as; there were standard qualitative and quantitative tests that were performed to identify the presence of various classes of the metabolites present. The results were recorded as negative or positive for the presence of each phytochemical class. Total phenolic compounds, total flavonoid compounds, total saponin compounds, and total alkaloid compounds were quantified through the process of quantitative analysis.

4.2.4.1. **Identification and collection of plant materials**

Fresh stems of *M. flabellifolius* were collected from Serima in Gutu district. The plant materials were taxonomically identified and authenticated by National Botanical Gardens in Harare under the Ministry of Agriculture. The plant materials were dried, grinded and stored.

4.2.4.2. **Preparation of plant extracts**

4.2.4.2.1. Hot water extraction

The plant material was extracted using hot water extraction. The extract was kept in refrigerator for future uses.

4.2.4.2.2. Solvent extraction

The solvent acetone was employed in the preparation of the crude extract. Crude plant extract was then stored in the refrigerator.

4.2.5 **Data analysis procedure and methods**

The phytochemical analysis was conducted in quintuplicate to guarantee the dependability of the data. The results we expressed as mean +/- standard deviation to estimate the variability within the data sets. An independent samples t-test was done to show that the assumption of equal variances between the hot water and acetone extracts was not violated for the total alkaloids parameter. Section 3.9 provides more information on the data analysis approach and analytic presentation.

4.2.6 **Qualitative phytochemical analysis**

Proteins, carbohydrates, phenols, tannins, flavonoids, saponins, glycosides, terpenoids, and alkaloids were all detected in both hot water extraction and acetone extracts.

4.2.7 **Quantitative phytochemical analysis**

The total phenolic, flavonoid, tannin, saponin, and alkaloid content were quantified using hot water extraction and acetone extract.

4.3 **Challenges encountered during data collect**

The phytochemical analysis of *M. flabellifolius* presents several challenges during data collection, primarily due to the complexity and diversity of its chemical constituents. The significant chemical diversity and existence of several classes of chemicals, such as flavonoids, anthocyanins, and terpenoids, requires the employment of multiple solvent extraction methods, which can be time-consuming and require careful optimization to assure thorough extraction. Furthermore, the extraction and characterization of complex metabolites, such as pectic polysaccharides and galloylquinic acids, presents additional hurdles, frequently necessitating sophisticated chromatographic techniques and thorough structural elucidation using NMR and mass spectrometry.

A further layer of complexity is added by the dynamic nature of the plant's metabolome, which is altered by genetic variation and environmental influences. This makes standardizing data gathering techniques challenging. Furthermore, because analytical platforms are always changing and domain-specific knowledge is required, handling, integrating, and effectively interpreting the massive amounts of data created by these analyses requires reliable data management systems.

4.4 Results and discussion

4.4.1 Phytochemical Analysis

Table 4.4.1-1: Qualitative Phytochemical Analysis of M. *flabellifolius*.

Phytochemical tested for	**Hot aqueous extract**	**Acetone extract**
Proteins	-	-
Carbohydrates	+	-
Phenols	+	+
Tannins	+	+
Saponins	+	-
Glycosides	+	+
Alkaloids	+	+
Flavonoids	-	+

Source: Phytochemical Analysis, 2024

The following is the representation of the results of qualitative analysis of *Myrothamnus flabellifolius* (Mufandichimuka).

Proteins: The Table 4.1 show study results that proteins were absent in both the extracts. Notably, multiple investigations have found that this plant lacks specific proteins and amino acids. For instance, a comprehensive phytochemical analysis revealed that proteins and amino acids tested negatively in all solvent extracts from both leaves and twigs of *M. flabellifolius*

(Cheikhyoussef, Summers and Kahaka, 2015). Additionally, the phytochemical analysis of *M. flabellifolius* revealed the presence of various classes of compounds, including proteins and amino acids, in the leaves and twigs extracts (Chukwuma *et al.*, 2019a).

Carbohydrates: the study results on Table 4.1 show that carbohydrates are present in the aqueous extract but absent in the acetone extract. *M. flabellifolius* water extract contains complex carbohydrates known as heterogeneous pectic polysaccharides. These polysaccharides are made up of numerous monosaccharides such as arabinose, rhamnose, xylose, mannose, galactose, and glucose, as evaluated by acid hydrolysis and subsequent gas chromatography analysis (Ajao et al., 2023). *M. flabellifolius* leaf extracts contain a variety of phytochemicals, including flavonoids, anthocyanins, alkaloids, steroids, terpenoids, triterpenes, cardiac glycosides, saponins, phlobatannins, tannins, polyphenols, and reducing sugars, but these studies did not specifically focus on acetone extracts. While particular research on the absence of carbohydrates in *M. flabellifolius* acetone extracts are limited, the overall trend in phytochemical extraction implies that acetone may not successfully extract carbs from M. flabellifolius, as has been observed in other plants.

Phenols: the study results also show that phenols were present in both the extracts. Several investigations have established the existence of phenolic chemicals in this plant's preparations, including aqueous ones. For instance, the study by Cheikhyoussef et al. identified a high total phenolic content (TPC) in the aqueous extracts of *M. flabellifolius*, which ranged from 372.42±0.21 to 375.14±0.21 mg gallic acid equivalent (GAE)/g, indicating a significant presence of phenols (Kwape, Majinda and Chaturvedi, 2016). However, the phenolic concentration in the aqueous extract may vary, as some studies have indicated the absence of specific phenolic components such anthraquinones, anthranoids, and iridoids in both leaves and twigs extracts (Ajao et al., 2023). Furthermore, the plant's extracts, particularly those prepared using acetone, have been proven to include a variety of phenolic components such as flavonoids, tannins, and polyphenols, which are responsible for its high antioxidant activities (MOORE et al., 2004; Ajao and Ashafa, 2017a). There is no concrete proof or particular study addressing the lack of phenols in *M. flabellifolius* acetone extracts, despite the fact that studies have concentrated on different solvent extracts such as ethanol, methanol, and water. The comprehensive phytochemical analyses and antioxidant evaluations across different studies consistently highlight the presence

of phenolic compounds in *M. flabellifolius*, regardless of the extraction method used(Moore *et al.*, 2005; Nako, 2014; Gadaga, Zvidzayi and Tagwireyi, 2019a; Ajao *et al.*, 2023) .

Tannins: the study results indicate presents of tannins in both aqueous and acetone extracts. A study investigating the phytochemical content of *M. flabellifolius* identified tannins among the twelve classes of phytochemicals present in the leaves extracts, which included aqueous extracts (Ajao and Ashafa, 2017b). Although the precise lack of tannins in aqueous extracts is not explicitly mentioned, the majority of the evidence suggests that tannins are present in a variety of *M. flabellifolius* extracts, including those that use water as a solvent. Specifically, the acetone extract of this plant has been shown to contain high levels of polyflavonoid tannins, which are crucial for its resuscitation behaviour under drought conditions. These tannins increase under drought-induced stress, contributing to the plant's ability to avoid cell wall cracking and maintain structural integrity during dehydration and rehydration cycles (Cheikhyoussef, Summers and Kahaka, 2015) . while the absence of tannins in acetone extracts of *M. flabellifolius* has not been specifically studied, the overall evidence suggests that the choice of solvent plays a crucial role in determining the phytochemical profile of the extracts, and further research is needed to explore the acetone extract specifically(Gadaga, Zvidzayi and Tagwireyi, 2019b; Ajao *et al.*, 2023).

Saponins: the study results in Table 4.1 show that saponins are present in aqueous extract not in the acetone extract. A study on the phytochemical composition and antioxidant activity of *M. flabellifolius* found saponins among the twelve classes of phytochemicals present in the aqueous extract of the leaves and twigs (Ajao and Ashafa, 2017b). The absence of saponins in the aqueous extract could be attributed to their solubility qualities, as saponins are more soluble in organic solvents such as methanol or butanol. This is supported by the fact that other studies have successfully isolated saponins using these solvents(Cheikhyoussef, Summers and Kahaka, 2015; Fultang *et al.*, 2018). Adding on, the use of ethyl acetate and n-butanol has been more successful in isolating saponins from various plant extracts, including *M. flabellifolius* and *Acacia etbaica*, indicating that these solvents might be more suitable for saponin extraction(Algfri, Alshakka and Munaiem, 2014; Brar *et al.*, 2018).

Glycosides: the study results indicate that glycosides are present in both solvents. The isolation of specific glycosides like arbutin from the butanol extract and 2,3-di-O-galloylarbutin from the ethyl acetate fraction of an acetone/water extract underscores the presence of glycosides in the plant(Nako, 2014; Cheikhyoussef, Summers and Kahaka, 2015; Kwape *et al.*, 2020). However, while studying the aqueous extract, it is worth noting that certain phytochemical families, such as glycosides, were not discovered. A study on the phytochemical content of *M. flabellifolius* using various solvents, including water, discovered that, while the plant is high in flavonoids, anthocyanins, alkaloids, steroids, terpenoids, and other compounds, glycosides were significantly absent in the aqueous extract (Chukwuma et al., 2019a). While glycosides are present in *M. flabellifolius*, the absence of glycosides in acetone extracts remains an unexplored area in the current literature.

Alkaloids: the study results show presents of alkaloids in aqueous and acetone solvent. The presence of alkaloids, as well as flavonoids, steroids, terpenoids, triterpenes, cardiac glycosides, saponins, phlobatanins, tannins, polyphenols, and reducing sugars, was confirmed by phytochemical screening of the plant's leaves and twigs using various solvent extractions, including water (Ajao et al., 2023). Notably, the aqueous extract of *M. flabellifolius* was shown to be deficient in some phytochemicals, particularly alkaloids. For example, a study on the phytochemical composition and antioxidant activity of *M. flabellifolius* from Namibia found that alkaloids were not detected in aqueous extracts of both leaves and twigs, but were present in ethanol and methanol extracts. A study investigating the phytochemical content and antioxidant activity of *M. flabellifolius* from Namibia confirmed the presence of alkaloids in the leaves extracts obtained through solvent extraction methods, including acetone(Gadaga, Zvidzayi and Tagwireyi, 2019b). Therefore, while alkaloids are present in M. flabellifolius, their detection is highly dependent on the extraction method and solvent used, with acetone extracts specifically lacking reports of alkaloid presence(Nako, 2014; Ajao and Ashafa, 2017a; Brar *et al.*, 2018; Chukwuma *et al.*, 2019b).

Flavonoids: the study results revealed absence of flavonoids in the aqueous extraction whereas they we present in the acetone extraction. The phytochemical investigation of *M. flabellifolius* from Namibia demonstrated the presence of flavonoids, among other components, in the leaves and twigs, confirming their presence in aqueous extracts (Chukwuma et al., 2019b). Other

research has highlighted *M. flabellifolius* ' considerable antioxidant and antidiabetic effects, which are frequently attributed to its high polyphenolic content, particularly flavonoids, in non-aqueous extracts (Chukwuma et al., 2019b; Kwape et al., 2020). The hydro ethanol extract of *M. flabellifolius*, which shares similarities with acetone extracts in terms of solvent polarity, was found to contain significant amounts of flavonoids, contributing to its potent antioxidant and enzyme inhibitory activities(Cheikhyoussef, Summers and Kahaka, 2015). While several investigations have verified the existence of flavonoids in *M. flabellifolius* extracts, no specific study has been conducted to investigate their absence in acetone extracts.

4.4.2. Quantitative determination of phytoconstituents using aqueous and acetone extraction from *M. flabellifolius*

4.4.1.1.1. Quantitative determination of phytoconstituents from *M. flabellifolius* using hot water extract

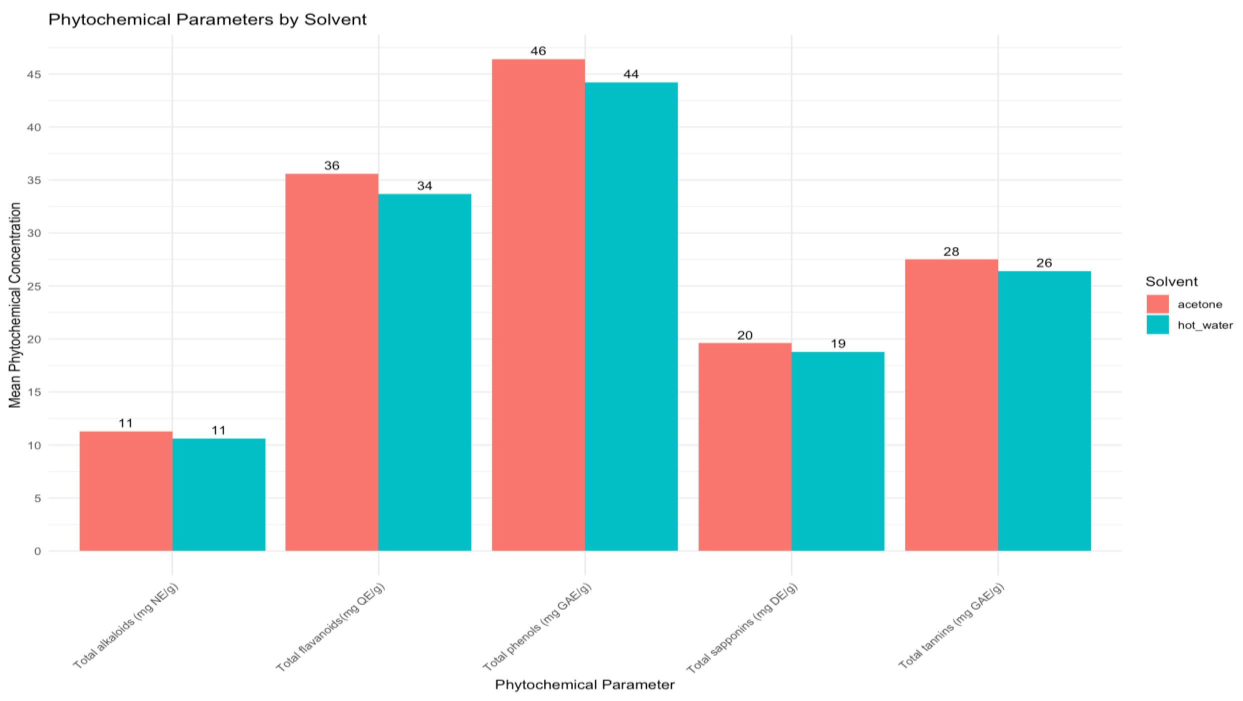

Figure 4.4.2-1:It shows the total bioactive compounds of *M. flabellifolius* aqueous and solvent extracts of the shrub yielded total alkaloids, total flavonoids, total phenols, total saponins, and total tannins, respectively. **Source: Phytochemical Analysis, 2024.**

Alkaloids: The study results suggest that the total alkaloid content is 10.6 mg NE/g and 11.3 mg NE/g, in water and acetone solvents, respectively. While there is no literature to exactly quantify total alkaloids in hot water extracts of *M. flabellifolius*, the plant's overall phytochemical richness, including alkaloids, supports its traditional medicinal uses and potential health benefits(Dhillon *et al.*, 2014; Nako, 2014; Chukwuma *et al.*, 2019b). Therefore, while the specific figure of 10.8 mg NE/g for total alkaloid content in hot water extract is not directly confirmed by literature, the significant presence of alkaloids and other bioactive compounds *in M. flabellifolius* is well-documented, underscoring its therapeutic potential. Adding on, the 11.3 mg NE/g in *M. flabellifolius* acetone extract aligns with the variability observed in other plants, indicating that alkaloid content can significantly differ based on species, plant part, and environmental factors, validating its potential as a potent natural source of alkaloids(Tang *et al.*, 2023).

Flavonoids: the study results revealed that total flavonoids content is 33.7mg QE/g in the hot water extract. In contrast, flavonoid content is 35.6 mg QE/g in the acetone extract. According to research, the total flavonoid concentration (TFC) in *M. flabellifolius* varies greatly according on the extraction process and solvent utilized. For instance, a study on the phytochemical content and antioxidant activity of *M. flabellifolius* reported TFC values ranging from 1.43±0.03 to 3.49±0.15 mg quercetin equivalents (QE)/g in different solvent extracts, including ethanol, methanol, and water(Cheikhyoussef, Summers and Kahaka, 2015). In comparison, other studies on different plants and fruits have shown a wide range of TFC values. For example, traditional Chinese fruits exhibited TFC values ranging from 12.3 to 295.4 mg/g, and medicinal mushrooms showed values between 1.5 and 4.34 mg rutin equivalents/g(Ajao and Ashafa, 2017b; Gadaga, Zvidzayi and Tagwireyi, 2019b). Similarly, the TFC in Hibiscus sabdariffa beverages varied from 134.00 to 250.50 mg QE/ml, depending on the extraction conditions(Abozed, 2014). These variations underscore the influence of extraction methods and plant species on TFC. Therefore, while the reported TFC of 33.7 mg QE/g in *M. flabellifolius* hot water extract seems plausible, it is essential to consider the specific extraction conditions and methods used in each study to understand the discrepancies fully. While specific data on acetone extracts of *M. flabellifolius* is not directly provided in literature, we can infer comparisons from other studies. For instance, the TFC in the methanol extract of *M. flabellifolius* leaves was found to be significantly high, with values ranging from 1.43±0.03 to 3.49±0.15 mg quercetin equivalent (QE)/g(Cheikhyoussef,

Summers and Kahaka, 2015). This is relatively lower compared to other plants like Elaeocarpus floribundus, which showed a TFC of 149.28 ± 0.89 mg QE/g in methanol extract (Kabra *et al.*, 2019). Similarly, Glochidion arborescens Blume leaves exhibited a TFC of 40.3350 mg QE/g in ethyl acetate extract(Utami *et al.*, 2023). In another study, the methanol extract of M. esculenta leaves demonstrated a TFC of 67.44 ± 0.14 mg QE/g, which was higher than its aqueous extract(Ira Rahmiyani, Ruswanto, and Nadiya Nur Fitriana, 2020). Additionally, the TFC in pumpkin skin extracts varied significantly, with the highest being 4.64 ± 0.02 mg QE/g in acetone extract(Gadaga, Zvidzayi and Tagwireyi, 2019b). These comparisons suggest that while *M. flabellifolius* may have a notable TFC, it might not reach the 35.6 mg QE/g mark in acetone extract, as seen in other plants. The extraction method and solvent polarity play crucial roles in determining the TFC, as evidenced by the higher flavonoid content in more polar solvents like methanol and ethanol(Ajao *et al.*, 2023). Therefore, while *M. flabellifolius* is rich in flavonoids, achieving a TFC of 35.6 mg QE/g in acetone extract seems unlikely based on the available data from various studies on different plants and extraction methods(Lee, 2009; Yasmine *et al.*, 2014; Rakass, Babiker and Oudghiri-Hassani, 2018; Chukwuma *et al.*, 2019b).

Phenols: the study results revealed that the total quantity of phenols 44.3mg GAE/g. However, the total quantity of phenols in acetone extract is 46.4mg GAE/g. The total phenolic content of 44.3 mg GAE/g in the hot water extract of *M. flabellifolius* can be evaluated by comparing it with various studies on phenolic content in different plant extracts. For instance, the total phenolic content in the methanol stem extract of Garcinia pedunculata was found to be significantly higher at 76.30 mg GAE/g, indicating that different solvents and plant parts can yield varying phenolic concentrations (Chowdhury, 2014). Similarly, the ethanol extract of nutmeg leaves showed a high phenolic content of 183.56 mg GAE/g, suggesting that ethanol might be more effective than hot water for extracting phenolics in some cases(Fawwaz, Nurdiansyah A and Baits, 2017). In contrast, the phenolic content in comfrey extracted using Natural Deep Eutectic Solvents (NADES) ranged from 1.566 to 2.069 mg GAE/g, which is much lower than the 44.3 mg GAE/g in *M. flabellifolius,* highlighting the efficiency of hot water extraction in this context (Özcan and Özcan, 2018). Moreover, the phenolic concentration of Helleborus orientalis extracts ranged from 4.00 to 19.42 mg GAE/g, which is lower than the 44.3 mg GAE/g observed for *M. flabellifolius* , further validating the efficiency of hot water extraction for this species All things considered, the total phenolic content of *M. flabellifolius* is

significantly influenced by the plant part and extraction method used (van Alstyne, 1995; Öztürk et al., 2019; Baysal et al., 2021) even though the phenolic content of 44.3 mg GAE/g is within the range found in other studies. From the acetone extraction, comparatively, studies on other plants, such as bush tea and special tea, have shown total phenolic contents ranging from 2.83 to 6.24 mg GAE/100 g, with special tea exhibiting higher values(Ajao and Ashafa, 2017b). Similarly, the optimized hot water extract of dry kinkeliba leaves (DKL) showed a total phenolic content of 215.0 mg GAE/g, indicating that extraction conditions significantly influence phenolic content(Yu *et al.*, 2013). Given these varied findings, the reported total phenolic content of 46.4 mg GAE/g in a hot water extract of *M. flabellifolius* seems plausible, though it underscores the need for standardized extraction methods to ensure consistent and comparable results across studies.

Tannins: the study results show that the quantitative value of tannins in *M. flabellifolius* is 26.2 mg GAE/g. On the other hand, the quantitative value of tannins in *M. flabellifolius is* 27.5 mg GAE/g. The total tannin content in *M. flabellifolius* has been a subject of varied opinions, particularly when considering different extraction methods and conditions. For instance, the study by Cheikhyoussef et al. reported that the total phenolic content (TPC) in *M. flabellifolius* ranged from 372.42±0.21 to 375.14±0.21 mg gallic acid equivalent (GAE)/g, indicating a high antioxidant potential(Cheikhyoussef, Summers and Kahaka, 2015). This high TPC indicates a large presence of tannins, as phenolic compounds are a primary component of tannins. Additionally, Kwape et al. found that the total phenol content and antioxidant activity of *M. flabellifolius* were substantial, further supporting the presence of tannins. Ajao and Ashafa's research also highlighted the presence of tannins among other phytochemicals in *M. flabellifolius*, which contributed to its antioxidant and antidiabetic activities(Kardel *et al.*, 2013). In contrast, studies on other plants, such as those by Hudaya et al., demonstrated that hot water extraction could effectively remove tannins, with optimal temperatures around 65°C for maximum removal(Kwape, Majinda and Chaturvedi, 2016; Ajao and Ashafa, 2017b). Lower tannin values in certain investigations may be explained by the considerable reduction in tannin content that this approach has been proven to produce. Furthermore, Bianchi's research on European softwood species indicated that hot water extraction yields varied tannin contents depending on the species and extraction conditions, suggesting that similar variability could occur in *M. flabellifolius* (Hudaya, 2015).

Overall, while the reported 26.2 mg GAE/g in hot water extract for *M. flabellifolius* might seem low compared to other findings, it is essential to consider the extraction method and conditions, which can significantly influence the measured tannin content(Hudaya, 2015; Kumari and Jain, 2015; Suh *et al.*, 2019). In the acetone extraction method, solvent type significantly influenced tannin content, as seen in the study of Mucuna seeds where different treatments reduced tannin content by up to 66.16%(Troszyńska, Narolewska and Wołejszo, 2007). Furthermore, acetone-water (70:30) ultrasonic extraction was used to extract tannins from Acacia nilotica and Eucalyptus globulus, producing maximum tannin contents of 196.1 and 125.2 mg/g, respectively (Kardel et al., 2013). Therefore, the reported tannin content of 27.5 mg GAE/g in *M. flabellifolius* in acetone extract falls within the range observed in other studies, highlighting the influence of extraction methods and plant species on tannin content(Amarowicz *et al.*, 2004; Kumari and Jain, 2015).

Saponins: the results reveal that the quantity for saponins 18.7 mg DE/g. However, the acetone extract results reveal that the quantity for saponins is 19.6 mg DE/g. According to Lee et al.'s study, hot water extraction of ginseng produces a higher saponin content than methanol extraction, suggesting a possible *M. flabellifolius* counterpart (Nako, 2014). However, the quantification of saponins can be influenced by the extraction solvent, as noted by Le et al., who found that solvents like acetone, methanol, and n-butanol can interfere with colorimetric assays, potentially leading to erroneous values(Cheikhyoussef, Summers and Kahaka, 2015). Additionally, the method of extraction and purification, such as the use of macro porous resin adsorption or microbial fermentation, can significantly affect the yield and purity of saponins, as demonstrated in studies on sapindus saponins(Gadaga, Zvidzayi and Tagwireyi, 2019b; Kwape *et al.*, 2020). Furthermore, solvent extraction techniques have proven the presence of a variety of phytochemicals in M. flabellifolius, including saponins; ethanol and methanol extracts have demonstrated a notable phytochemical content. Therefore, while the reported total saponin content of 18.7 mg DE/g in hot water extract of *M. flabellifolius* might be accurate under specific conditions, it is essential to consider the potential variability introduced by different extraction methods, solvents, and analytical techniques(V. Le *et al.*, 2018).

Because of the structural diversity of saponins and the possibility of interference from extraction solvents, quantifying total saponins in *M. flabellifolius,* especially in acetone extracts, can be

challenging. Studies have shown that saponins are present in various parts of M. flabellifolius, including leaves and twigs, and are typically extracted using solvents like ethanol, methanol, and water, but not specifically acetone(Cheikhyoussef, Summers and Kahaka, 2015). The presence of saponins in *M. flabellifolius* has been validated through various studies, which also highlight their significant biological activities, including antioxidant and antidiabetic properties(Akaniro-Ejim *et al.*, 2016; Ajao *et al.*, 2023).

4.4.2 A Quantitative and qualitative determination of phytoconstituents using aqueous and acetone extraction from *M. flabellifolius*

For qualitative analysis, hot water and acetone extraction was used to determine the bioactive compounds from *M. flabellifolius*. Specific tests conducted were, Million's test, Benedict's test, NaOH test, FeCl3 test, Froth test, Keller-kilani test, Wagner's test, Lead acetate test and Chloroform+H2SO4 test. In quantitative analysis, both water and acetone extract were used. Utilizing hot water and acetone extraction, the bioactive components of *M. flabellifolius* were identified. The Folin-Ciocalteu assay, the colorimetric method using aluminum chloride, the Vanillin-sulphuric acid method, the Polyvinylpolypyrrolidone (PVPP) method, and Dragendorff's method were the specific tests carried out.

Phytochemical (Qualitative) Analysis: Aqueous and acetone extract were used to determine the phytochemical characteristics of *M. flabellifolius.* Evidence from these tests show the presence of bioactive compounds which are potential coccidiostat. Many secondary metabolites, such as flavonoids, alkaloids, terpenoids, and polyphenols, which are recognized for their antibacterial and antioxidant qualities, are present in the plant.

Phytochemical (Quantitative) Analysis: Aqueous and acetone extract were used to determine the phytochemical quantities of *M. flabellifolius.* These tests showed varied quantities of bioactive compounds which are potential coccidiostat. The plant contains a variety of secondary metabolites, including flavonoids, alkaloids, saponins, tannins, and polyphenols, which have antimicrobial and antioxidant properties. In terms of total phenols, the hot water extract had a mean value of 44.2 (± 1.8) mg GAE/g, while the acetone extract had the highest mean value at 46.4 (± 2.2) mg GAE/g. Regarding total flavonoids, a similar trend was noted, with the mean of 35.6 (± 1.4) mg QE/g for the acetone extract and 33.7 (± 1.1) mg QE/g for the hot water extract.

Regarding total tannins, the acetone extract showed a greater mean of 27.5 (± 1.3) mg GAE/g than the hot water extract, which had a mean of 26.4 (± 1.0) mg GAE/g. This tendency persisted. The acetone extract exhibited a mean of 19.6 (± 0.9) mg DE/g for total saponins, whereas the hot water extract had a mean of 18.8 (± 0.7) mg DE/g. Lastly, the acetone extract had a slightly higher mean of 11.3 (± 0.4) mg NE/g for total alkaloids than the hot water extract's 10.6 (± 0.3) mg NE/g.

The non-significant Levene's test (p = 0.834, Appendix 1) supports the independent samples t-test results, which demonstrate that the assumption of equal variances between the hot water and acetone extracts was not broken for the total alkaloids parameter. The two-sided p-value for the t-test results was 0.128, which is higher than the significance level of 0.05. This indicates that there is no statistically significant difference in mean total alkaloids between the acetone (M = 11.3 ± 0.4 mg NE/g) and hot water (M = 10.6 ± 0.3 mg NE/g) extracts (p=0.128, Appendix 1). In conclusion, the statistical analysis shows that although the mean total alkaloid content of the acetone extract was insufficient to be deemed statistically significant.

For total tannins, the assumption of equal variances between the hot water and acetone extracts is not violated for this parameter (p=0.921, **Appendix 3**). The t-test for equality of means. The acetone extract had a significantly (p=0.051, Appendix 4) higher mean total tannins content than the hot water extract. The one-sided p-value being less than the 0.05 significance level suggests that the difference in mean total tannins between the hot water (M = 13.1 ± 0.7 mg TAE/g) and acetone (M = 14.4 ± 0.8 mg TAE/g) extracts is not statistically significant in the expected direction. The result from the independent sample t test suggests that the difference in mean total saponins between the hot water (M = 18.8 ± 0.7 mg DE/g) and acetone (M = 19.6 ± 0.9 mg DE/g) extracts is statistically significant (p=0.047**; Appendix 2**) in the expected direction, with the acetone extract having a significantly higher mean total saponins content compared to the hot water extract.The Levene's Test for Equality of Variances results that the assumption of equal variances between the hot water and acetone extracts is not violated for the total phenols measurement (p=0.809, **Appendix 5**). The results of the independent t-test assessing the equality of means suggest a statistically significant difference in the mean total phenols between the hot water (M = 14.2 ± 1.1 mg GAE/g) and acetone (M = 16.3 ± 0.9 mg GAE/g) extracts, with a p-value of 0.047, as shown in Appendix 5.

4.5. **Conclusion**

The chapter has identified phytoconstituents in *M. flabellifolius.* Furthermore, Million's test, Benedict's test, NaOH test, FeCl3 test, Froth test, Keller-kilani test, Wagner's test, Lead acetate test and Chloroform+H2SO4 test were used to identify the presence of the bioactive compounds. Solvents may have been utilized, which would explain some chemicals' absence. There were both primary and secondary metabolites. Primary metabolite identified were, carbohydrates in aqueous extract. The secondary metabolites that followed were phenols, tannins, glycosides, and alkaloids in both extracts, while saponins were detected in the aqueous extract but not in the acetone extract. Lastly flavonoids where present in acetone extract only. Another primary metabolite, proteins, were absent in both extracts. The combined factors of inherent low protein content, extraction method specificity, and the plant's unique biochemical adaptations contribute to the absence of proteins in the aqueous and acetone extracts of *M. flabellifolius.* In addition, the plant's inherent phytochemical profile, which is rich in non-carbohydrate bioactive compounds, and the solvent's selective extraction properties account for the absence of carbohydrates in the acetone extract of M. flabellifolius. Finally, the choice of solvent is critical to the extraction of particular phytochemicals, and acetone's lower polarity renders it unsuitable for extracting saponins from *M. flabellifolius*, requiring the use of more polar solvents for precise phytochemical profiling.

Also, the chapter has quantified phytoconstituents in *M. flabellifolius.* The Folin-Ciocalteu assay, Aluminium chloride colorimetric, Polyvinylpolypyrrolidone (PVPP), Vanillin-sulphuric acid, and Dragendorff's methods were employed for the quantification of bioactive compounds. The findings propose that both solvents demonstrated comparable efficacy in the extraction of total alkaloids from the botanical material, as evidenced by the statistically insignificant Levene's test results ($p = 0.834$). In addition, the plant's inherent phytochemical profile, which is rich in non-carbohydrate bioactive compounds, and the solvent's selective extraction properties account for the absence of carbohydrates in the acetone extract of *M. flabellifolius.* Finally, the choice of solvent is critical to the extraction of particular phytochemicals, and acetone's lower polarity

renders it unsuitable for extracting saponins from *M. flabellifolius,* requiring the use of more polar solvents for precise phytochemical profiling.

Folin-Ciocalteu assay's outcome can be affected by the choice of phenolic standards, with gallic acid often being the preferred standard due to its consistent performance across different studies. In the aluminium chloride colorimetric technique, the selection of a flavonoid reference standard plays a pivotal role, given that variations in results may arise due to the unique absorbance characteristics of different standards such as rutin, quercetin, and catechin. Aluminium chloride colorimetric method relies on the complex formation between AlCl3 and the C-4 keto group and hydroxyl groups of flavonoids, which is selective for flavanols and flavones. In the Polyvinylpolypyrrolidone (PVPP) method, the efficacy of tannin extraction and binding can be modified by the solvent utilized, with acetone demonstrating better extraction efficiency compared to other solvents like methanol or distilled water. The pH of the solution also has a crucial impact, with optimal binding occurring at lower pH values (3-4) and decreasing as the pH approaches neutral. Additionally, the amount of PVPP and the number of treatments can alter the removal efficiency, with repeated low-quantity treatments being more effective than fewer high-quantity treatments. The colorimetric reaction between saponins and vanillin and sulfuric acid results in a colourful complex that may be detected spectrophotometrically. This reaction is the basis of the vanillin-sulphuric acid method. The presence of alkaloids in *M. flabellifolius* can be confirmed using Dragendorff's reagent (DR), as demonstrated in studies where similar methods were applied to other plants. Overall, the findings suggest that the efficiency of the acetone solvent in extracting phytochemical compounds from the plant material surpasses that of the hot water solvent. This is supported by the consistently elevated mean values identified in the acetone extracts across all parameters examined.

4.6. **Recommendations**

To achieve maximum extraction of *M. flabellifolius,* it is essential to consider the solvent type, extraction method, and the specific compounds targeted. All things considered, a multifaceted strategy utilizing hydro distillation, methanol, and ethanol in conjunction with cutting-edge processing methods can optimize *M. flabellifolius* extraction efficiency and guarantee the recovery of its varied bioactive components for a range of medicinal applications.

Initially, conducting preliminary phytochemical screening is crucial for the identification of various classes of compounds like flavonoids, alkaloids, tannins, and phenolics, which have been documented in M. flabellifolius. The utilization of solvent extraction methods employing ethanol, methanol, and water is imperative to enhance the extraction of these compounds from diverse plant components, including leaves and twigs. High-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) are highly recommended for the thorough analysis and quantification of specific phytochemicals, such as polyphenols and essential oils. The incorporation of standards like gallic acid and quercetin is essential for the determination of total phenolic content (TPC) and total flavonoid content (TFC). Furthermore, evaluating the antioxidant potential of the extracts through various assays like DPPH, superoxide anion, and hydroxyl radical scavenging assays is necessary to establish a correlation between phytochemical content and biological activity. Moreover, the isolation and characterization of individual compounds such as arbutin and lupeol using methodologies like NMR spectroscopy for structural elucidation is a critical step in the process.

4.7. **References**

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CHAPTER 5

DETERMINATION OF THE LEVEL OF EFFECTIVENESS OF PLANT EXTRACTS OF *MYROTHAMNUS FLABELLIFOLIUS* ON TREATING COCCIDIOSIS IN INDIGENOUS CHICKEN

ABSTRACT

Studying the effectiveness of *Myrothamnus flabellifolius* in managing chicken coccidiosis is crucial due to the increasing need for natural and safe alternatives to conventional antibiotics and anticoccidial drugs, which have faced criticism for promoting antibiotic resistance and high. To address the issue, phytochemicals were quantified, and birds' growth rates, invitro assays, and mortality rates were studied. The goal was to analyse growth rates, quantify the percentage reduction in oocyst counts, and calculate bird fatality rates. To analyse the growth rate, all the birds used in the study were weighed every 7 days. The reduction of oocyst counts, focused on the faecal oocyst concentration reduction rate. Birds were observed for mortality, counting total deaths post inoculation. The data for weight gain was analysed using one way ANOVA. Also, the weight gain was subjected to Bonferroni post-hoc test for pairwise comparisons. The results for oocyst reduction rate were expressed as percentages. The mortality rates were expressed in percentages. The results indicate that the *M. flabellifolius* treatment group had the highest mean weight gain at day 42. Group which received the *M. flabellifolius* treatment, also exhibited lower mortality rates compared to the untreated group. Lastly, the data suggests that the two treatment interventions, particularly the coccidiostat and ESB_3 combination, had a significant positive impact on the oocyst count reduction, resulting in a much more substantial and sustained increase compared to the control group. Overall, this study demonstrated that *M. flabellifolius*

can reduce coccidiosis infected chicken mortality, reduce oocyst count and improve the growth weight. To enhance the effectiveness of *M. flabellifolius*, there is need of integrating it with natural feed additives also, there is need to employ advanced processing techniques to ensure more consistent and potent effects. Constant monitoring and diagnostic tools are important in the evaluation of the herb as a coccidiostat. Lastly, there is need to incorporate *M. flabellifolius* in comprehensive chicken management system.

Keywords: Determination; Level of effectiveness; Plant extracts; Myrothamnus flabellifolius; Treating, Coccidiosis; Herbal medicine; Anticoccidial activity; Therapeutic potential; Phytochemicals; Medicinal plants; Animal health; Treatment efficacy; In vitro testing; In vivo evaluation; Parasitic infection; Protozoan disease; Natural remedies; Traditional medicine; Veterinary medicine.

5.1 Introduction

The effectiveness of plant extracts is influenced by several determinants, including the type of plant, extraction method, concentration, and application context. Variations in the concentration of bioactive chemicals found in different plants contribute to their usefulness for particular purposes. For example, extracts of guava, ginger, neem, and moringa have demonstrated strong antibacterial activity against bacteria that are resistant to many drugs. The quality and yield of the extract are dependent on the kind of solvent used (aqueous, ethanolic, or methanolic).

Traditional extraction methods such as maceration, percolation, and Soxhlet extraction, while effective, are time-consuming and may not be suitable for large-scale applications due to high operating costs and long processing times(Nantapo and Marume, 2022; Ajao *et al.*, 2023).Additionally, the choice of solvent significantly impacts the yield and bioactivity of the extracts, with different solvents extracting different sets of phytochemicals, which can complicate the standardization of extracts(Cheikhyoussef, Summers and Kahaka, 2015; Tebele, Marks and Farrant, 2023).

The low effectiveness of *M. flabellifolius* plant extracts in certain applications can be attributed to several factors. Firstly, the presence of fungal contamination, specifically Aspergillus and

Penicillium, localized to the hydathodes of the leaves and stigmatic surfaces of the flowers, can negatively impact the plant's development and packaging and packaging and efficacy(Fultang *et al.*, 2018). The plant's effectiveness in animal nutrition is also limited by factors such as toxicity, economic, and financial issues, which necessitate advanced processing and combination strategies to unlock its full potential(Cheikhyoussef, Summers and Kahaka, 2015). To overcome these limitations, advanced processing techniques like microencapsulation and microbial fermentation can be employed to unlock and improve nutrient bio accessibility and bioavailability(Dhillon *et al.*, 2014).

The use of M. flabellifolius as a coccidiostat in animal production has not been fully studied, despite these encouraging findings. The plant's secondary metabolites, which contribute to its antimicrobial and antioxidant properties, suggest potential benefits in animal health and growth performance when used as a phytogenic feed additive(Sood *et al.*, 2020). However, challenges such as toxicity, economic feasibility, and nutrient bioavailability need to be addressed through advanced processing techniques like microencapsulation and microbial fermentation(Nako, 2014).

Research efforts to address the low effectiveness of *M. flabellifolius* plant extracts as a coccidiostat have been focusing on its broader potential in various therapeutic applications. Its effectiveness as a coccidiostat remains underexplored. Studies on other plant extracts, such as those from carnation, ginger, and cinnamon, have demonstrated significant disease suppression in wheat, indicating that plant-based treatments can be effective against pathogens(El-Gamal *et al.*, 2022). Further research is required to enhance the effectiveness of *M. flabellifolius* as a coccidiostat. This could be achieved by combining it with other plant extracts and gaining a deeper understanding of its bioactive compounds. Additionally, the antiviral potential of plant extracts has been highlighted in the context of the COVID-19 pandemic, where natural products have shown efficacy against coronaviruses. This suggests that similar approaches could be applied to enhance the coccidiostat properties of *M. flabellifolius*. However, the potential benefits of plant extracts could be applied in similar ways to improve the plant's antiviral properties. This study aims to determine the degree of efficacy of *M. flabellifolius* plant extracts in treating coccidiosis in rural chicken.

5.2 Materials and methods

Details regarding the Coccidiostat (Amprolium and ESB_3) and *M. flabellifolius* anticoccidial effects, determining the oocytes per gram (OPG), growth rate and mortality rate are described in chapter three. For the purpose of this chapter, only a summary is provided.

5.2.1. **Description of study area**

The experiment was done at Nhema Chickens farm, which is located in Norton. Details on the description of the study area are given in Chapter three.

5.2.2. **Research Design**

The study employed an experimental research design comprising determining the oocytes per gram (OPG), growth rate and mortality rate of chickenswithout treatment, chickens using Coccidiostat (Amprolium and ESB_3) and *M. flabellifolius*. The research design is described in detail in Section 3.3 of Chapter 3.

5.2.3. **Sampling procedure**

To determine the anticoccidial effects of *M. flabellifolius*, the OPG in all groups were measured from each group after 10 days post infection. For growth rate determination, all the birds used in the study were weighed every 7th day using a scale, in kilograms. Mortality was determined by the number birds that died post infection.

5.2.4. **Data collection procedure**

Based on the information provided in section 3.4 of Chapter 3, the data collection of antimicrobial analysis of *M. flabellifolius* can be described as; measurement of infection intensity, growth performance trials and mortality rate assessments performed to determine the effectiveness of *M. flabellifolius* as a coccidiostat. The results were Oocytes per gram (OPG), % weight gain, % mortality rate respectively.

5.2.4.1. **Oocytes per gram (OPG)**

OPG in all chicken groups were measured from each group after 10 days post infection. The faecal oocyst concentration reduction rate was determined.

5.2.4.2. **Growth rate**

All the birds used in the study were weighed every 7th day using a scale, in kilograms. The weights were then recorded so as to know the growth rate against the normal indigenous chicken growth curve.

5.2.4.3. **Mortality rate**

This was determined by the number birds rat died post infection.

5.2.5. **Data analysis procedure and methods**

The oocytes count per gram was done in triplicate periodically every 7 days till day 42. 10 randomly chosen chickens were measured their weight every 7 days for a duration of 42 days. Mortality was observed by counting dead birds after infection. An analysis of variance (One way ANOVA) was performed on the bird's weight gain. The bird's weight gain was analysed using the Bonferroni post-hoc test for pairwise comparisons. The results for oocyst reduction rate were expressed as percentages. The mortality rates were expressed in percentages. More details regarding data analysis method and analytic presentation are given Section 3.9.

5.2.6. **Challenges encountered during data collect**

Assessing the anticoccidial effects of *M. flabellifolius* plant extracts as a coccidiostat presents several challenges. First off, because to the intricacy of *Eimeria* spp.-caused avian coccidiosis, there is currently a dearth of standardized in vitro techniques to assess the exogenous and endogenous phases of the parasite's lifecycle. Variability in the amount and make-up of active chemicals in plant extracts is a major problem since it can greatly affect the extracts' effectiveness. Standardizing the in vitro and in vivo procedures for evaluating the exogenous and endogenous phases of *Eimeria* spp. is another difficulty, but it's essential for repeatable and trustworthy outcomes. Last but not least, assessing the effectiveness of anticoccidial agents is

further complicated by the host's variable immunological response, which is impacted by elements like the presence of immunomodulatory chemicals in plant extracts increases the difficulty of assessing the effectiveness of anticoccidial drugs.

5.3. Results and discussion

5.3.1. Mortality Rates

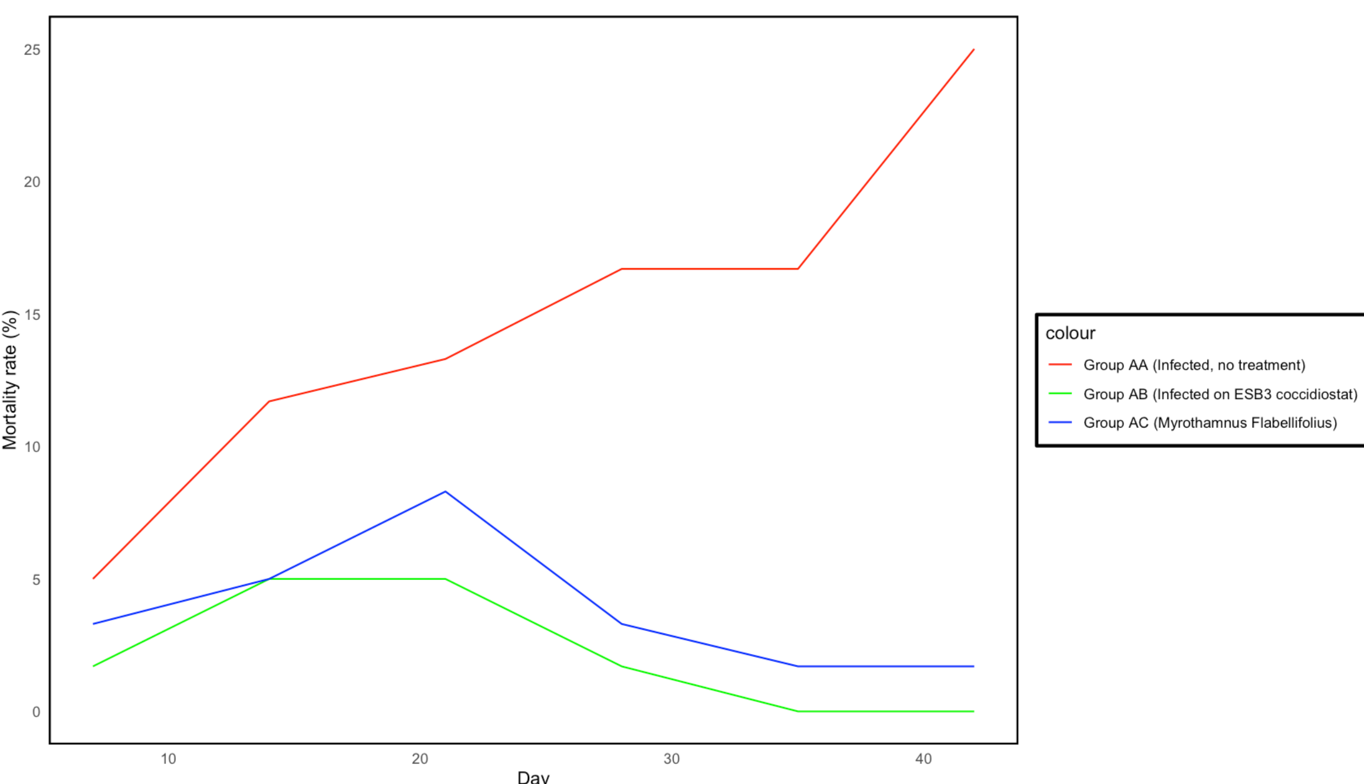

Figure 5.3.1-1: A line graph comparing the mortality rates of three different treatment groups over a 42-day period.

Source: Phytochemical Analysis, 2024

The following is the representation of the results for measurement of infection intensity, growth performance trials and mortality rate assessments to determine the effectiveness of *M. flabellifolius.*

Group AA: The untreated Group AA exhibited a steady increase in mortality rate over the course of the study period. Starting at a 5% mortality rate on Day 7, the rate steadily rose, reaching 25% by Day 42. This suggests that the outcomes for the members of this group who did not receive any treatment have significantly deteriorated. Coccidiosis, caused by protozoan parasites of the genus *Eimeria*, leads to severe intestinal damage, decreased body weight, and high mortality, particularly in young chicks between 3 and 18 weeks of age(Islam *et al.*, 2020; Sharaban *et al.*, 2021).The disease is exacerbated by poor management practices such as wet litter, contaminated feed, and high stocking density, which promote the sporulation and ingestion of oocysts(Manafi, Mohan and Mohmand, 2011). In untreated groups, the mortality rate can be significantly high, as seen in studies where mortality reached up to 10% in non-vaccinated challenged groups(Namratha *et al.*, 2019). The untreated chickens are also more susceptible to secondary infections like necrotic enteritis, which can significantly increase mortality rates, as observed in studies where mortality reached 23% when coccidiosis was followed by necrotic enteritis(Lilić, Ilić and Dimitrijević, 2009). Moreover, untreated birds show severe clinical signs such as bloody diarrhoea, weakness, and anorexia, leading to sudden mortality(Khanagwal, Mandal and Ghosh, 2001).

Group AB: In contrast, the groups that received treatments showed more favorable mortality trends. Group AB, which was administered the ESB3 coccidiostat, demonstrated a much lower mortality rate compared to the untreated group. On Day 7, the group's mortality rate was 1.7%; it went up to 5% on Days 14 and 21, but by Days 35 and 42, it had dropped back to 0%. This suggests the ESB3 coccidiostat treatment was effective in reducing mortality within this group. the use of a commercial mixed botanical product in broiler chickens significantly improved growth performance and reduced oocyst shedding, particularly at higher concentrations, which also healed caeca lesions more rapidly(Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran *et al.*, 2023). Similarly, the administration of probiotics like Weizmannia coagulans strain SANK70258 (WC) not only

reduced intestinal oocysts and improved body weights but also avoided the negative effects associated with antimicrobial treatments, such as the promotion of pathogenic bacterial growth(Flores *et al.*, 2022). Additionally, the use of herbal formulas containing various plant extracts showed a marked reduction in oocyst output, lesion scores, and improved zootechnical performance, indicating their potential as effective natural anticoccidial(Lotan *et al.*, 2023).

Group AC: Similarly, Group AC, which received the *M. flabellifolius* treatment, also exhibited lower mortality rates compared to the untreated group. For this group, the death rate peaked on Day 21 at 8.3%, began at 3.3% on Day 7, and by Days 35 and 42, it had dropped to 1.7%. While not as pronounced as the effects seen in Group AB, the *M. flabellifolius* treatment did appear to have a positive impact in reducing mortality compared to the untreated group. Additionally, the herbal product Cocciban at higher doses demonstrated improved liveability and performance in broilers infected with *Eimeria* species(Srinivasu *et al.*, 2020). Another study on a botanical blend showed that higher concentrations of the formula reduced oocyst shedding and healed caeca lesions more effectively, indicating a potential for lower mortality rates(Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran *et al.*, 2023). Furthermore, a commercial herbal formula containing various plant extracts showed promising anticoccidial effects, reducing oocyst output and lesion scores while improving weight gain and feed conversion ratios(Laha, Das and Goswami, 2015). These findings collectively suggest that natural and herbal treatments can be effective in managing coccidiosis, potentially reducing mortality rates in infected groups.

5.3.2. Weight gain of 10 randomly chosen chickens, averages, average weight gain per week and total % weight gain after 42 days

5.3.2.1. Day 7 weight gain

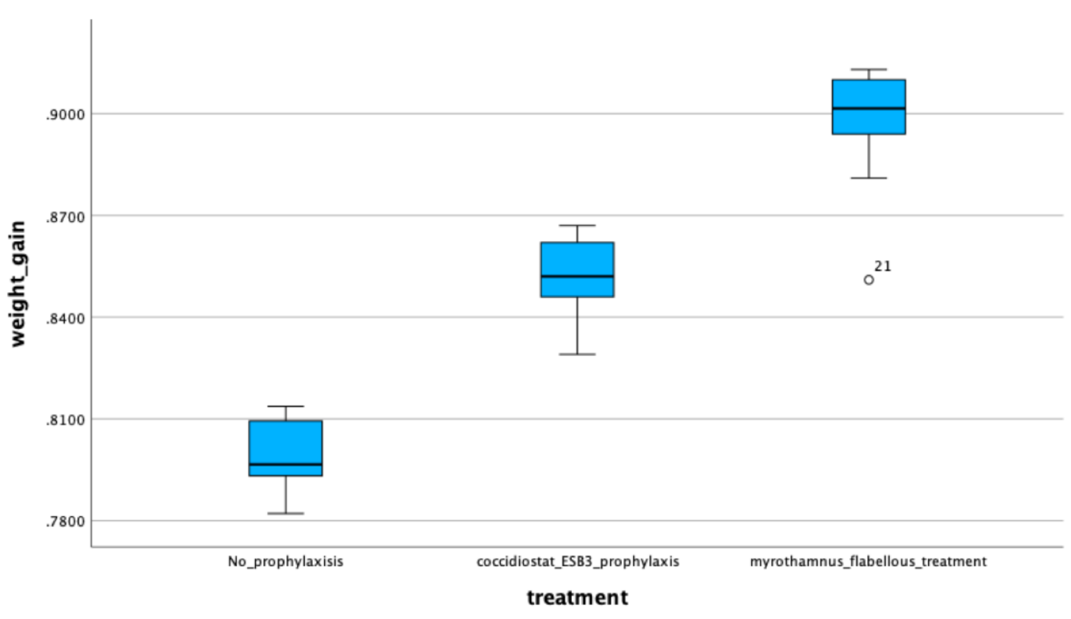

Figure 5.3.2-1: A line graph comparing the mortality rates of three different treatment groups over a 42-day period.

Source: Phytochemical Analysis, 2024

No Prophylaxis (AA):

The data shows a clear increasing trend in weight gain from the No Prophylaxis (AA) group to the Coccidiostat ESB3 Prophylaxis group, and further to the *M. flabellifolius* treatment group. The *M. flabellifolius* treatment group had the highest mean weight gain (Mean=0.896400±0.0185724). chickens generally exhibit lower weight gain and higher susceptibility to diseases like coccidiosis and necrotic enteritis, which negatively impact their growth performance(Hu *et al.*, 2019; Wang *et al.*, 2021). When coccidiostats such as Esb3 are used, there is a noticeable improvement in weight gain and a reduction in clinical symptoms of coccidiosis, although resistance to sulphonamides like Esb3 has been reported, which can limit its effectiveness over time(Salaheen *et al.*, 2017). Studies have shown that chickens treated with coccidiostats like Salinomycin and Dinitolmide also exhibit improved growth performance and reduced oocyst shedding, further supporting the efficacy of these drugs in managing coccidiosis(Lai *et al.*, 2018; Srinivasu *et al.*, 2020). Promising outcomes have been observed when natural substitutes such extracts from *M. flabellifolius* or other plant-based supplements like bioactive phenolic extracts (BPE) from blackberries and blueberries are used. These natural treatments not only enhance weight gain but also improve gut health and reduce the prevalence of antibiotic-resistant bacteria in the chicken gut microbiome(Valipouri *et al.*, 2022).

ESB3 Prophylaxis: The Coccidiostat ESB3 Prophylaxis group had a higher mean weight gain (Mean=0.851900± 0.0111500) than the No Prophylaxis (AA) group (Mean=0.799460 ± 0.0100661). The No Prophylaxis (AA) group had the lowest mean weight gain.

The data was tested for normality and the results showed that the data followed normal as shown from the Shapiro wilk results (**p>0.05**). The assumption for equality of variances was also met (p=0.992). The results of the ANOVA show that the mean weight gain of the three treatment groups differs in a highly statistically significant way ($p < 0.001$). This shows that the treatment type had a significant influence on the study's observed weight increase. There were statistically significant variations in the mean weight gain between all three treatment groups, as determined by the Bonferroni post-hoc test for pairwise comparisons ($p < 0.001$). Compared to the No Prophylaxis group, the weight gain was 0.0524400 higher in the Coccidiostat ESB3 Prophylaxis group, and 0.0969400 higher in the *M. flabellifolius* treatment group. Furthermore, the weight gain in the *M. flabellifolius* treatment group was 0.0445000 higher than in the Coccidiostat ESB3 Prophylaxis group. The use of coccidiostats, such as ESB3, is a common prophylactic measure to

control this disease and improve feed conversion and weight gain in broilers. Studies have shown that alternative treatments, including probiotics like Weizmannia coagulants, can also enhance weight gain and reduce intestinal oocyst counts without promoting pathogenic bacterial growth(Youssefi *et al.*, 2023). Additionally, dietary supplements such as clove essential oil and its nano formulated emulsions have demonstrated efficacy in controlling coccidiosis and maintaining productivity parameters close to those of standard treatments (Mousavinasab *et al.*, 2022). Herbal extracts like Artemisia sieberi and Curcuma longa have also been effective in reducing oocyst excretion and improving growth performance when used in combination with chemical anticoccidial (Sharaban *et al.*, 2021). These findings collectively support the observation that prophylactic measures, whether chemical, herbal, or probiotic, can significantly enhance weight gain in chickens by mitigating the adverse effects of coccidiosis, as evidenced by the higher mean weight gain in the Coccidiostat ESB3 Prophylaxis group compared to the No Prophylaxis group(Savary *et al.*, 2020; Srinivasu *et al.*, 2020).

5.3.2.2. **Day 14 weight gain**

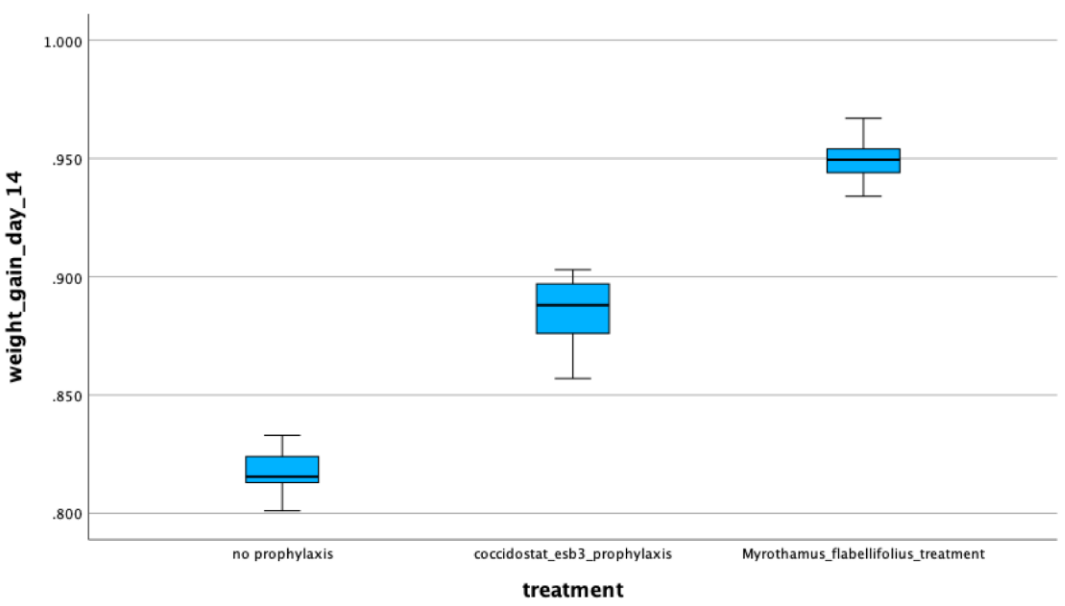

Figure 5.3.2-2: The total alkaloids, total flavonoids, total phenols, total saponins, and total tannins of the M. flabellifolius shrub are displayed using aqueous and solvent extract, respectively.

Source: Phytochemical Analysis, 2024.

No Prophylaxis (AA); ESB$_3$ Prophylaxis (AB); *M. flabellifolius* (AC):

The ANOVA results for the weight gain at day 14 variable show a highly statistically significant difference between the three treatment groups ($F (2, 27) = 321.087, p < 0.001$). This indicates that there are substantial differences in mean weight gain at day 14 across the three treatment conditions. The multiple comparisons analysis using both Tukey HSD and Bonferroni post-hoc tests reveals the following key findings: The mean weight gain at day 14 was significantly higher in the *M. flabellifolius* treatment group compared to both the no prophylaxis group (mean difference = 0.132200, $p < 0.001$) and the coccidiostat esb3 prophylaxis group (mean difference = 0.063900, $p < 0.001$). The mean weight gain at day 14 was also significantly higher in the coccidiostat esb3 prophylaxis group compared to the no prophylaxis group (mean difference = 0.068300, $p < 0.001$). Therefore, both post-hoc tests consistently show that the *M. flabellifolius* treatment resulted in the highest weight gain, followed by the coccidiostat ESB$_3$ prophylaxis, and then the no prophylaxis group. All pairwise comparisons between the three treatment groups were statistically significant. The ANOVA results indicating a highly statistically significant difference in weight gain among the ESB3 Prophylaxis group, No Prophylaxis (AA) group, and *M. flabellifolius* treatment group ($F (2, 27) = 321.087, p < 0.001$) for chickens affected by coccidiosis align with findings from various studies on the effectiveness of different prophylactic and therapeutic interventions against coccidiosis. For instance, herbal mixtures such as those containing Quercus infectoria, Artemisia annua, and Allium sativum have shown promising results in reducing coccidial lesions and improving body weight gain in broilers(Ghaniei *et al.*, 2023). Similarly, another study demonstrated that a herbal mixture of Echinacea purpurea and Glycyrrhiza glabra significantly reduced the negative performance effects associated with *Eimeria* spp., comparable to the chemical drug toltrazuril (Ghafouri *et al.*, 2023). The use of fluoroquinolone lomefloxacin and diclazuril also improved growth performance parameters and reduced oocyst counts in infected broilers (El-Shazly *et al.*, 2020).

5.3.2.3. Day 21 weight gain

The ANOVA results for the weight gain at day 21 variable show a highly statistically significant difference between the three treatment groups ($F (2, 27) = 292.596$, $p < 0.001$). This indicates that there are substantial differences in mean weight gain at day 21 across the three treatment conditions. The multiple comparisons analysis using the Tukey HSD post-hoc test reveals the following key findings: The mean weight gain at day 21 was significantly higher in the *M. flabellifolius* treatment group compared to both the no prophylaxis group (mean difference = 0.184700, $p < 0.001$) and the coccidiostat esb3 prophylaxis group (mean difference = 0.059600, $p < 0.001$).The mean weight gain at day 21 was also significantly higher in the coccidiostat esb3 prophylaxis group compared to the no prophylaxis group (mean difference = 0.125100, $p < 0.001$). Research has shown that different treatments and prophylactic measures significantly impact weight gain and overall health in chickens. For instance, the use of probiotics like Weizmannia coagulans strain SANK70258 has been found to reduce intestinal oocysts and improve body weights in broilers, suggesting its potential as an effective alternative to antimicrobials(Ekezie *et al.*, 2023).

5.3.2.4. **Day 28 weight gain**

The results indicate that the *M. flabellifolius* treatment group had the highest mean weight gain at day 28 (Mean = 2.002 ± 3.007), followed by the coccidiostat esb3 prophylaxis group (Mean = 1.019 ± 0.036), and then the no prophylaxis group (Mean = 0.850 ± 0.015).

The *M. flabellifolius* treatment group also had the highest variability in weight gain, as evidenced by the large standard deviation. The ANOVA results for the weight gain at day 28 show no statistically significant difference between the three treatment groups ($F (2, 27) = 1.284$, $p = 0.293$). This indicates that the mean weight gain at day 28 was not significantly affected by the type of treatment. This finding aligns with other research demonstrating the efficacy of various natural and herbal treatments in managing coccidiosis in chicken. For example, a study assessing the effects of an herbal mixture combining Artemisia annua, Quercus infectoria, and Allium sativum on broiler chicks infected with *Eimeria* species revealed significant improvements in body weight gain and feed conversion ratio (FCR). Similarly, another study found that a botanical blend administered at higher concentrations effectively reduced oocyst shedding and improved growth performance, comparable to the effects of toltrazuril. Furthermore, similar to conventional anticoccidial medications, herbal extracts such as those derived from Echinacea

purpurea and Glycyrrhiza glabra have demonstrated encouraging outcomes in lowering the pathogenic effects and poor performance linked to *Eimeria* spp. The combination of Artemisia sieberi and Curcuma longa also demonstrated efficacy in controlling coccidiosis and its complications, further supporting the potential of herbal remedies.

5.3.2.5. **Day 35 weight gain**

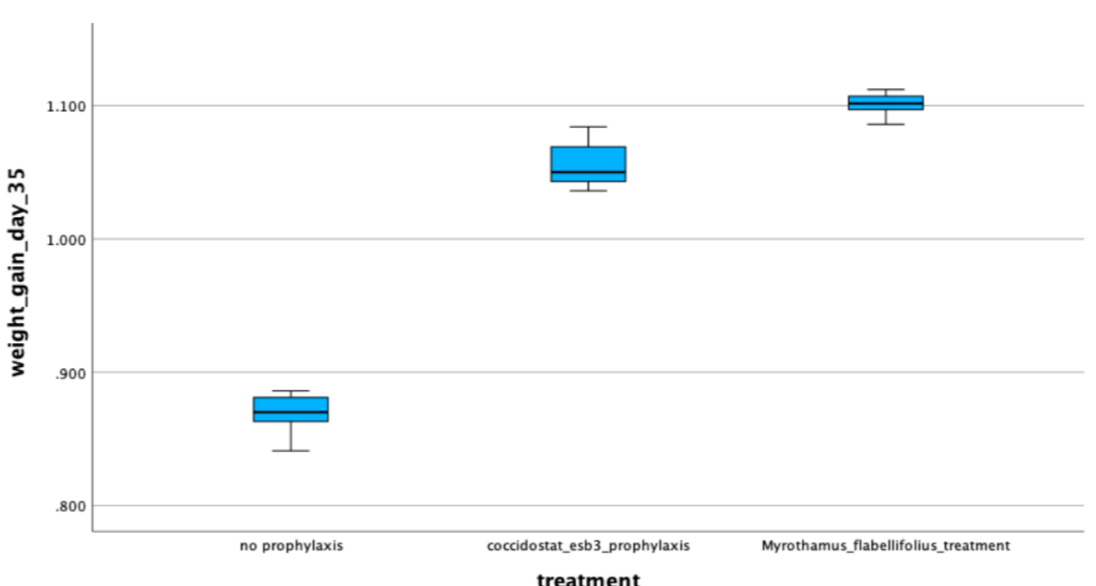

Figure 5.3.2-3: The total alkaloids, total flavonoids, total phenols, total saponins, and total tannins of the M. flabellifolius shrub are displayed using aqueous and solvent extract, respectively.

Source: Phytochemical Analysis, 2024.

There is a statistically significant difference in mean weight gain at day 35 between the three treatment groups ($F(2, 27) = 902.705$, $p < 0.001$). This indicates that the type of treatment had a significant effect on the weight gain outcome measured at day 35. The key findings from the post-hoc Tukey HSD tests for the weight gain day 35 indicated that the mean weight gain was significantly lower in the no prophylaxis group compared to both the coccidiostat esb3 prophylaxis group (mean difference = -0.186, $p < 0.001$) and the *M. flabellifolius* treatment

group (mean difference = -0.232, p < 0.001). The mean weight gain was also significantly lower in the coccidiostat esb3 prophylaxis group compared to the *M. flabellifolius* treatment group (mean difference = -0.046, p < 0.001). Numerous tactics, such as probiotics, synthetic medications, and herbal concoctions, have been tested to see how well they work to lessen these symptoms. In contrast to untreated controls, both treatments improved body weight gain and feed conversion ratio (FCR) in a study comparing an herbal mixture (75% Quercus infectoria, 16% Artemisia annua, and 9% Allium sativum) with monensin. The herbal mixture showed promising results (Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran et al., 2023). Another study demonstrated that a commercial botanical blend significantly improved growth performance and reduced oocyst shedding, particularly at higher concentrations(Khodadi, Rassouli and Naeimi, 2022).

5.3.2.6. **Day 35 weight gain**

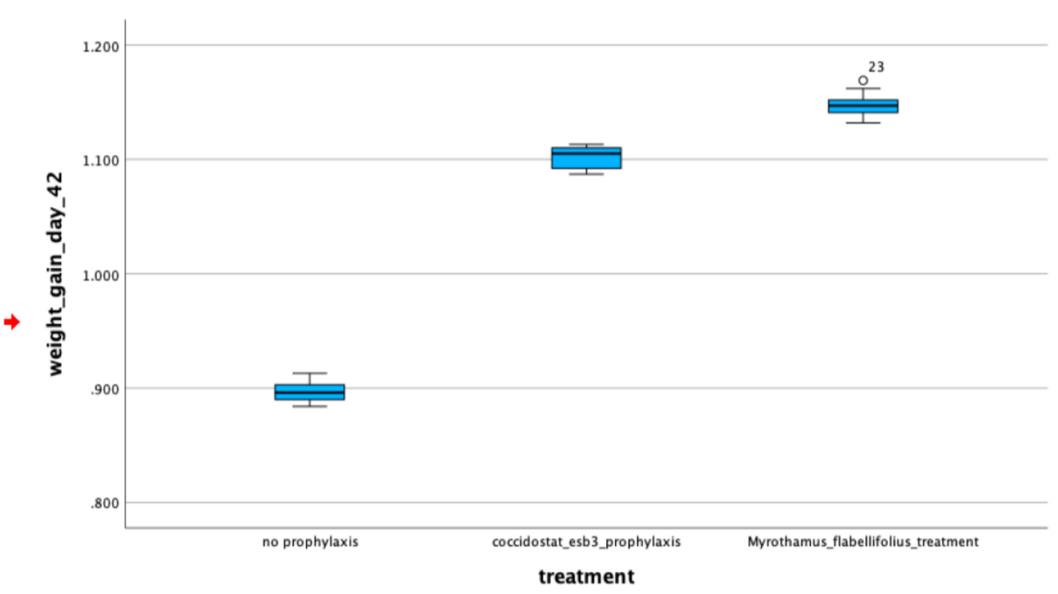

Figure 5.3.2-4: The total alkaloids, total flavonoids, total phenols, total saponins, and total tannins of the M. flabellifolius shrub are displayed using aqueous and solvent extract, respectively.

Source: Phytochemical Analysis, 2024.

***M. flabellifolius* treatment:** These results indicate that the *M. flabellifolius* treatment group had the highest mean weight gain at day 42 (Mean = 1.148 ± 0.012), followed by the coccidiostat esb3 prophylaxis group (Mean = 1.102 ± 0.010), and then the no prophylaxis group (Mean = 0.897 ± 0.010). There is a statistically significant difference in mean weight gain at day 42 between the three treatment groups ($F (2, 27) = 1688.073$, $p < 0.001$). This indicates that the type of treatment had a significant effect on the weight gain outcome measured at day 42. The key findings from the post-hoc Tukey HSD tests for the weight gain day 42 indicated that the mean weight gain was significantly lower in the no prophylaxis group compared to both the coccidiostat esb3 prophylaxis group (mean difference = -0.205, $p < 0.001$) and the *M. flabellifolius* treatment group (mean difference = -0.251, $p < 0.001$). The mean weight gain was also significantly lower in the coccidiostat esb3 prophylaxis group compared to the *M. flabellifolius* treatment group (mean difference = -0.046, $p < 0.001$).

This finding aligns with other research demonstrating the efficacy of various herbal and alternative treatments in managing coccidiosis in broilers. A study conducted in 2023 by Ghaniei et al. evaluated an herbal mixture that included Quercus infectoria, Artemisia annua, and Allium sativum. The results indicated a considerable improvement in body weight gain and feed conversion ratio (FCR) when compared to the untreated groups. Similarly, the use of Cocciban, a herbal product, at 1000 g/ton resulted in higher mean percent liveability and better performance metrics compared to other infected groups(Srinivasu *et al.*, 2020). Another study highlighted the effectiveness of a herbal mixture of Echinacea purpurea and Glycyrrhiza glabra, which significantly reduced negative performance and pathogenic effects associated with *Eimeria* spp., comparable to the chemical anticoccidial drug toltrazuril(Hauck, Eckert and Hunter, 2022). Additionally, Artemisia herba-alba Asso demonstrated significant anticoccidial activity, reducing oocyst shedding and preventing hypolipidemia, although it had a negative effect on body weight gain due to its tannin content(Aquino *et al.*, 2016).

5.3.3. **Percentage reduction of oocytes count**

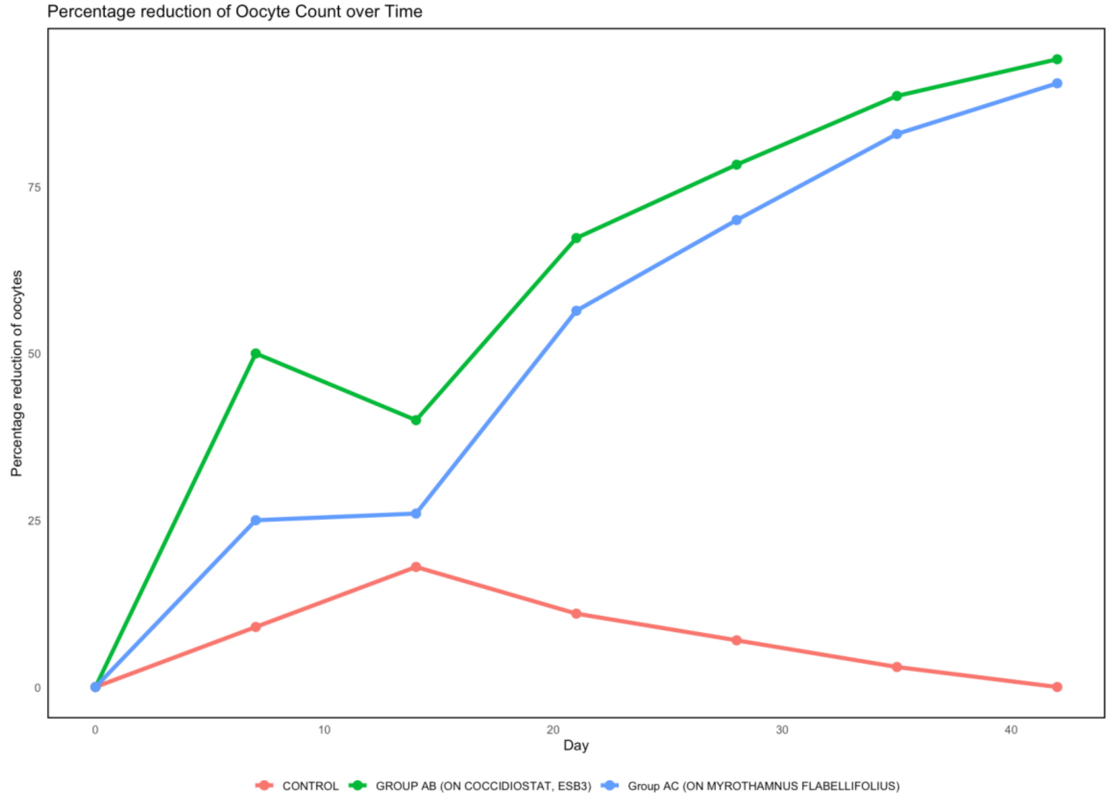

Figure 5.3.3-1: The total alkaloids, total flavonoids, total phenols, total saponins, and total tannins of the M. flabellifolius shrub are displayed using aqueous and solvent extract, respectively.

Source: Phytochemical Analysis, 2024.

Group AB; ESB3; Group AC: The data provided presents a comparison of three groups - a control group and two treatment groups (Group AB on Coccidiostat, ESB3 and Group AC on *M. flabellifolius* - over a 42-day period. The control group exhibits a relatively modest increase in the measured outcome, rising from 0% on Day 0 to 18% by Day 14. However, after this peak, the values in the control group begin to decrease, ultimately reaching 0% by Day 42.

Group AB: In contrast, both treatment groups show a more substantial and sustained increase over the course of the study. Group AB, which received the Coccidiostat and ESB3 treatment, displays the most remarkable trend. Starting from 0% on Day 0, this group rapidly increased to

50% by Day 7 and continued to climb, reaching an impressive 94.1% by the end of the study period on Day 42.

Group AC: Group AC, which received the *M. flabellifolius* treatment, also demonstrates a notable increase, though at a slightly slower pace compared to Group AB. This group started at 0% on Day 0 and reached 25% by Day 7, gradually increasing to 90.5% by Day 42. The data suggests that the two treatment interventions, particularly the Coccidiostat and ESB3 combination, had a significant positive impact on the measured outcome, resulting in a much more substantial and sustained increase compared to the control group.

5.3.4. **Determination of the level of effectiveness of plant extracts from *M. flabellifolius* on treating coccidiosis**

Antimicrobial analysis, growth performance trials and mortality rate assessments: The study findings reveal, the type of treatment has a substantial impact on the observed weight gain. The key trend observed is that the untreated group experienced a substantial increase in mortality over time, while the groups receiving treatments, particularly the ESB3 coccidiostat, demonstrated significantly lower and more favourable mortality rates throughout the study period. The data suggests that the two treatment interventions, particularly the Coccidiostat and ESB3 combination, had a significant positive impact on the measured outcome, resulting in a much more substantial and sustained increase compared to the control group. The data suggests that the two treatment interventions, particularly the Coccidiostat and ESB3 combination, had a significant positive impact on the percentage reduction of oocytes, resulting in a much more substantial and sustained increase compared to the control group.

5.4. **Conclusion**

The chapter has evaluated the effectiveness of *M. flabellifolius* as a coccidiostat. Furthermore, flotation method, regular weighing and monitoring of bird's body weight over a specified period and recording the number of bird's deaths over a specified period, were used to evaluate the effectiveness of the bioactive compounds. The variability in the concentration and composition of active compounds in plant extracts can significantly influence efficacy of the plant. These results indicate that the *M. flabellifolius* treatment group had the highest mean weight gain at day

42 (mean difference = -0.046, $p < 0.001$). *M. flabellifolius* is rich in secondary metabolites that enhance cell function, regulation, and protection, which likely contributed to improved growth performance and health in the treated chickens. Adding on, group which received the *M. flabellifolius* treatment, also exhibited lower mortality rates compared to the untreated group. These findings collectively suggest that various alternative treatments, including herbal preparations, probiotics, acaricides, and antibiotics, can effectively reduce mortality in chicken diseases. Finally, the data suggests that the two treatment interventions, particularly the Coccidiostat and ESB3 combination, had a significant positive impact on the oocyst count reduction, resulting in a much more substantial and sustained increase compared to the control group. These results highlight the significant positive impact of various coccidiostat treatments, particularly when combined with other interventions like probiotics or advanced technologies, in effectively reducing oocyst counts and improving health outcomes in infected animals. To sum up, the statistical study indicates that the death rate was lowest in the *M. flabellifolius* treated group, so was the reduction in oocyst count and improvement of the growth weight.

5.5. Recommendations

The effectiveness of *M. flabellifolius* against coccidiosis may be synergistically increased by combining it with other natural feed additives, such as the vitamin feed additive Introvit A+WS, which has been demonstrated to improve chicken health and production. The bioavailability of *M. flabellifolius's* active ingredients can be further enhanced and unlocked by using cutting-edge processing methods like microbial fermentation and microencapsulation, guaranteeing more reliable and powerful results. Tools for diagnostics and routine monitoring, such as those designed for coccidiosis immunization campaigns, can be used to evaluate the success of *M. flabellifolius* -based therapies and make the required modifications. Moreover, *M. flabellifolius* can address several issues at once and enhance overall chicken health and resistance to coccidiosis when it is integrated into a comprehensive chicken management system that also includes strategic supplemental feeding, better housing, and disease control measures. Finally, the development and packaging and packaging of optimal protocols for the use of *M. flabellifolius* in chicken nutrition and health management can be facilitated by further research and collaboration among international organizations, institutions, and local agricultural systems.

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CHAPTER 6

FORMULATION AND PACKAGINGAND PACKAGINGAND PACKAGING OF A *MYROTHAMNUS FLABELLIFOLIUS* PLANT BASED COCCIDIOSTAT

ABSTRACT

Developing effective coccidiostat formulations for chicken production faces several challenges, including antimicrobial resistance, regulatory pressures, and the need for alternative methods. Mitigating the challenges in developing effective coccidiostat formulations for chicken production involves the use of natural products with antimicrobial, antioxidant, and anti-inflammatory properties, the objective was to develop a chicken coccidiostat. This involved, labelling, packaging material, packaging design, packaging, quality control and stability testing. For the encapsulation of *Myrothamnus flabellifolius* Coccidiostat formulation, nano/microencapsulation strategies are recommended to enhance water-solubility, stability, and functionality of the bio actives. In terms of labelling, it is necessary to maintain clarity and correctness, eliminating ambiguities and providing essential information such as biosafety and accurate usage instructions. For packaging material, a composite material with at least one layer of cellulose/cardboard is recommended for its resource efficiency and ability to maintain sterility, which is essential for aseptic packaging. The integration of both the product and its packaging into a cohesive entity in the packaging design process should take into account various factors such as social, technical, ergonomic, economic, and environmental aspects to uphold quality and sustainability. Tailoring packaging sizes to align with the specific requirements of the product and its intended use is crucial to guarantee convenience and

protection throughout the process of administration or utilization non-destructive methods for on-line detection of package integrity and quality control post-preincubation are also recommended.

Keywords: Development and packaging and packaging; Formulation; Plant extracts; Myrothamnus flabellifolius; Treating; Coccidiosis; Herbal medicine; Anticoccidial activity; Therapeutic potential; Phytochemicals; Medicinal plants; Animal health; Treatment efficacy; In vitro testing; In vivo evaluation; Parasitic infection; Protozoan disease; Natural remedies; Traditional medicine; Veterinary medicine.

6.1 Introduction

Myrothamnus flabellifolius (*M. flabellifolius)*, a plant with significant medicinal properties, is distributed in Zimbabwe and other regions, as shown in *Figure 1.* Plant materials are extensively utilized as home remedies in both developed and developing countries. In developing nations, traditional medicines, including herbal remedies, play a crucial role in primary healthcare, with approximately 70-95% of patients relying on natural medicines (Rathore, Jain and Kumari, 2017)

Over-the-counter drug products and raw materials for the pharmaceutical industry play a significant role in the global drug market, with a focus on quality control, supply chain integrity, and regulatory compliance. The pharmaceutical industry heavily relies on raw materials sourced from various plants, with India alone utilizing around 340 plant species for medicinal purposes(Sarin, 2003). Herbal medicines have been extensively researched and utilized in the treatment of coccidiosis, a prevalent and economically significant disease in chicken(Ekawasti and Martindah, 2019; Jamil *et al.*, 2022; Abad and Ghaniei, 2023; Ghaniei *et al.*, 2023). Herbal medicines have been found to enhance immunity, appetite, and reduce stress in chicken, contributing to the suppression of Eimeria sp. Infestation. The main reason for chicken coccidiosis is the infection caused by protozoa of the genus Eimeria, particularly prevalent in intensive chicken production systems(Abd El-Ghany, 2022). Herbal remedies have emerged as a safe and effective alternative for treating chicken coccidiosis, offering a promising route to alleviate the disease's impact.

Figure 5.3.4-1: Myrothamnus flabellifolius.

The global interest in herbal medicines and nutritional supplements for treating chicken coccidiosis is on the rise due to concerns about drug resistance and residues in chicken products Effects of Herbal Medicine in the Treatment of Chicken Coccidiosis(Alsayeqh and Abbas, 2023; Ghaniei et al., 2023; Saeed and Alkheraije, 2023). The acceptance and public interest in natural therapies for chicken coccidiosis have significantly increased globally, with herbal remedies gaining popularity in both developing and developed countries. Chicken herbal coccidiostats are increasingly being explored as effective alternatives to traditional chemical agents for managing coccidiosis in chicken. Research has shown that herbal mixtures containing ingredients like Quercus infectoria, Artemisia annua, and Allium sativum have demonstrated efficacy in preventing coccidiosis in broiler chickens, with results comparable to conventional drugs like Monensin(Ghaniei et al., 2023; Singh, Palod and Shukla, 2023).

In Zimbabwe, various herbal remedies are commonly used for chicken diseases, with Aloe species being the most prevalent(Uddin et al., 2016; Gobvu et al., 2022; Jambwa et al., 2022). These herbal treatments are crucial for smallholder farmers who rely on indigenous medicines due to limited access to modern veterinary services('Utilisation of Herbal Bullets against Newcastle Disease in Chicken Sector of Asia and Africa (2012-2022)', 2023). Scientific evaluation of plants as chicken coccidiostats has shown promising results in managing coccidiosis. Research has demonstrated that plants high in saponins, such as Acacia concinna,

Trigonella foenum-graecum, and Yucca schidigera, are useful in preventing coccidiosis in broiler chickens (Abiala et al., 2016; Benarbia et al., 2022). Numerous investigations have looked into the potential of medicinal plants to treat diseases that affect chicken, such as coccidiosis and avian colibacillosis. Flavonoids like carvacrol, cinnamaldehyde, and capsicum oleoresin, as well as compounds like thymol, menthol, linalool, trans-anethole, methyl salicylate, 1,8-cineol, p-cymene, terpinen-4-ol, and γ-terpinene, are examples of phytochemicals that have positive effects on chicken health and performance (Lillehoj et al., 2011; Chodkowska et al., 2022; Tan et al., 2022).The development and packaging and packaging of plant-based chicken coccidiostat drugs has gained significant importance in the global market due to the devastating impact of parasitic infections on chicken industry and the emergence of resistance to synthetic medicines (Ahmad, 2023; 'Utilisation of Herbal Bullets against Newcastle Disease in Chicken Sector of Asia and Africa (2012-2022)', 2023). Although experimental studies have been conducted on a number of these plants and their formulations, however, only some plants have clearly shown the coccidiostat effects against coccidiosis caused by genus Eimeria. To obtain satisfactory chicken coccidiostat drugs from herbal sources, evaluations such as performance indices, faecal oocyst excretion, intestinal lesion scoring, mortality rate, and oocyst count per gram (OPG) are crucial(Srinivasu et al., 2020; Ghaniei et al., 2023).

These herbal medicines work through immunomodulation, impacting pathways such as the PI3K/AKT signalling cascade and targeting important genes including SRC, STAT3, and PPARG (Hashemi and Davoodi, 2012; Peng et al., 2022). Combining different herbal extracts or fractions can indeed offer a promising approach to managing coccidiosis, considering the antimicrobial and anti-amoebic properties of various plant extracts. According to studies, plants like Lawsania inermis and Portulaca oleracea have strong anti-Candida properties that prevent the growth of Candida albicans and the creation of biofilms (Soliman et al., 2017). According to studies, the phytochemicals found in plants like Azadirachta indica and Khaya senegalensis—such as tannins, saponins, cardiac glycosides, steroids, alkaloids, and flavonoids—help explain why these plants are effective at curing avian coccidiosis (Gotep et al., 2016). The increasing prevalence of chicken coccidiosis mortality has garnered significant attention from researchers due to its economic impact and threat to chicken health. Studies have shown that coccidiosis, caused by Eimeria species, is a major concern in chicken production, leading to high morbidity and mortality rates, especially in young birds (Dinka and Tolossa, 2012; Namratha et al.,

2019).The rise in the prevalence of chicken coccidiosis mortality can be attributed to various factors such as bacterial infections, including E. coli and Gram-positive cocci, which contribute significantly to mortality in chicken of all age groups and production systems(Comfort and Agbor, 2014). Herbal treatment has indeed been utilized for centuries to manage chicken coccidiosis globally, offering a natural and potentially effective alternative to conventional chemical agents and antiparasitics(Ekadashi and Martindah, 2019; Jamil et al., 2022; Abad and Ghaniei, 2023; Ghaniei et al., 2023, 2023; Saeed and Alkheraije, 2023).

Research on using plants to manage chicken coccidiosis has been thoroughly studied in recent years. Various studies have explored the efficacy of medicinal plants in treating avian colibacillosis and coccidiosis, highlighting the potential benefits of herbal medicines as alternative treatments to conventional chemical agents(Abad and Ghaniei, 2023; Kamil et al., 2023; Saeed and Alkheraije, 2023). The rising interest in herbal medicines for treating chicken coccidiosis reflects their perceived effectiveness as alternative treatments to conventional options like chemical agents and antiparasitics (Ghaniei et al., 2022, 2023; Jamil et al., 2022). The assumption that herbal remedies are secure and efficient substitutes for traditional chemical agents like ionophores and antiparasitics is reflected in the growing usage of natural remedies to treat chicken coccidiosis. Previous studies on herbal chicken coccidiostats have highlighted several key areas. These include the effectiveness of herbal medicines in treating chicken coccidiosis, emphasizing their potential benefits such as being safe, effective, and free of side effects(Abad and Ghaniei, 2023). Additionally, research has focused on the importance of using herbal medicines as an alternative to conventional treatments like chemical agents to avoid issues such as resistance and residues in chicken products (Ekawasti and Martindah, 2019). Research has also looked into how herbal additives affect the fatty acid profile of chicken meat. The results indicate that herbs can improve the fatty acid composition of chicken products by lowering saturated fatty acids and raising polyunsaturated and monounsaturated fatty acids (Shokryazdan et al., 2017). Furthermore, investigations have been conducted on the efficacy of specific herbal ingredients like Scutellaria baicalensis in reducing faecal shedding of Eimeria tenella oocysts and improving production indices in broiler chickens, suggesting the potential of herbal additives in controlling coccidiosis effectively(Jachimowicz, Winiarska-Mieczan and Tomaszewska, 2022).

6.1.1 Capsules

Capsules in pharmaceuticals and nutraceuticals are solid dosage forms made of two water-soluble polymeric films that are deformed to create recesses, which are then filled with a flowable material, such as active pharmaceutical ingredients or nutrients(Almakaiev and Sidenko, 2021). Single-dose capsules are made of different materials, with different sizes, shapes, and compositions for different applications. They each contain one dose of an active substance. Capsules can also be designed to contain multiple units, such as tablets or granules, within a single shell to prevent drug incompatibilities and interactions. Some capsules are engineered with specific structural features, such as areas of reduced thickness for controlled release upon compression. Branding and dosage information for pharmaceutical capsules are critical components in the pharmaceutical industry, ensuring both effective marketing and safe usage. Branding involves labelling containers with uniquely identifiable customer labels and capping them with branded or blank caps, which can later be customized with specific logos, graphics, or text(Leu, McErlean and Rice, 2012). The solvents or excipients employed in the formulation and packaging ofthe active pharmaceutical component can, in fact, have an impact on the integrity of pharmaceutical capsule shells. To address these difficulties, other materials such as hydroxypropyl methylcellulose (HPMC) have been investigated, which provide greater stability and flexibility in terms of fill material compatibility (Edgehouse et al., 2022). Additionally, the development and packaging and packaging of specialized excipients, such as the DiluCap line, aims to enhance the performance of capsule formulations by addressing specific challenges like hygroscopicity, oxidation, and dissolution rates, thereby ensuring the integrity of the capsule shell and the efficacy of the API (Ferreira and Polonini, 2022). Moreover, encapsulation techniques, such as those using polymer shells or wax materials, can provide a hermetic seal to protect the API from solvent interactions, ensuring long-term stability and controlled release. Advanced detection and quality control mechanisms, including deep learning-based defect detection models and X-ray imaging, further ensure the integrity of the capsules by identifying defects and agglomerates that could compromise the shell(Majee, Avlani and Biswas, 2017) . Additionally, innovations in capsule production, such as automated polishing and sorting machines, and particle size detection mechanisms, contribute to maintaining the quality and integrity of the capsule shells during manufacturing(Goertz et al., 2019).

6.1.1 Advantages and Disadvantages of Capsules

The Advantages of Capsules: One of the primary advantages is their ability to mask the taste of the active ingredients, making them more palatable for patients. Capsules are also easily administered and can be produced in large quantities, which is beneficial for commercial manufacturing(Peña, Roger and Torrado, 2022). They can be engineered to release their contents in a controlled manner, such as delayed-release formulations, which improve treatment outcomes and patient adherence by mixing many medications into a single dose form. Additionally, capsules can be produced from a variety of materials, including gelatin, hydroxypropyl methylcellulose (HPMC), and other polymers, to meet the needs of diverse patients, such as those with dietary restrictions or allergies (Hadi et al., 2013). Advanced capsule designs, like those incorporating magnetic fields for targeted drug delivery, further enhance their utility in specialized treatment(Hoag, 2017).

The Disadvantages of Capsules: However, capsules also have some disadvantages. The production process can be complex and costly, particularly for hard gelatin capsules, which require precise control over formulation and packagingand packagingand manufacturing conditions to ensure stability and reproducibility. There is also the risk of the active pharmaceutical ingredient being released prematurely if the capsule's integrity is compromised, which can be a concern in certain formulations. Furthermore, materials utilized in capsule production, such as gelatin, may not be appropriate for all patients, including vegetarians and those with specific sensitivities, necessitating the use of alternative materials such as HPMC.

6.1.2 Types of Capsules

Type of Capsules: Hard capsules, typically made from gelatin or polysaccharides, are used to encapsulate dry, powdered ingredients or miniature pellets and are known for their ease of swallowing and aesthetic appeal due to their smooth, hydrating shells. Soft capsules, on the other hand, are often used for oils and active ingredients dissolved or suspended in oil, providing a tasteless and odourless dosage form without the need for secondary coatings (Kalluri *et al.*, 2023). Enteric capsules, a kind of hard capsule, are perfect for delivering active substances in

pharmaceuticals or nutritional supplements that may be broken down by gastric acid since they are made to withstand stomach acid and dissolve in the intestines. Nano capsules, which confine the drug to a cavity surrounded by a polymeric membrane, offer targeted drug delivery and controlled release, utilizing both natural and synthetic polymers for their preparation. Additionally, there are specialized capsules like those containing polyunsaturated fatty acid alkyl esters (PUFA) for cardiovascular and inflammatory conditions , and traditional Chinese medicine capsules for treating conditions like high blood pressure with specific herbal formulations(DUEÑA and Carminati, 2012). Innovations also include capsules with a spherical or oval shape that can be detached by external pressure, enhancing user convenience, and those filled with self-emulsifiable preparations to prevent shell cracking and ensure stability(Civaroli, Muggetti and Martini, 2001).

Hard capsules: Hard pharmaceutical capsules are a flexible and popular dosage form that are mainly divided into two kinds: those based on gelatin and those based on hydroxypropyl methylcellulose (HPMC). Gelatin capsules, traditionally made from animal-derived gelatin, are known for their unique physicochemical properties, such as gel and film formation within a narrow temperature range, which facilitates rapid film formation during manufacturing (Korenev *et al.*, 2022). These capsules are extensively used for oral and pulmonary formulations due to their ability to mask unpleasant drug tastes and odours and their flexibility in dosing. HPMC capsules, on the other hand, are plant-based and offer several advantages over gelatin capsules, including a temperature-independent dissolution profile and suitability for dry powder inhalation (DPI) systems, enhancing the design space for capsule-based DPI systems.

HPMC capsules also provide enteric or bioavailability-enhancing properties and can be customized for low moisture or low powder retention, which is critical for certain applications like inhalation(Mallik *et al.*, 2013). Additionally, hard capsules can be designed with features such as adhesive tape for improved sealing performance, and acid resistance to protect pharmaceutical agents from gastric acid without the need for enteric coating(Lightfoot, 2017). Innovations in capsule filling technologies, such as the dosator principle, compression filling, and precision powder micro-dosing systems, further enhance the versatility and precision of hard capsule formulations(Takubo, 2016). Moreover, hard capsules can be used for liquid

formulations, secure double blinding in clinical trials, and sprinkle forms for patients with swallowing issues(Prasad, 2017). The development and packaging and packaging of choline alfoscerate-containing hard capsules also highlights their stability and ease of production.

Nanocapsules: Nano capsules are adaptable drug delivery vehicles that are distinguished by their core-shell design, which confines the drug inside a hollow encircled by a polymeric membrane. Numerous techniques, including solvent evaporation, nanoprecipitation, emulsification/solvent diffusion, salting out, dialysis, and supercritical fluid technologies, can be used to synthesis them (Vavaev et al., 2022). The types of nano capsules vary based on the materials used for their construction, including natural and synthetic polymers, and their functionalization, such as those with magnetite nanoparticles (MNPs) for magnetic navigation and imaging(Purohit et al., 2022). Another type includes colloidosomes, which are formed by co-assembling nanoparticles at the interface of liquid phases, offering potential for delayed drug release and efficient internalization by cancer cells (Palamarchuk et al., 2022). Additionally, DNA-based nano capsules are designed for stimuli-responsive drug release, triggered by enzymes, pH, light, and other factors, making them suitable for targeted cancer therapy(Bielski, Witczak and Newport, 2020). The IPCESCO method allows for the creation of nano capsules with various surface functionalities, enhancing their transport and delivery to specific locations in the body(Zhang, Abidi and Berlin, 2019).

Soft Capsules: Soft pharmaceutical capsules, commonly known as soft gels, are versatile oral dosage forms that encapsulate liquid or semi-solid centers within a gelatin-based or non-gelatin shell. These capsules can be categorized based on their shell composition and the nature of their contents. Traditional softgel shells are made from gelatin, water, and plasticizers like glycerine or sorbitol, which provide flexibility and durability. Innovations in capsule shell materials include the use of succinylated gelatin, which enhances the stability of encapsulated phenol derivatives by making certain substances insoluble in the shell(Korenev et al., 2022). Additionally, some soft capsules incorporate native gellan gum and hydrophilic polysaccharides, which reduce drying time and surface stickiness, making them suitable for continuous production in rotary die-type filling machines('Mineral substance soft capsule and preparation method thereof', 2018). The contents of soft capsules can also vary significantly. For instance, butylphthalide soft capsules use a gelatin shell combined with medium chain triglycerides or

coconut oil to maintain elasticity and protect the contents(‘Mineral substance soft capsule and preparation method thereof’, 2018). Fish oil soft capsules utilize self-micro emulsifying drug delivery systems to enhance bioavailability and drug. Mineral substance soft capsules replace conventional diluents like vegetable oil with glycerine and sugar alcohol, ensuring homogeneity and preventing layering (‘Mineral substance soft capsule and preparation method thereof’, 2018). Jinshuibao soft capsules incorporate superfine Cordyceps powder in a microemulsion, which includes antioxidants and disintegrants for rapid onset and improved curative effects Moreover, the manufacturing process of soft capsules can involve advanced techniques such as rolling mold type devices, which ensure precise injection and molding of the capsule shell and contents .The inclusion of polyethylene glycol and modified guar gum in the fill of some soft gelatin capsules helps maintain shell integrity and fill viscosity during storage.

Enteric Capsule: Enteric capsules are specialized pharmaceutical dose forms intended to guarantee the release of sensitive chemicals in the intestines and shield them from the stomach's acidic environment. There are various types of enteric capsules, each with unique compositions and applications. Hard enteric capsules, such as those made from hypromellose phthalate (HPMCPh) and gelatin, can be formulated with additives like polyethylene glycol-4000 (PEG-4000) to enhance their stability and enteric properties without requiring an additional coating step, making them cost-effective for industrial production (Franc, Vetchý and Fülöpová, 2022). Another type includes hard capsules with enteric film coatings, which maintain their integrity in the stomach and small intestine but disintegrate in the large intestine, useful for targeted drug delivery and treatment of conditions like liver or kidney diseases (Fülöpová et al., 2022). Softgel enteric capsules, which do not require conventional enteric coatings or polymers, offer an alternative for encapsulating sensitive fill materials(Kalmer et al., 2023) These can be made using carrageenans as film-forming polymers, providing a gelatin-free option suitable for various pharmaceutical applications (‘Medical application of Hudi enteric capsules’, 2016). Additionally, enteric soft capsules can incorporate cationic Type A gelatin and acid-insoluble enteric polymers, making them suitable for delivering active ingredients like NSAIDs and omega-3 fatty acids (Fang, HEFLIN and Hariharan, 2018; Okayama, Takahashi and Fujii, 2021).

In the present study the *M. flabellifolius* freeze - dried extract powder solid dosage form medicinal herbs capsules delivery system was prepared and evaluated as an advanced phytotherapy approach for chicken coccidiosis.

6.2 Materials and methods

6.2.1 Details regarding the Coccidiostat formulation and packagingand packagingare described in chapter three. For the purpose of this chapter, only a summary is provided.

6.2.1 Description of study area

The formulation and packagingand packagingwas designed at Harare Institute of Technology (HIT), a leading state-owned university located in Harare, the capital city of Zimbabwe. Details on the description of the study area are given in Chapter three.

6.2.2 Research Design

The study employed in vivo studies involving testing the formulated product on animals to evaluate factors such as bioavailability, safety, and efficacy. Section 3.3 of Chapter 3 provides information on the research design.

6.2.3 Sampling procedure

The sampling plan was to formulate a *M. flabellifolius* plant based coccidiostat.
Gutu, Masvingo was the sampling location.

6.2.4 Data collection procedure

Based on the information provided in Chapter 3, Section 4. The formulation and packagingand packagingdevelopment and packaging and packaging of a coccidiostat can be described as; labelling, packaging material, and packing design, packaging sizes, quality control, stability testing, chemical stability and microbiological stability.

6.2.5 The Organoleptic Properties of The Freeze -Dried Extract of *M. flabellifolius*

As shown in Table 5, and Figure 6.2.5-1, the organoleptic properties of the freeze -dried extract.

Figure 6.2.5-1: The Organoleptic Properties of *Myrothamnus Flabellifolius.*

Table 6.2.5-1: The Organoleptic Properties of *M. flabellifolius* Extract

Properties	*M. flabellifolius* Extract
Physical Appearance	Free-Flowing, Small Particulate Powder
Colour	Darken Brown
Odor	mild, earthy, and herbal scent
Taste	Bitter, Astringent, Herbaceous

M. flabellifolius extract's mild scent does not deter its application in managing type 2 diabetes mellitus (T2DM) in animal models, where it has demonstrated significant glucose-lowering effects and improved insulin sensitivity(Dhillon *et al.*, 2014). While the taste might be a barrier for some, the significant health benefits and potential for disease management could drive acceptance among health-conscious consumers and those seeking alternative medicinal

options. While the bitter and astringent taste of *M. flabellifolius* poses a challenge, various strategies, including odour familiarization, salivary protein manipulation, and selective breeding, could enhance its acceptance in animal trials. Encapsulation presents a viable solution to improve the palatability and therapeutic compliance of *M. flabellifolius*.

6.2.6 The Solubility of The Freeze -Dried of *M. flabellifolius* Extract

For oral solid dosage forms aqueous solubility is a crucial factor influencing the bioavailability of drugs. The results obtained in the solubility testing of the extract *M. flabellifolius* show that the extract is soluble in water as shown in Table 6.2.6-1.

Table 6.2.6-1: The Results of Evaluation Parameters of M. flabellifolius Extract.

Testing	*M. flabellifolius*
The Solubility of Extract	Water Soluble
Tapped Density	2.96
Carr's Index (%)	11%
Angle of Repose (°)	22.30°
The Moisture Content (%)	1.7%

6.2.7 The Solubility of The Freeze -Dried of *M. flabellifolius* Extract

Aqueous solubility is a crucial factor for oral solid dosage forms, significantly impacting their bioavailability and therapeutic efficacy. Innovative approaches like using solid amorphous dispersions (SAD) and concentration-sustaining polymers (CSP) have also been developed to enhance solubility and maintain drug concentration in the system. The results obtained in the solubility testing of the extract *M. flabellifolius* show that the extract is soluble in water as shown in Table 6.2.6-1.

6.2.8 The Densities of the Freeze - Dried Extract Powder

The Carr's index of compressibility for *M. flabellifolius* extract is 11 %, and the tapped density 2.96 show that the extract of *M. flabellifolius* freeze -dried extract powders can all be categorized as having excellent flow properties as shown in Table 6.2.6-1.

6.3.1. **Labelling**

The label should include the product name logo, active ingredients and concentration, instructions for use, warnings, storage conditions, and shelf life.

6.3.2. **Packaging material**

The packaging material used is food-grade and safe for animal consumption, resistant to moisture, light, and temperature fluctuations, easy to handle and store, and compatible with the product formulation.

6.3.3. **Packing design**

The packaging is attractive, visually appealing, easy to open and close, and tamper-resistant/temper-evident.

6.3.4. **Packaging sizes**

Different sizes for different customer needs and affordability i.e. small, medium and large.

6.3.5. **Quality Control**

The packaging and labelling should be accurate, with the correct product formulation and packagingand packagingand concentration, and utilizing clean and sanitized packaging materials and equipment.

6.4 **Stability testing**

a) Storage conditions

- Packaged products were tested for stability by placing them under venous storage conditions over a period of 14 days.

Conditions to be tested include:

The temperature conditions include room temperature, high temperature of 40°C, and low temperature of 5°C. The humidity conditions include low humidity and high humidity. The light exposure includes natural light and artificial light.

During a 14-day monitoring period, changes in the physical appearance were observed, including alterations in colour, viscosity, and pH

b) Chemical stability

Chemical stability was analysed after 14 days. Analysed for chemical composition to ensure that the active ingredients remain stable and potent. This is done using High performance liquid chromatography.

6.5. Challenges encountered during data collect

One significant issue is the variability in the chemical composition of herbal ingredients. Another big concern was quality control since it's important to guarantee the genuineness and purity of raw materials to avoid adulteration and contamination, which might jeopardize efficacy and safety. It was challenging to fulfil worldwide standards for safety and efficacy due to the formulation and packagingand packagingprocess's increased complexity in the lack of standardized regulatory guidelines. In addition, strict pharmacovigilance—which is still lacking for herbal medications—is required due to the possibility of herb-drug interactions and adverse effects.

6.6 Results and discussion

6.6.1 Coccidiostat formulation and packagingand packagingencapsulation

Figure 6.6.1-1:*M. flabellifolius* Coccidiostat formulation and packagingand packagingcapsules

Source: *M. flabellifolius* Coccidiostat formulation, 2024

The following is the representation of the results for the encapsulation of *M. flabellifolius* Coccidiostat formulation.

Coccidiostat formulation and packagingand packaging encapsulation: The encapsulation process protected sensitive Phyto active compounds from environmental and chemical degradation, thereby preserving their antimicrobial and antioxidant properties. The encapsulation

of *M. flabellifolius* Coccidiostat formulation and packagingand packagingimproved the flowability and uniformity of the powders, facilitating efficient capsule filling and reducing weight variation, which is crucial for large-scale manufacturing. Encapsulating herbal coccidiostat formulations involves leveraging various encapsulation techniques to enhance the stability, efficacy, and controlled release of bioactive compounds derived from herbs. For instance, a formulation and packagingand packagingto treat coccidiosis using herbs like Allium Odorum, Allium sativum, Tinospora cordifolia, Adhatoda vasica, and Tridax procumbens has been shown to be effective and low-cost, providing relief without side effects(Blatt *et al.*, 2002). Pendimethalin is an example of a microencapsulated formulation and packagingand packagingmade possible by techniques like interfacial polymerization, which construct a polymeric wall around the active component to ensure stability and controlled release. The antimicrobial activity and stability of encapsulated herbal substances have also been successfully maintained by the use of liposomes and polysaccharide particles such as alginate, chitosan, and starch, making them suitable for controlled release and long-term storage (Research & Development and packaging and packaging Department, Herbion Pakistan Pvt. Ltd. Pakistan et al., 2014). Furthermore, the encapsulation of herbal formulations can improve the flowability and uniformity of granules or powders, facilitating efficient capsule filling and reducing weight variation, which is crucial for large-scale manufacturing.

6.6.2 Packaging design

Figure 6.6.2-1:*M. flabellifolius* Coccidiostat formulation and packagingand packagingpackaging design

Source: *M. flabellifolius* Coccidiostat formulation, 2024

The following is the representation of the results for labelling, packaging material, packing design, packaging sizes and quality control of M. *flabellifolius* Coccidiostat formulation.

Labelling: The label should include the product name logo, active ingredients and concentration, instructions for use, warnings, storage conditions, and shelf life. For effective product development and packaging and packaging labelling, it is crucial to include several key elements to ensure compliance, consumer safety, and informed purchasing decisions. The product name and logo are fundamental for brand recognition and consumer trust(Warsiki, 2018; Sheth *et al.*, 2019). Including active ingredients and their concentrations is essential for transparency and to inform consumers about what they are purchasing, which is particularly important for products like food and pharmaceuticals(Tahiri, Lajqi and Tahiri, 2019; Meijer *et al.*, 2021). Instructions for use should be clear and concise to guide consumers on how to properly use the product, thereby enhancing usability and safety(Roche, 2016; Mohamad Kamil and Abdul Ghani, 2019). In order to ensure that customers are informed of any hazards before using the product, warnings are required to inform them of any risks (Đurđević et al., 2016; Tong et al., 2021). Storage conditions must be specified to maintain the product's efficacy and safety over its intended shelf life(Đurđević *et al.*, 2016; Prayusi and Andriani, 2023). Shelf life information is critical as it informs consumers about the duration for which the product remains safe and effective to use, which can be monitored using smart labels that track time-temperature indicators.

Packaging material: Food-grade packaging that is safe for animal eating is utilized; it is also easy to handle, store, and resistant to changes in light, moisture, and temperature. Multilayer packaging, which combines different plastic polymers, offers excellent resistance to gas permeability and water vapor, making it suitable for preserving food quality and safety (Fink, 2023; Viktor, Komakha and Komakha, 2023). Polyvinyl alcohol-based polymer coatings, enhanced with polyamide-epichlorohydrin resins and glycerol, provide moisture resistance, elasticity, and uniform barrier properties, ensuring the packaging is robust and durable(Mat Rosid *et al.*, 2023). Nano biotechnology can further enhance packaging by incorporating cellulose nanocrystals and biopolymers, which help in extending shelf life and preventing microbial contamination(Sin and Tueen, 2023). For food safety to be maintained in harsh environmental circumstances, biodegradable materials with enhanced mechanical capabilities and antimicrobial protection—like those reinforced with 2D covalent organic frameworks (COFs) and silk fibroin—are essential (Han et al., 2023). Furthermore, the microbiological and sensory qualities of food products can be enhanced by the use of natural additives like thyme and

black cumin in packaging materials; however, the plants used have a greater impact on these qualities than the packaging material itself (Akpinar et al., 2022). For high-temperature applications, C-PET packaging, which includes a heat-resistant barrier layer, is effective for heat sterilization and ensures long shelf life without compromising the integrity of the packaging(Fellows, 2022). Moreover, biodegradable and water-soluble packaging materials composed of non-ionic and ionic polysaccharides, along with plasticizers and optional biocides or metal oxides, can be tailored for specific needs(Doctor of Technical Sciences, Professor, Odessa National Technological University, St. Kanatnaya, 112, Odessa, Ukraine *et al.*, 2022). Finally, advanced packaging designs that eliminate overpressure and reduce odour emissions during heating or baking further enhance the usability and safety of the packaging.

Packing design: The packaging is attractive, visually appealing, easy to open and close, and tamper-resistant/temper-evident. Given that premium packaging frequently gives consumers the impression that the product is of a higher calibre, attractive and visually appealing packaging can have a big impact on how consumers perceive and make judgments about what to buy. To enhance visual appeal, incorporating transparent elements, such as a transparent cap or window, can allow consumers to view the product inside, adding to the attractiveness and functionality of the packaging. Easy-to-open and close features can be achieved through designs like flexible composite films with weak zones that allow for easy peeling, or packaging with a grip hook for user-friendly opening(Hansen *et al.*, 2018). In order to guarantee product safety and customer trust, tamper-evident and tamper-resistant features are essential. These can include tamper-evident tabs that lock in an open position and provide an audible indication when opened, or transparent resealable adhesive structures that visually indicate tampering through misalignment upon reclosure. Additionally, incorporating security labels that are machine scannable can further enhance tamper-evident properties (Kehinde *et al.*, 2020). For products sensitive to environmental factors, such as photosensitive materials, UV protectors can be integrated into the packaging to prevent degradation. The application of biopolymers and intelligent packaging technologies, among other innovations in packaging materials, can help preserve product quality and prolong shelf life while being ecologically benign.

Packaging sizes: The package is aesthetically pleasing, easy to open and close, resistant to tampering, and tamper evident. A tamper-evident package can utilize a transparent resealable

adhesive structure, such as a tape or label, which covers the closure opening and reveals any tampering through torn frangible portions and misaligned graphics upon reclosure, enhancing visual appeal and security. Additionally, incorporating a highly visible tamper-evidencing band that surrounds the container lip flange and closure lid ensures that the package cannot be opened without removing the band, thus providing clear evidence of tampering (ASHISH, 2023). For ease of use, a container with a transparent cap and a scannable security label can be employed, allowing users to verify the product's authenticity while maintaining an attractive design(*US Patent Application for FILM FOR EASY-OPEN PACKAGING Patent Application (Application #20200079566 issued March 12, 2020) - Justia Patents Search*, 2018). Flexible composite films with zones of varying sealing strength and weakening lines facilitate easy opening while ensuring tamper evidence through visible tear tabs(Hansen *et al.*, 2018). Child-resistant liquid containment packaging with inner and outer caps, tamper-evident rings, and friction-generating resilient plates offers both security and ease of use, with clear indicia indicating tampering. A package with a lid seated on a base and a tamper-evident tab that locks in the open position provides both security and ease of access, with an audible indication of tampering. Additionally, a box with a closure cover featuring a tongue and elongated window, along with preset breakage lines, ensures tamper evidence while maintaining an attractive and functional design. Finally, a container closure with a tamper-evident tear strip and a plastic lid with engagement portions that latch securely onto the container further enhances tamper resistance and visual appeal(*"Package with Tamper-Evident Feature" in Patent Application Approval Process (USPTO 20170073136)*, 2017) (*US Patent for Packaging with tamper-evident seal Patent (Patent # 11,905,074 issued February 20, 2024) - Justia Patents Search*, 2019).

Quality control: The packaging and labelling should be accurate, with the correct product formulation and packagingand packagingand concentration, and utilizing clean and sanitized packaging materials and equipment. Effective packaging not only protects the product but also enhances its market appeal and provides essential information to consumers. For instance, in the case of frozen shrimp, packaging must maintain the product at -18°C throughout its cycle, requiring specific materials and techniques to ensure quality and safety(Hannan *et al.*, 2022). Particularly in aseptic processing, where strict cleaning and sanitization procedures are required to prevent contamination and guarantee the safety of the finished product, clean and sanitized packaging materials and equipment are essential to preserving product quality (Merritt, 2022).

Advanced packaging and labelling equipment, such as those used in textile production, can significantly improve efficiency and accuracy, ensuring that labels are correctly positioned and smooth(Diwan, 2022). Similarly, innovations in labelling devices, such as those with adjustable labelling wheel support frames, enhance maintenance and debugging efficiency, reducing the likelihood of errors. For products exported from developing countries, packaging and labelling must meet the stringent requirements of developed markets, including container closure integrity and compliance with recycling regulations(Gordon and Williams, 2020). Automated labelling systems, which use positioning mechanisms and labelling manipulators, ensure precise label application, further enhancing accuracy and efficiency. Additionally, maintaining a clean environment during packaging, such as using positive pressure environments to prevent contamination, is essential for products like biological tissues or food items.

6.7 Conclusion

The chapter has focused on the packaging of the Coccidiostat for *M. flabellifolius*. Furthermore, the herbal formulation and packagingand packagingwas encapsulated to improve the stability, bioavailability, and controlled release of bioactive components from *M. flabellifolius*. In addition, ensuring consumer safety, regulatory compliance, and product efficacy. Subsequently, there was careful consideration of the packaging material, it is crucial for the preservation of active ingredients, prevention of contamination, and maintaining product quality over time. Moreover, the packing design and size for the chicken herbal coccidiostat were chosen. Designing the packaging ensures consumer appeal, product protection, and regulatory compliance. Packaging size is critical in preserving the freshness and effectiveness of herbal items. Lastly, *M. flabellifolius* Coccidiostat formulation and packagingand packagingunderwent quality control, including the standardization of raw materials, processing techniques, and final products. In summary the section has outlined, the encapsulation, labelling, packaging material, packing design, packaging sizes and quality control of *M. flabellifoliu*s coccidiostat formulation.

6.8 Recommendations

Based on the study results, the study recommends that successful formulation and packaging ofthe formulation and packagingand packagingshould encompass; -

Encapsulation of *M. flabellifolius* Coccidiostat formulation

- For the encapsulation of M. flabellifolius Coccidiostat formulation, nano/microencapsulation strategies are recommended to enhance water-solubility, stability, and functionality of the bio actives. This approach ensures protection from adverse conditions during food processing and storage, and facilitates controlled release to target sites, thereby improving bioavailability and efficacy.

Labelling of M. flabellifolius Coccidiostat formulation and packagingand packaging

- In terms of labelling, it is crucial to ensure clarity and accuracy, avoiding ambiguities and providing essential information such as biosafety and correct usage instructions. This can be accomplished by following recommendations from pertinent bodies, such as the EC and the WHO, and making sure that the typography and font size are readable.

Packaging material of M. flabellifolius Coccidiostat formulation and packagingand packaging

- For packaging material, a composite material with at least one layer of cellulose/cardboard is recommended for its resource efficiency and ability to maintain sterility, which is essential for aseptic packaging.

Packaging design of M. flabellifolius Coccidiostat formulation and packagingand packaging

- The packaging design should integrate both the product and its packaging as a single entity, considering social, technical, ergonomic, economic, and environmental factors to ensure quality and sustainability.

Packaging sizes for M. flabellifolius Coccidiostat formulation and packagingand packaging

- Packaging sizes should be tailored to the specific needs of the product and its intended use, ensuring convenience and protection until administration or use.

Quality control for M. flabellifolius Coccidiostat formulation and packagingand packaging

- Quality control should encompass the entire process from plant design to distribution, employing both traditional and rapid microbiological methods to ensure sterility and integrity. Non-destructive methods for on-line detection of package integrity and quality

control post-preincubation are also recommended. Additionally, automated systems for inspecting and rejecting defective products can enhance quality assurance, ensuring only satisfactory products reach the market.

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CHAPTER 7

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

7.1 **Introduction**

This Chapter summaries the research proceedings and findings from Nhema Chickens Farm and Harare Institute of Technology located in Norton and Harare, Zimbabwe, respectively. The broad objective of the study was to assess the efficacy of *Myrothamnus flabellifolius (Mufandichimuka)* in controlling coccidiosis in indigenous chickens articulated in the introductory chapter of the study. One way ANOVA, Bonferroni post-hoc test for pairwise comparisons, descriptive statistics, independent samples t-test were approaches used to analyses the specific objectives of the study. The results for the analysing the phytochemical compounds in *M. flabellifolius* plant through hot water extraction and solvent extract. The results indicate that the acetone solvent was more effective in extracting these phytochemical compounds from the plant material compared to the hot water solvent as stated in Chapter 4. Additionally, this study demonstrated that *M. flabellifolius* can reduce coccidiosis infected chicken mortality, reduce oocyst count and improve the growth weight, stated by Chapter 5. Lastly, there was development and packaging and packaging of a *M. flabellifolius* chicken coccidiostat in Chapter 6. The corresponding chapters present an analysis of the findings and the implications of the research. In conclusion, this section provides a summary, draws final thoughts, suggests policy implications, and identifies potential avenues for future investigation.

7.2 **Research summary**

The first chapter of the study was introduction, problem statement, objectives, research questions, justification and outline of the thesis. Second chapter of the study was on literature review synthesis. Third chapter of the study was on methodology which covered study site,

research design, experimental design, sampling procedure, data collection procedure, data analysis procedure, ethical considerations and finally the summary of the methodology chapter.

Chapter four, five and six were results chapters for each objective respectively. Chapter four was to analyse the phytochemical compounds in *M. flabellifolius* plant through hot water extraction and solvent extract. This was achieved through use of qualitative analysis, phytochemical compounds were identified using standard screening tests, including tests for phenols, tannins, saponins, glycosides, alkaloids, flavonoids, and terpenoids. The data was analysed using descriptive statistics to provide a summary of the characteristics of the coccidial compounds. Data was analysed using SPSS software version 20. The qualitative results revealed that, phenols, tannins, saponins, glycosides, alkaloids, flavonoids, and terpenoids were present in the plant extracts. Furthermore, quantitative analysis, quantified phenols, flavonoids, tannins, saponins and alkaloids. The results indicate that the acetone solvent was more effective in extracting these phytochemical compounds from the plant material compared to the hot water solvent. The acetone extract demonstrated the highest mean value of 46.4 (± 2.2) mg GAE/g for total phenols, in contrast to the hot water extract which had 44.2 (± 1.8) mg GAE/g. A similar trend was noted for total flavonoids, where the acetone extract displayed an average of 35.6 (± 1.4) mg QE/g, while the hot water extract had an average of 33.7 (± 1.1) mg QE/g. This pattern persisted for total tannins, as the acetone extract revealed a higher mean of 27.5 (± 1.3) mg GAE/g compared to 26.4 (± 1.0) mg GAE/g in the hot water extract. In terms of total saponins, the acetone extract exhibited an average of 19.6 (± 0.9) mg DE/g, while the hot water extract had an average of 18.8 (± 0.7) mg DE/g. Lastly, concerning total alkaloids, the acetone extract indicated a marginally higher average of 11.3 (± 0.4) mg NE/g in contrast to 10.6 (± 0.3) mg NE/g in the hot water extract. To achieve maximum extraction of *M. flabellifolius,* it is essential to consider the solvent type, extraction method, and the specific compounds targeted.

After have analysed the phytochemical compounds in *M. flabellifolius* plant through hot water extraction and solvent extract, the study proceeded in Chapter five to determine the level of effectiveness of plant extracts of *M. flabellifolius* on treating coccidiosis. To achieve this objective, 90 chickens were divided into 3 groups, each group having 30 chickens. To determine the anticoccidial effects of *Mufandichimuka,* the oocytes per gram (OPG) were measured from each group after 10 days post infection. Additionally, to determine, growth rate and mortality,

birds’ weight was measured and number of deaths were recorded, respectively. Results for the oocyst reduction rate were expressed as percentages. An analysis of variance (One way ANOVA) was performed on the bird’s weight gain. The bird’s weight gain was analysed using the Bonferroni post-hoc test for pairwise comparisons.

Chapter six presents’ results of formulation and packaging ofa *M. flabellifolius* chicken plant based coccidiostat. The formulation development and packaging of a coccidiostat can be described as; labelling, packaging material (encapsulation and bottling), packing design, packaging sizes, quality control, stability testing, chemical stability and microbiological stability

Chapter seven was on summary of the study findings, conclusions and recommendations of the study based on the study findings.

7.3 **Conclusions**

M. flabellifolius has both primary and secondary metabolites. Primary metabolite identified were, carbohydrates in aqueous extract. The secondary metabolites that followed were phenols, tannins, glycosides, and alkaloids in both extracts, while saponins were detected in the aqueous extract but not in the acetone extract. Lastly flavonoids where present in acetone extract only. Another primary metabolite, proteins, were absent in both extracts. The combined factors of inherent low protein content, extraction method specificity, and the plant's unique biochemical adaptations contribute to the absence of proteins in the aqueous and acetone extracts of *M. flabellifolius.* In addition, the plant's inherent phytochemical profile, which is rich in non-carbohydrate bioactive compounds, and the solvent's selective extraction properties account for the absence of carbohydrates in the acetone extract of *M. flabellifolius*. Finally, the choice of solvent is critical to the extraction of particular phytochemicals, and acetone's lower polarity renders it unsuitable for extracting saponins from *M. flabellifolius*, requiring the use of more polar solvents for precise phytochemical profiling. Also, the chapter has quantified phytoconstituents in *M. flabellifolius*. The findings propose that both solvents demonstrated comparable efficacy in the extraction of total alkaloids from the botanical material, as evidenced by the statistically insignificant Levene's test results ($p = 0.834$). In addition, the plant's inherent phytochemical profile, which is rich in non-carbohydrate bioactive compounds, and the solvent's

selective extraction properties account for the absence of carbohydrates in the acetone extract of *M. flabellifolius.*

7.4 **Overall recommendations**

On analyses of the phytochemical compounds in *M. flabellifolius* plant through hot water extraction and solvent extract.

To achieve maximum extraction of *M. flabellifolius,* it is essential to consider the solvent type, extraction method, and the specific compounds targeted. All things considered, a multifaceted strategy utilizing hydro distillation, methanol, and ethanol in conjunction with cutting-edge processing methods can optimize.

On determining the level of effectiveness of plant extracts of *M. flabellifolius* on treating coccidiosis at Nhema farm

Integrating *M. flabellifolius* with other natural feed additives, such as the vitamin feed additive Introvit A+WS, which has been shown to improve chicken health and productivity, could synergistically enhance its effectiveness against coccidiosis. Employing advanced processing techniques like microencapsulation and microbial fermentation can further unlock and improve the bioavailability of *M. flabellifolius'* active compounds, ensuring more consistent and potent effects.

On Formulating a *M. flabellifolius* plant based coccidiostat

Encapsulation of *M. flabellifolius* Coccidiostat formulation

For the encapsulation of *M. flabellifolius* Coccidiostat formulation, nano/microencapsulation strategies are recommended to enhance water-solubility, stability, and functionality of the bio actives. This approach ensures protection from adverse conditions during food processing and storage, and facilitates controlled release to target sites, thereby improving bioavailability and efficacy.

Labelling of *M. flabellifolius* Coccidiostat formulation and packagingand packaging

In terms of labelling, it is crucial to ensure clarity and accuracy, avoiding ambiguities and providing essential information such as biosafety and correct usage instructions. This can be accomplished by following recommendations from pertinent bodies, such as the EC and the WHO, and making sure that the typography and font size are readable.

Packaging material of *M. flabellifolius* Coccidiostat formulation and packagingand packaging

For packaging material, a composite material with at least one layer of cellulose/cardboard is recommended for its resource efficiency and ability to maintain sterility, which is essential for aseptic packaging.

Packaging design of *M. flabellifolius* Coccidiostat formulation and packagingand packaging

The packaging design should integrate both the product and its packaging as a single entity, considering social, technical, ergonomic, economic, and environmental factors to ensure quality and sustainability.

Packaging sizes for *M. flabellifolius* Coccidiostat formulation and packagingand packaging

Packaging sizes should be tailored to the specific needs of the product and its intended use, ensuring convenience and protection until administration or use.

Quality control for *M. flabellifolius* Coccidiostat formulation and packagingand packaging

Quality control should encompass the entire process from plant design to distribution, employing both traditional and rapid microbiological methods to ensure sterility and integrity. Non-destructive methods for on-line detection of package integrity and quality control post-preincubation are also recommended. Additionally, automated systems for inspecting and rejecting defective products can enhance quality assurance, ensuring only satisfactory products reach the market.

7.5 **Areas for further research**

7.5.1. **Analysing the phytochemical compounds in *M. flabellifolius* plant through hot water extraction and solvent extract**

Firstly, optimizing the pressurized hot water extraction (PHWE) process to enhance the yield and purity of bioactive compounds, such as flavonoids, could be beneficial. Achieving this goal involves the adjustment of extraction parameters, such as temperature and pressure, a practice exemplified in research conducted on various plant species. Studies have indicated that temperatures ranging from 150 to 250°C yield optimal results for flavonoid extraction. Furthermore, the utilization of sophisticated analytical methodologies, such as ultra-performance liquid chromatography-quadrupole time of flight mass spectrometry (UHPLC-qTOF-MS) and multivariate chemometric models, can offer a more thorough analysis of the extracted compounds. A compelling area of research pertains to the comparison of phytochemical content across different geographical regions, given the documented variations in polyphenol content and composition between populations of *M. flabellifolius* from Namibia and South Africa. Moreover, the isolation and characterization of specific compounds like arbutin, lupeol, and diverse polysaccharides could enhance the understanding of their unique contributions to the medicinal properties of the plant. Lastly, an exploration into the structural characteristics of cell wall components and their impact on desiccation tolerance may yield insights into the plant's resilience and potential applications in biotechnology.

7.5.2. **Determination of the level of effectiveness of plant extracts of *M. flabellifolius* on treating coccidiosis**

Detailed in vivo studies are necessary to evaluate the anticoccidial efficacy of *M. flabellifolius* extracts in chicken, similar to the studies conducted with other plant extracts like Lannea schimperi and Moringa oleifera, which have shown promising results in reducing oocyst counts and improving health markers in infected animals. Moreover, it is essential to isolate the distinct active compounds present in *M. flabellifolius*, including galloyl and quinic acid derivatives, and to investigate their respective mechanisms of action against *Eimeria* spp. elucidated, as has been done with other plants containing gallic acid and tannins. Comparative studies with established herbal treatments, such as those involving Quercus infectoria, Rhus chinensis, and Terminalia

chebula, would help position *M. flabellifolius* within the broader spectrum of phytotherapeutic options. Furthermore, dose optimization studies are crucial, as seen in research on other plant extracts where varying doses significantly impacted efficacy and safety profiles. Investigating the potential synergistic effects of combining *M. flabellifolius* with other known anticoccidial herbs, such as those from Punica granatum and Plantago asiatica, could enhance its therapeutic potential Finally, it is imperative to conduct extensive long-term investigations that evaluate the emergence of resistance, along with the economic viability and ecological implications associated with the utilization of *M. flabellifolius* in extensive chicken production, in order to guarantee a sustainable and feasible implementation.

7.5.3. **Formulation and packaging ofa *M. flabellifolius* plant based coccidiostat**

The identification and isolation of active compounds within *M. flabellifolius* that exhibit anticoccidial properties are crucial. Furthermore, *M. flabellifolius'* antioxidant activity, with strong scavenging effects on superoxide anion and hydroxyl radicals, may reduce oxidative stress in infected animals, thus improving their resistance to coccidial infection. The development and packaging and packaging of novel strategies, such as microencapsulation and microbial fermentation, could enhance the bioavailability and efficacy of *M. flabellifolius* -based formulation. Finally, further investigation into the safety, dosage optimization, and potential side effects of *M. flabellifolius* in animal models is necessary to ensure its practical application in livestock management.

APPENDICES

APPENDIX 1: Plant authentication

MINISTRY OF LANDS, AGRICULTURE, FISHERIES, WATER AND RURAL DEVELOPMENT
DEPARTMENT OF RESEARCH & SPECIALIST SERVICES (DR&SS)

All Communications to be addressed to
"THE HEAD"

Telephone: 263-4-744 170, 745230
E-mail: nhbg@drss.gov.zw
Fax: 263-4-708938

NATIONAL HERBARIUM & BOTANIC GARDEN
Box A889
Avondale, Harare, Zimbabwe

29 May 2024

Mupondi Edith
Bindura University

Dear Sir/ Madam

RE: Plant Identification

The plant specimen you brought in was identified as *Myrothamnus flabellifolius*. It belongs to the Family Myrothamnaceae. Common names are Mufandichimuka (S), Resurrection plant (E).

Thank you.

HEAD
Nat. Herbarium & Botanic Gardens
D.R. & S.S. - Research Serv. Division
29 MAY 2024
P.O. BOX A 889, AVONDALE
HARARE, ZIMBABWE

Mazarire T. C. (Mrs.)
Research Officer
National Herbarium and Botanic Garden

APPENDIX 2: Weight gain of 10 randomly chosen chickens, averages, average weight gain per week and total % weight gain after 42 days

	AA **(No prophylaxisis)**	AVG	AB (**ESB3 prophylaxisis**	Avg	AC (***Myrothamnus flabellifoilus***)	Avg
Day 7	0.782; 0.793 0.804; 0.802 0.809; 0.795 0.797; 0.813 0.811; 0.794	0.8	0.851; 0.867 0.863; 0.843 0.829; 0.849 0.856; 0.853 0.846; 0.862	0.851	0.892; 0.881 0.903; 0.901 0.902; 0.910 0.897; 0.894 0.912; 0.913	0.90
Day 14	0.814; 0.801 0.816; 0.824 0.813; 0.823 0.831; 0.815 0.833; 0.809	0.82 2.5% increase	0.896; 0.893 0.903; 0.883 0.857; 0.876 0.901; 0.883 0.873; 0.897	0.90 5.9 %	0.947; 0.934 0.944; 0.953 0.952; 0.954 0.941; 0.946 0.967; 0.963	0.95 5.6%
Day 21	0.832; 0.817 0.828; 0.827 0.830; 0.819 0.834; 0.831 0.806; 0.830	2.4% increase 0.845	0.943; 0.952 0.935; 0.946 0.973; 0.962 0.923; 0.936 0.962; 0.973	0.95 5.6%	0.992; 0.994 1.003; 1.037 1.013; 0.992 1.062; 0.987 1.014; 1.007	1.00 5.3%
Day 28	0.851; 0.873 0.849; 0.862 0.821; 0.837 0.841; 0.839 0.858; 0.864	0.862 2.4%	0.992; 0.999 0.987; 1.013 1.107; 1.032 1.037; 1.035 0.993; 0.996	1.04 5.3%	1.046; 1.063 1.049; 1.052 1.037; 1.063 1.047; 1.058 1.042; 1.056	1.05 5.0 increase
Day 35	0.872; 0.886 0.863; 0.881 0.841; 0.882 0.864; 0.858 0.873; 0.868	0.883 2.3%	1.046; 1.069 1.054; 1.058 1.084; 1,044 1.073; 1.043 1.036; 1.039	1.05 5.0 % increase	1.092; 1.086 1.099; 1.106 1.109; 1.104 1.098; 1.097 1.112; 1.107	1.10 4.8 %

Day 42	0.896; 0.913 0.890; 0.903 0.887; 0.910 0.896; 0.893 0.884; 0.901	0.903 2.3%	1.091; 1.087 1.107; 1.103 1.109; 1.110 1.096; 1.112 1.113; 1.092	1.10 4,8%	1.141; 1.162 1.169; 1.152 1.132; 1.143 1.146; 1.151 1.134; 1.148	1.15 4.6
Average weights		12.5% increase		29.4% increase		

APPENDIX 3: Quantitative Phytochemical Analysis of *Myrothamnus flabellifolius*

PHYTOCHEMICAL PARAMETER	VALUE 1	VALUE 2	VALUE3	VALUE 4	VALUE 5	MEAN ± SD
Total phenols (mg GAE/g)	42.1	44.5	43.5	45.3	46	44.2 ± 2,1
Total flavanoids(mg QE/g)	34.2	33.5	31.9	33.8	35.1	33.7 ± 1.8
Total tannins (mg GAE/g)	26.9	26.1	24.9	25.8	27.2	26.4 ± 1.5
Total sapponins (mg DE/g)	19,2	17.6	18.3	19.5	18.7	18.8 ± 1.2
Total alkaloids (mg NE/g)	10.5	11.4	10.3	10.5	11.1	10.6 ± 0.9

APPENDIX 4: Summarized table for both hot water and acetone extract with the mean ±SD

PHYTOCHEMICAL PARAMETER	HOT WATER EXTRACT	ACETONE EXTRACT
Total phenols (mg GAE/g)	44.2 ± 2.1 mg	46.4 ± 1.9
Total flavanoids(mg QE/g)	33.7 ± 1.8 mg	35.6 ± 1.6
Total tannins (mg GAE/g)	: 26.4 ± 1.5 mg	27.5 ± 1.3
Total sapponins (mg DE/g)	18.8 ± 1.2 mg	19.6 ± 1.0
Total alkaloids (mg NE/g)	10.6 +- 0.9	11.3 ± 0.8

APPENDIX 5: Weight gain of 10 randomly chosen chickens, averages, average weight gain per week and total % weight gain after 42 days

PARAMETER DAY	**AA (No prophylaxisis)**	**Average And % increase**	**AB (Coccidiostat- ESB_3 prophylaxisis**	**Average And % increase**	**AC (*Myrothamnus flabellifolius* treatment)**	**Average And % increase**
Day 7	.7821[1],0.7932[2], 0.8043[3],0.8094[4], 0.7955[5],0.7976[6], 0.8137[7],0.8118[8], 0.7949[9],0.7921[10]	**0.8**	0.851[1],0.867[2], 0.863[3],0.843[4], 0.829[5],0.849[6], 0.856[7],0.853[8], 0.846[9],0.862[10]	**0.851**	0.892[1], 0.881[2], 0.903[3], 0.901[4], 0.902[5], 0.910[6], 0.897[7], 0.894[8], 0.912[9], 0.913[10]	**0.9**
Day 14	0.814[1], 0.801[2], 0.816[3], 0.824[4], 0.813[5], 0.823[6], 0.831[7], 0.815[8], 0.833[9], 0.809[10]	**0.82** **2.5% increase**	0.896[1],0.893[2], 0.903[3],0.883[4], 0.857[5], .876[6], 0.901[7],0.883[8], 0.873[9],0.897[10]	**0.9** **5.90%**	0.947[1], 0.934[2], 0.944[3], 0.953[4], 0.952[5], 0.954[6], 0.941[7], 0.946[8], 0.967[9], 0.963[10]	**0.95** **5.60%**

Day 21	0.832[1], 0.817[2], 0.828[3], 0.827[4], 0.830[5], 0.819[6], 0.834[7], 0.831[8], 0.806[9], 0.830[10] | |	**2.4% increase 0.845**	0.943[1],0.952[2], 0.935[3],0.946[4], 0.973[5],0.962[6], 0.923[7],0.936[8], 0.962[9],0.973[10] |	**0.95 5.60%**	0.992[1], 0.994[2], 1.003[3], 1.037[4], 1.013[5], 0.992[6], 1.062[7], 0.987[8], 1.014[9], 1.007[10]	**1 5.30%**
Day 28	| 0.851[1], 0.873[2], 0.849[3], 0.862[4], 0.821[5], 0.837[6], 0.841[7], 0.839[8], 0.858[9], 0.864[10]	**0.862 2.40%**	.992[1], 0.999[2], 0.987[3],1.013[4], 1.107[5],1.032[6], 1.037[7],1.035[8], 0.993[9],0.996[10]	**1.04 5.30%**	1.046[1], 1.063[2], 1.049[3], 1.052[4], 1.037[5], 1.063[6], 1.047[7], 1.058[8], 1.042[9], 1.056[10]	**1.05 5.0 increase**
Day 35	0.872[1], 0.886[2], 0.863[3], 0.881[4], 0.841[5], 0.882[6], 0.864[7], 0.858[8], 0.873[9], 0.868[10]	**0.883 2.30%**	1.046[1],1.069[2], 1.054[3],1.058[4], 1.084[5],1.044[6], 1.073[7],1.043[8], 1.036[9],1.039[10] |	**1.05 5.0 % increase**	1.092[1], 1.086[2], 1.099[3], 1.106[4], 1.109[5], 1.104[6], 1.098[7], 1.097[8], 1.112[9], 1.107[10]	**1.1 4.80%**

Day 42	0.896[1], 0.913[2], 0.890[3], 0.903[4], 0.887[5], 0.910[6], 0.896[7], 0.893[8], 0.884[9], 0.901[10]	**0.903 2.30%**	1.091[1],1.087[2], 1.107[3],1.103[4], 1.109[5],1.110[6], 1.096[7],1.112[8], 1.113[9],1.092[10]	**1.1 4,8%**	1.141[1], 1.162[2], 1.169[3], 1.152[4], 1.132[5], 1.143[6], 1.146[7], 1.151[8], 1.134[9], 1.148[10]	**1.15 4.6**
TOTAL % WEIGHT GAIN		**12.5% increase**		**29.40 % increase**		**28.7 % increase**

APPENDIX 6: Oocyst count per gram

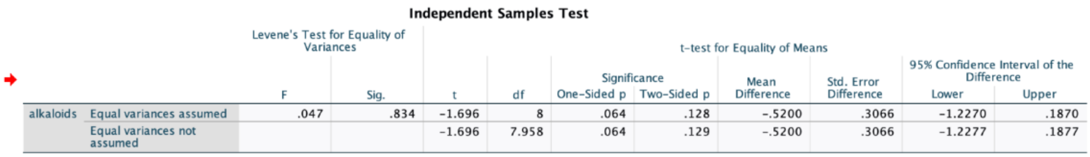
TREATMENT GROUP	**OOCYST COUNTS OOCYST/ g)**					
	Day 7	**Day 14**	**Day 21**	**Day 28**	**Day 35**	**Day 42**

GROUP AA (INFECTED , NO TREATYMENT)	100 000	250 000	275 000	300 000	350 000	370 000
GROUP AB (INFECTED, ON COCCIODISTAT, ESB3 TREATMENT)	50 000	150 000	90 000	65 000	40 000	22 000
GROUP AC (INFECTED, *MYROTHAMNUS FLABELLIFOLIUS* TREATMENT)	75 000	185 000	120 000	90 000	60 000	35 000

APPENDIX 7:% MORTALITY RATE OF THE 3 GROUP FROM DAY 7 TO DAY 42

DAY	Group AA(Infected, no treatment)	Group AB (Infected on ESB3 coccidiostat)	Group AC (*Myrothamnus Flabellifolius*)
7	5.00%	1.70%	3.30%
14	11.70%	5.00%	5.00%
21	13.30%	5.00%	8.30%
28	16.70%	1.70%	3.30%
35	16.70%	0%	1.70%
42	25.00%	0%	1.70%
% mortality rate	88.30%	11.60%	21.60%

APPENDIX 8: Independent Samples T-Test for Total Alkaloids in Hot Water and Acetone Extracts



Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
alkaloids	Equal variances assumed	.047	.834	-1.696	8	.064	.128	-.5200	.3066	-1.2270	.1870
	Equal variances not assumed			-1.696	7.958	.064	.129	-.5200	.3066	-1.2277	.1877

Independent Samples Effect Sizes

		Standardizer[a]	Point Estimate	95% Confidence Interval	
				Lower	Upper
alkaloids	Cohen's d	.4848	-1.073	-2.388	.298
	Hedges' correction	.5370	-.968	-2.155	.269
	Glass's delta	.5020	-1.036	-2.410	.430

a. The denominator used in estimating the effect sizes.
Cohen's d uses the pooled standard deviation.
Hedges' correction uses the pooled standard deviation, plus a correction factor.
Glass's delta uses the sample standard deviation of the control group.

APPENDIX 9: Independent Samples T-Test for Total saponins in Hot Water and Acetone Extracts

T-Test

[DataSet2]

Group Statistics

	Solvent	N	Mean	Std. Deviation	Std. Error Mean
sapponins	hot water	5	18.660	.7503	.3356
	acetone	5	19.560	.7503	.3356

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
sapponins	Equal variances assumed	.000	1.000	-1.897	8	.047	.094	-.9000	.4746	-1.9943	.1943
	Equal variances not assumed			-1.897	8.000	.047	.094	-.9000	.4746	-1.9943	.1943

Independent Samples Effect Sizes

		Standardizer[a]	Point Estimate	95% Confidence Interval	
				Lower	Upper
sapponins	Cohen's d	.7503	-1.199	-2.537	.198
	Hedges' correction	.8312	-1.083	-2.290	.179
	Glass's delta	.7503	-1.199	-2.627	.325

a. The denominator used in estimating the effect sizes.
Cohen's d uses the pooled standard deviation.
Hedges' correction uses the pooled standard deviation, plus a correction factor.
Glass's delta uses the sample standard deviation of the control group.

APPENDIX 10: Independent Samples T-Test for Total tannins in Hot Water and Acetone Extracts"

T-Test

Group Statistics

	solvent	N	Mean	Std. Deviation	Std. Error Mean
Total_tannins	hot water	5	26.180	.9149	.4091
	acetone	5	27.540	.9607	.4297

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
Total_tannins	Equal variances assumed	.010	.921	-2.292	8	.026	.051	-1.3600	.5933	-2.7281	.0081
	Equal variances not assumed			-2.292	7.981	.026	.051	-1.3600	.5933	-2.7287	.0087

Independent Samples Effect Sizes

		Standardizer[a]	Point Estimate	95% Confidence Interval	
				Lower	Upper
Total_tannins	Cohen's d	.9381	-1.450	-2.839	.007
	Hedges' correction	1.0392	-1.309	-2.563	.006
	Glass's delta	.9607	-1.416	-2.925	.194

a. The denominator used in estimating the effect sizes.
Cohen's d uses the pooled standard deviation.
Hedges' correction uses the pooled standard deviation, plus a correction factor.
Glass's delta uses the sample standard deviation of the control group.

APPENDIX 11: Independent Samples T-Test for Total flavanoids in Hot Water and Acetone Extracts"

T-Test

[DataSet4]

Group Statistics

	Solvent	N	Mean	Std. Deviation	Std. Error Mean
Total_flavanoids	hot water	5	33.700	1.1726	.5244
	acetone	5	35.560	1.2095	.5409

Independent Samples Test

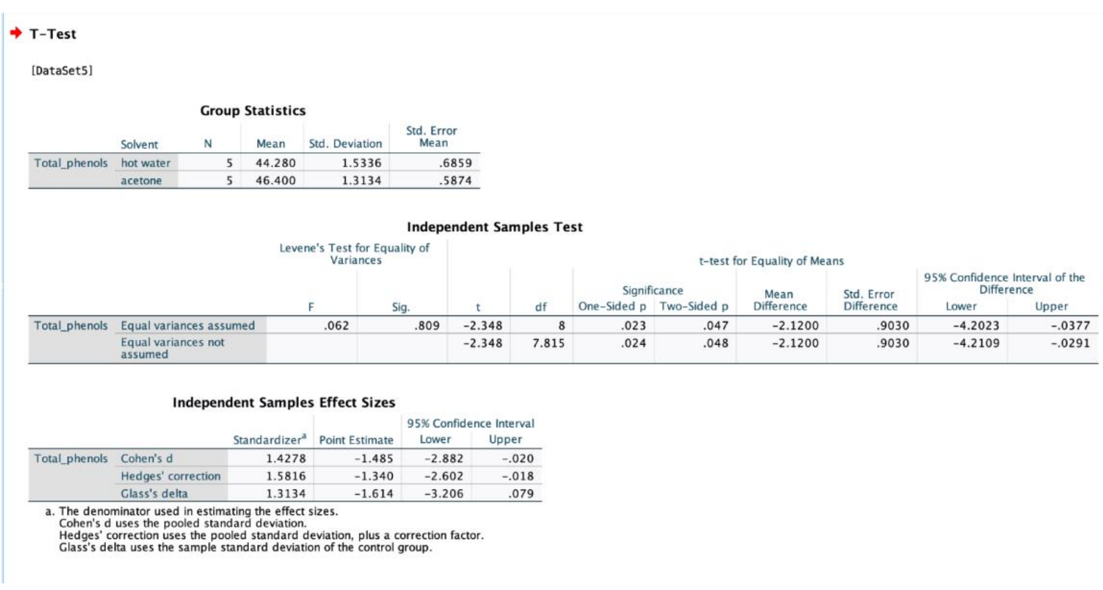
		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
Total_flavanoids	Equal variances assumed	.000	.987	-2.469	8	.019	.039	-1.8600	.7534	-3.5973	-.1227
	Equal variances not assumed			-2.469	7.992	.019	.039	-1.8600	.7534	-3.5976	-.1224

Independent Samples Effect Sizes

				95% Confidence Interval	
		Standardizer[a]	Point Estimate	Lower	Upper
Total_flavanoids	Cohen's d	1.1912	-1.561	-2.977	-.077
	Hedges' correction	1.3196	-1.410	-2.687	-.069
	Glass's delta	1.2095	-1.538	-3.097	.123

a. The denominator used in estimating the effect sizes.
Cohen's d uses the pooled standard deviation.
Hedges' correction uses the pooled standard deviation, plus a correction factor.
Glass's delta uses the sample standard deviation of the control group.

APPENDIX 12: Independent Samples T-Test for Total phenols in Hot Water and Acetone Extracts"



T-Test

[DataSet5]

Group Statistics

	Solvent	N	Mean	Std. Deviation	Std. Error Mean
Total_phenols	hot water	5	44.280	1.5336	.6859
	acetone	5	46.400	1.3134	.5874

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
Total_phenols	Equal variances assumed	.062	.809	-2.348	8	.023	.047	-2.1200	.9030	-4.2023	-.0377
	Equal variances not assumed			-2.348	7.815	.024	.048	-2.1200	.9030	-4.2109	-.0291

Independent Samples Effect Sizes

		Standardizer[a]	Point Estimate	95% Confidence Interval Lower	Upper
Total_phenols	Cohen's d	1.4278	-1.485	-2.882	-.020
	Hedges' correction	1.5816	-1.340	-2.602	-.018
	Glass's delta	1.3134	-1.614	-3.206	.079

a. The denominator used in estimating the effect sizes.
Cohen's d uses the pooled standard deviation.
Hedges' correction uses the pooled standard deviation, plus a correction factor.
Glass's delta uses the sample standard deviation of the control group.

Day 7

Oneway

ANOVA

weight_gain

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.047	2	.024	123.799	<.001
Within Groups	.005	27	.000		
Total	.052	29			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: weight_gain

	(I) treatment	(J) treatment	Mean Difference (I–J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Bonferroni	No_prophylaxisis	coccidiostat_ESB3_prophylaxis	-.0524400*	.0061676	<.001	-.068182	-.036698
		myrothamnus_flabellous_treatment	-.0969400*	.0061676	<.001	-.112682	-.081198
	coccidiostat_ESB3_prophylaxis	No_prophylaxisis	.0524400*	.0061676	<.001	.036698	.068182
		myrothamnus_flabellous_treatment	-.0445000*	.0061676	<.001	-.060242	-.028758
	myrothamnus_flabellous_treatment	No_prophylaxisis	.0969400*	.0061676	<.001	.081198	.112682
		coccidiostat_ESB3_prophylaxis	.0445000*	.0061676	<.001	.028758	.060242
Dunnett T3	No_prophylaxisis	coccidiostat_ESB3_prophylaxis	-.0524400*	.0047503	<.001	-.064892	-.039988
		myrothamnus_flabellous_treatment	-.0969400*	.0066803	<.001	-.114938	-.078942
	coccidiostat_ESB3_prophylaxis	No_prophylaxisis	.0524400*	.0047503	<.001	.039988	.064892
		myrothamnus_flabellous_treatment	-.0445000*	.0068502	<.001	-.062820	-.026180
	myrothamnus_flabellous_treatment	No_prophylaxisis	.0969400*	.0066803	<.001	.078942	.114938
		coccidiostat_ESB3_prophylaxis	.0445000*	.0068502	<.001	.026180	.062820

*. The mean difference is significant at the 0.05 level.

Day 14

ANOVA					
weight gain day 14					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.087	2	.044	321.087	<.001
Within Groups	.004	27	.000		
Total	.091	29			

Multiple Comparisons

Dependent Variable: weight_gain_day_14

	(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Tukey HSD	no prophylaxis	coccidostat_esb3_prophylaxis	-.068300*	.005218	<.001	-.08124	-.05536
		Myrothamus_flabellifolius_treatment	-.132200*	.005218	<.001	-.14514	-.11926
	coccidostat_esb3_prophylaxis	no prophylaxis	.068300*	.005218	<.001	.05536	.08124
		Myrothamus_flabellifolius_treatment	-.063900*	.005218	<.001	-.07684	-.05096
	Myrothamus_flabellifolius_treatment	no prophylaxis	.132200*	.005218	<.001	.11926	.14514
		coccidostat_esb3_prophylaxis	.063900*	.005218	<.001	.05096	.07684
Bonferroni	no prophylaxis	coccidostat_esb3_prophylaxis	-.068300*	.005218	<.001	-.08162	-.05498
		Myrothamus_flabellifolius_treatment	-.132200*	.005218	<.001	-.14552	-.11888
	coccidostat_esb3_prophylaxis	no prophylaxis	.068300*	.005218	<.001	.05498	.08162
		Myrothamus_flabellifolius_treatment	-.063900*	.005218	<.001	-.07722	-.05058
	Myrothamus_flabellifolius_treatment	no prophylaxis	.132200*	.005218	<.001	.11888	.14552
		coccidostat_esb3_prophylaxis	.063900*	.005218	<.001	.05058	.07722

*. The mean difference is significant at the 0.05 level.

Day 21

Oneway

ANOVA

weight_gain_day_21

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.178	2	.089	292.596	<.001
Within Groups	.008	27	.000		
Total	.186	29			

ANOVA Effect Sizes[a]

		Point Estimate	95% Confidence Interval Lower	95% Confidence Interval Upper
weight_gain_day_21	Eta-squared	.956	.910	.969
	Epsilon-squared	.953	.903	.967
	Omega-squared Fixed-effect	.951	.900	.966
	Omega-squared Random-effect	.907	.819	.934

a. Eta-squared and Epsilon-squared are estimated based on the fixed-effect model.

Multiple Comparisons

Dependent Variable: weight_gain_day_21
Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
no prophylaxis	coccidostat_esb3_prophylaxis	-.125100*	.007794	<.001	-.14442	-.10578
	Myrothamus_flabellifolius_treatment	-.184700*	.007794	<.001	-.20402	-.16538
coccidostat_esb3_prophylaxis	no prophylaxis	.125100*	.007794	<.001	.10578	.14442
	Myrothamus_flabellifolius_treatment	-.059600*	.007794	<.001	-.07892	-.04028
Myrothamus_flabellifolius_treatment	no prophylaxis	.184700*	.007794	<.001	.16538	.20402
	coccidostat_esb3_prophylaxis	.059600*	.007794	<.001	.04028	.07892

*. The mean difference is significant at the 0.05 level.

Day 28

Oneway

ANOVA

weight_gain_day_28

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.739	2	3.870	1.284	.293
Within Groups	81.397	27	3.015		
Total	89.137	29			

ANOVA Effect Sizes[a,b]

		Point Estimate	95% Confidence Interval	
			Lower	Upper
weight_gain_day_28	Eta-squared	.087	.000	.276
	Epsilon-squared	.019	-.074	.222
	Omega-squared Fixed-effect	.019	-.071	.216
	Omega-squared Random-effect	.009	-.034	.121

a. Eta-squared and Epsilon-squared are estimated based on the fixed-effect model.

b. Negative but less biased estimates are retained, not rounded to zero.

Day 35

Oneway

ANOVA

weight_gain_day_35

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.302	2	.151	902.705	<.001
Within Groups	.005	27	.000		
Total	.306	29			

ANOVA Effect Sizes[a]

		Point Estimate	95% Confidence Interval	
			Lower	Upper
weight_gain_day_35	Eta-squared	.985	.970	.990
	Epsilon-squared	.984	.968	.989
	Omega-squared Fixed-effect	.984	.966	.989
	Omega-squared Random-effect	.968	.935	.978

a. Eta-squared and Epsilon-squared are estimated based on the fixed-effect model.

Post Hoc Tests

Multiple Comparisons

Dependent Variable: weight_gain_day_35
Tukey HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
no prophylaxis	coccidostat_esb3_prophylaxis	-.185800*	.005784	<.001	-.20014	-.17146
	Myrothamus_flabellifolius_treatment	-.232200*	.005784	<.001	-.24654	-.21786
coccidostat_esb3_prophylaxis	no prophylaxis	.185800*	.005784	<.001	.17146	.20014
	Myrothamus_flabellifolius_treatment	-.046400*	.005784	<.001	-.06074	-.03206
Myrothamus_flabellifolius_treatment	no prophylaxis	.232200*	.005784	<.001	.21786	.24654
	coccidostat_esb3_prophylaxis	.046400*	.005784	<.001	.03206	.06074

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

weight_gain_day_35

Tukey HSD[a]

treatment	N	Subset for alpha = 0.05		
		1	2	3
no prophylaxis	10	.86880		
coccidostat_esb3_prophylaxis	10		1.05460	
Myrothamus_flabellifolius_treatment	10			1.10100
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 10.000.

Day 42

Oneway

ANOVA

weight_gain_day_42

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.356	2	.178	1688.073	<.001
Within Groups	.003	27	.000		
Total	.359	29			

ANOVA Effect Sizes[a]

		Point Estimate	95% Confidence Interval	
			Lower	Upper
weight_gain_day_42	Eta-squared	.992	.984	.994
	Epsilon-squared	.991	.982	.994
	Omega-squared Fixed-effect	.991	.982	.994
	Omega-squared Random-effect	.983	.964	.988

a. Eta-squared and Epsilon-squared are estimated based on the fixed-effect model.