Bindura University of Science Education

Faculty of Science

Biological Sciences Department

Potential use of *Ziziphus mauritiana* (Indian jujube) extracts as natural preservatives in yoghurt

By

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A research project submitted in partial fulfilment of the requirements for the Bachelor of Science Honours Degree in Biotechnology.

June 2026

Approval form

The undersigned certify that have read the dissertation titled "Potential use of *Ziziphus mauritiana* (Indian jujube) extracts as natural preservatives in yoghurt and confirm that it is suitable for submission to the Biological Sciences Department, Faculty of Science and Engineering, for assessment.



Signature of student: Date: 18/06/26

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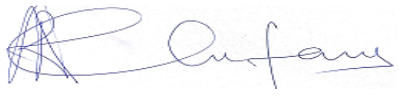
Signature:  Date: 19/06/2026

Dedication

To my family and friends. I cherish you all.

Acknowledgements

I would like to express my sincere gratitude to my supervisor, Mr. Alufasi for his guidance and encouragement throughout this project. I would also like to thank my family for their financial support, during the duration of the project and above all, I would like to thank the Lord almighty.

List of abbreviations

- CFU - Colony Forming Unit
- GRAS - Generally recognized As Safe
- HE - Hydroethanolic extracts
- MIC - Minimum Inhibitory Concentration
- NP - Natural Preservative
- PDA - Potato Dextrose Agar
- SD - Standard Deviation
- SP - Synthetic Preservative
- TNTC - Too Numerous To Count
- v/v - Volume per Volume

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Abstract

Growing consumer preference for natural food preservatives has driven research into plant-derived antimicrobial agents as alternatives to synthetic preservatives. This study evaluated the antimicrobial efficacy of *Ziziphus mauritiana* fruit extracts against yoghurt spoilage yeasts and molds and assessed their potential application in yoghurt. Aqueous, 50% hydroethanolic and 80% hydroethanolic extracts were prepared and tested for antimicrobial activity using the agar well diffusion method. The minimum inhibitory concentration (MIC) was determined using the broth microdilution technique. The aqueous extract was incorporated into the yoghurt samples, and compared with potassium sorbate and untreated control samples, over an accelerated storage period of 15 days. Lactic acid development, pH changes, yeast and mold count and sensory characteristics were monitored during this period. Findings indicated that all extracts exhibited antimicrobial activity against yeasts and molds, with extract type and concentration having a significant effect and interaction ($p < 0.05$). The 80% hydroethanolic extract displayed the highest antimicrobial activity, producing a maximum inhibition zone of 16.33 $\pm$ 1.15 mm at 100 % concentration, while the aqueous extract showed the lowest activity. The modal MIC value was 200 mg/mL and 18 ml of extract were added to 90 ml yoghurt samples. During the storage period, samples with *Z. mauritiana* extracts displayed reduced microbial growth, slower pH decline and lower lactic acid development compared to the untreated control. Sensory evaluation showed that yoghurt containing the plant extract maintained acceptable taste, texture, colour and consistency for a longer period than the control, however, potassium sorbate remained the most effective preservative, during the same storage period. Overall, the study concludes that *Z. mauritiana* extracts possess antifungal properties that can contribute to the preservation of yoghurt by inhibiting spoilage microorganisms and slowing physicochemical deterioration. Further research is recommended, to optimize extraction methods and application concentrations for improved preservative efficacy.

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Chapter 1

Introduction

1.0 Introduction

This chapter provides an overview of the study on the antimicrobial potential of *Z. mauritiana* fruits extracts and their potential application in yoghurt preservation. It highlights the importance of yoghurt preservation and the growing interest in natural plant-based alternatives to synthetic food preservatives. The chapter subsequently defines the research problem, aim, objectives, hypothesis, significance of the study, and the scope and delimitations of the research. These elements collectively establish the foundation and justification of the evaluation of *Z. mauritiana* as a potential natural preservative for inhibiting spoilage yeasts and molds in yoghurt and extending the product shelf-life.

1.1 Background to the study

Yoghurt is a highly sought-after and widely consumed fermented dairy product, due to its nutritional value, pleasant sensory characteristics and probiotic benefits. It is produced through the fermentation of milk by lactic acid bacteria, namely *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (Kwon et al., 2021). However, despite its acidic nature, it remains highly susceptible to microbial spoilage during storage, particularly by yeasts and molds. These spoilage microorganisms can survive and proliferate under acidic conditions and this results in undesirable changes in flavour, texture, appearance and overall product quality, and this consequently affects the yoghurt shelf life

In order to control spoilage and extend shelf life, commercial manufacturers utilize synthetic preservatives such as potassium sorbate and sodium benzoate because they can effectively inhibit the growth of yeasts and molds in dairy products, (Fandos & Dominguez, 2007; Karabulut et al., 2001; Li al., 2014). Although these preservatives are effective in inhibiting microbial spoilage, growing concerns regarding their long-term consumption, partnered with a demand for clean-label preservative-free foods, has driven research into alternatives (Kranjc, 2025). Numerous studies

have examined the effects of excessive consumption of these synthetic preservatives, and some results confirmed that the excessive consumption of artificial food additives is associated with gastrointestinal, respiratory, dermatological, and neurological side effects (Carocho et al., 2014; Randhawa & Bahna, 2009; Wilson & Bahna, 2005). Although viewed as secure, efficient and demonstrating reduced toxicity compared to alternative preservatives by Fandos & Dominguez, (2007); Karabulut et al., (2005) and Karabulut et al., (2001), other authors believe that the application of these preservatives negatively impacts human health (Kamankesh, Mohammadi, Tehrani, Ferdowsi, & Hosseini, 2013). Specifically, several instances of allergic reactions have been reported, including hives and asthma (Goren et al., 2015; Tfouni & Toledo, 2002). This literature has collectively stimulated interest in natural preservation systems.

Plant extracts have emerged as promising alternatives because they contain bioactive compounds such as phenolics, flavonoids, tannins and alkaloids that possess antimicrobial and antioxidant properties, (Cubero-Román et al., 2023; Javed et al., 2022). *Ziziphus. mauritiana*, locally known as masau is one such plant. It is a small, red-brown tropical fruit, native to southern Africa and popular in Zimbabwe. It is an important food source and income generator, particularly in rural areas and has also been utilized in traditional medicine, for treating various ailments. The different parts of the plant, such as the fruit, bark, roots and leaves, have been reported to contain phytochemicals with antioxidants, antimicrobial, anti-inflammatory and therapeutic properties. (Salamatou & Bayoï, 2024). Numerous studies have investigated the medicinal and antimicrobial properties of *Z. mauritiana*, however, limited information exists regarding its effectiveness against yoghurt spoilage yeasts and molds and its practical application in yoghurt preservation.

This study, therefore, aimed to evaluate the antimicrobial efficacy of the *Z. mauritiana* fruit extracts, against yoghurt spoilage yeasts and molds. Furthermore, the study investigated the potential application of the extracts as natural preservatives in yoghurt, by assessing their effects on microbial stability, physicochemical properties, sensory characteristics and shelf-life under accelerated storage conditions, that is, higher temperatures of 30^0-35^0 degrees Celcius.

1.2 Statement of the problem

The use of synthetic preservatives in yoghurt production has raised growing concerns regarding potential adverse health effects and consumer acceptance. On the other hand, yoghurt remains

highly susceptible to spoilage by yeasts and molds. Although plant extracts have demonstrated antimicrobial properties in various food systems, there is limited information regarding the effectiveness of *Ziziphus mauritiana* extracts as preservatives in yoghurt. There is therefore, a need to evaluate the antimicrobial activity of *Z. mauritiana* extracts and determine their potential to improve the microbiological stability and shelf-life of yoghurt.

1.3 Purpose of the study

1.3.1 Aim

The aim of this study was to evaluate the antimicrobial activity of *Ziziphus mauritiana* extracts and determine their effectiveness as natural preservatives for extending the shelf life of yoghurt.

1.3.2 Specific objectives

- To determine the antimicrobial activity of aqueous and hydroethanolic extracts of *Ziziphus mauritiana* against yoghurt spoilage yeasts and molds.
- To determine minimum inhibitory concentrations (MICs) of the extracts against spoilage microorganisms.
- To evaluate the effect of the extracts on yoghurt shelf life under accelerated storage conditions
- To compare the preservative effectiveness of the natural extract with potassium sorbate.

1.4 Research questions

- Do *Z. mauritiana* extracts possess antimicrobial activity against yoghurt spoilage yeasts and molds?
- What is the minimum inhibitory concentration of the extracts against spoilage microorganisms?
- Can *Z. mauritiana* extracts improve the microbiological stability of yoghurt during storage?
- How does the effectiveness of the *Z. mauritiana* extracts compare with that of potassium sorbate?

1.5 Hypotheses

Ziziphus mauritiana extracts have a preservative effect in yoghurt

1.6 Delimitations

The study was limited to the evaluation of *Z. mauritiana* fruit extracts only, as a natural preservative in yoghurt. Furthermore, only yeasts and molds isolated from yoghurt were considered in the antimicrobial assessment. Bacterial spoilage organisms and pathogenic microorganisms were not investigated. The study was also conducted using yoghurt samples prepared under laboratory conditions, and may not fully represent large or commercial-scale production systems. The study was conducted under accelerated shelf-life conditions, and therefore the long-term preservative effects in real time, were not evaluated. The phytochemical characterization and identification of the specific bioactive actives in the extracts were beyond the scope of the study.

1.7 Limitations

The study focused mainly on spoilage yeasts and molds. Other microorganisms that may influence the yoghurt quality and safety were not investigated, hence the antimicrobial activity observed cannot be generalized to all microorganism associated with yoghurt. The study evaluated selected extraction methods and treatment conditions only. Other extraction techniques, solvent systems, extract concentrations and formulation approaches were not investigated and may produce different antimicrobial effects.

1.8 Significance of the study

This research aimed to provide a reliable solution to the rising concerns about the implications of using synthetic and chemical preservations. Furthermore, it leverages an abundant local natural resource, to create a sustainable cost-effective bio-preservative, which ensures sustainable consumption and production patterns, thus reducing reliance on synthetic preservatives and extending shelf-life. The utilization of locally sourced *Z. mauritiana* supports the Education 5.0

agenda, which warrants heritage-based learning, and the use of locally sourced materials for problem solving and product development.

1.9 Definition of terms

- Natural preservative

A naturally occurring substance derived from plants, animals or microorganisms that inhibits microbial growth and prolongs food shelf life

- Synthetic preservative

A chemically manufactured compound added to food products to inhibit and delay spoilage.

- Minimum inhibitory concentration (MIC)

Lowest concentration of antimicrobial agent prevents visible growth of a microorganism under specified conditions

- Shelf life

The period during which a food product remains safe, acceptable and maintains its desired quality characteristics under specified conditions

- Antimicrobial activity

The ability of a substance to inhibit the growth or destroy microorganisms such as bacteria, yeasts and molds.

- Accelerated shelf-life testing

A method used to predict product stability by storing samples under elevated environmental conditions to increase the rate of deterioration

1.10 Study Synopsis

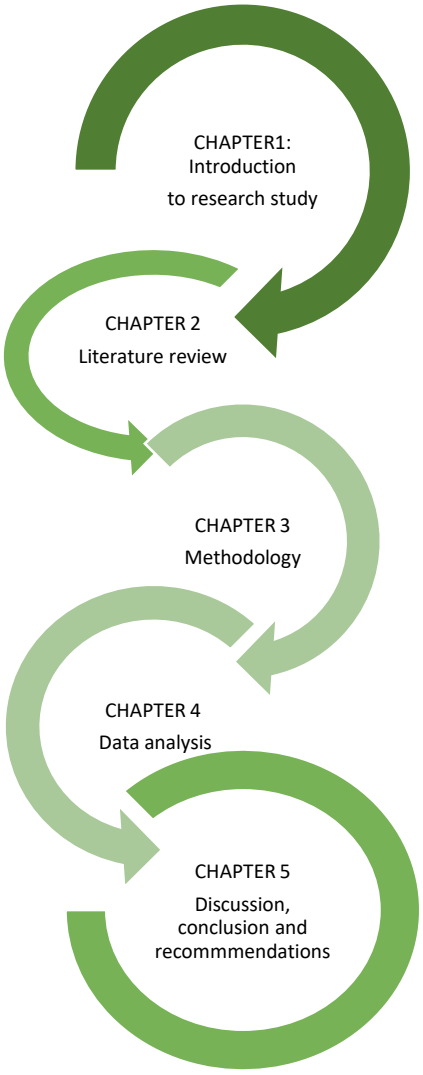

Figure 1. Study synopsis

1.11 Chapter summary

The chapter served as an introduction to the study. The study includes background information, problem statement, purpose, significance, research questions, limits, and scope. In addition, it describes the study's reasoning and motivation. The definitions of operational concepts and essential words are addressed. The second chapter examines the theoretical framework and provides a survey and review of related literature.

Chapter 2
Literature review

2.0 Introduction

A growing body of literature suggests that the modern food industry is undergoing a significant transformation driven by consumer demand for minimally processed, safe, and "clean-label" foods. Chemical preservatives such as sorbates, benzoates and natamycin, although effective, have faced growing consumer resistance, due to a rise in potential health concerns, resulting in consumer preference for natural alternatives. As a result, there has been lot of research into the development of natural preservatives. This chapter examines and reviews the existing literature on the development of natural preservatives, with the main focus on the potential of plant-derived extracts. It highlights their antimicrobial activity and effectiveness against potentially harmful microbiota, particularly food spoilage microorganisms, such as bacteria, yeasts and molds. In addition to that, this chapter evaluates the phytochemistry, antimicrobial activity, and the potential application of *Z. mauritiana* extracts, a locally sourced fruit, as natural preservatives in food.

2.1 History and definition of food preservation

Preservation can be defined as the practice of preventing food spoilage and contamination, by destroying or impeding the growth of spoilage microorganisms, slow oxidation and chemical reactions that lead to food decay. Historically, there have been many methods employed for the preservation of various foods. These methods include smoking, fermentation, salting, pasteurization and the use of chemical preservatives. Ariyamuthu et al. (2022) defines chemical preservatives as synthetic compounds that inhibit the growth of spoilage microorganisms, without essentially killing them, and further classifies them into two groups, namely:

1. Organic chemical preservatives such as sorbates, benzoates and acids
2. Inorganic chemical preservatives such as nitrites, sulphites and phosphates.

However, the chemical preservatives relevant to the undertaken topic of research are potassium sorbate and sodium benzoate, which are incorporated in commercial yoghurts.

2.2 Overview of yoghurt

Yogurt is a fermented dairy product, produced by the action of lactic acid bacteria, namely *Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptococcus thermophilus* (Kwon et al., 2021) and can be produced from various types of milk, including those from cow, goat, and plant sources These starter cultures ferment lactose to lactic acid, reducing pH and creating an environment that inhibits the growth of many spoilage and pathogenic microorganisms. Furthermore, these starter cultures contribute to the distinct yoghurt flavour, texture, nutritional value and digestibility (Tamine and Robinson, 2007). However, despite its acidic nature, yoghurt remains susceptible to spoilage by yeasts, molds, and post-processing contaminants during storage, and this affects its shelf-life. It is this limitation that necessitates additional preservation strategies to ensure safety and prolonged shelf-life by commercial manufacturers.

2.3 Negative impacts of chemical preservatives

Chemical preservatives have traditionally been used to inhibit microbial growth in food products, and extend their shelf-life. However, their application in food production is limited due to regulatory restrictions and rising consumer concerns. Sodium benzoate is a sodium salt which is commonly used as a chemical preservative in foods and it is found mainly in industrialized foods such as beverages and yoghurts. Sodium benzoate is considered safe by measurability agencies however there is still controversy over its effect on human health. Many effects like food allergy, food intolerance, cancer, multiple sclerosis, attention deficit, hyperactivity disorder, brain damage, nausea, cardiac disease among others have been reported. (Yadav & Gupta, 2021). Similarly, Carocho & Ferreira, (2013) and Singh & Singh, (2019) found many studies that have confirmed that the excessive consumption of synthetic food additives is related with gastrointestinal, respiratory and dermatological adverse reactions. Additionally, studies by Gören et al., (2015) and Hannuksela & Haahtela, (1987) highlighted cases of allergic reactions such as urticaria, asthma and cases of intolerance. Overall, the literature presents the problem that has resulted in a shift of research toward natural preservation systems that align with consumer expectations and sustainable food production practices.

2.4 Plant extracts, an attractive alternative

Plants have been used as medicine and preservatives as well as food flavourings, since ancient times. They can be used either fresh or dried and their utilisation encompasses various plant components such, roots, leaves, flowers, seeds, peels, tubers, or fruits. Plants have an abundance of secondary components namely; alkaloids, organosulfur compound, glycosides, flavonoids, tannins, phenolics, colorants, and resins (El-Aziz et al., 2023). Numerous studies have been carried out on these plant extracts and the findings demonstrate the antimicrobial and antioxidant activity of various plant extracts, and consequently, plant extracts have been proposed as antimicrobial agents and health-promoters to be included in a variety of food products (Silva et al., 2022). Total phenolic contents vary according to plant and extraction solvent, but are significant and have an in vitro correlation with antioxidant activity (Javed et al., 2022).

Ziziphus mauritiana, plant produces a small, red-brown fruit, and is native to the Southern African region and popular in Zimbabwe. It is rich in diverse bioactive compounds, including flavonoids, phenolic acids, tannins, saponins, alkaloids, and glycosides. Recent analytical studies and improved extraction methods have increased yields of polyphenols and antioxidant fractions, which are frequently the fractions with the strongest antibacterial properties.

2.5 Antimicrobial Activity of *Ziziphus mauritiana*

Numerous in vitro studies have demonstrated the antimicrobial efficacy of *Z. mauritiana* extracts against a broad spectrum of microorganisms, including Gram-positive bacteria, Gram-negative bacteria, and some fungi, such as *Sporocadaceae* (Akhtar et al., 2025), *Candida krusei* (Al-Bahrani et al., 2018) and *Mucor circinelloides* (Jangid & Begum, 2022). A study carried out by Salamatou & Bayoï (2024), found that *Z. mauritiana* extracts from the bark, leaves and fruit displayed antimicrobial activity against *S. aureus* and *S. epidermis.* Reported inhibition varies by extraction method, plant part, and target organism However, many studies reported stronger activity from methanolic or hydroalcoholic extracts and from bark or leaf extracts compared with fruit pulp (Asimuddin et al., 2020). The antimicrobial mechanisms are attributed primarily to phenolic compounds that disrupt cell membranes, interfere with enzyme systems, and cause leakage of

cellular contents. These properties suggest that *Z. mauritiana* extracts could function as natural antimicrobial agents in food systems.

2.6 Application of Plant Extracts in Yoghurt Systems

The incorporation of plant extracts into yoghurt has been widely studied as a strategy to improve functional value and shelf-life. In studies conducted by (Akan et al., 2022; Caleja et al., 2016; Jameel & Mohammed, 2021), plant extracts from various sources were added to yoghurts, and the findings suggested that they exhibited antimicrobial and antioxidant activity. Furthermore, other authors even noted an improved palatability and nutritional composition, and an extended shelf-life. However, some studies carried out using herbal and fruit extracts have reported both positive and negative effects depending on extract concentration. Similarly, findings by Lemoni et al. (2025), indicate that the sensory effects may restrict the use of plant extracts at concentrations required to achieve microbial inhibition and suggest that further research should be carried out for purposes of standardisation and reproducibility. Therefore, optimisation is critical when formulating plant-extract-enriched yoghurt. The viability of incorporating *Z. mauritiana* extracts into yoghurt matrices is demonstrated by a 2021 stirred-yoghurt study that used fruit extract specifically for *Z. mauritiana* and found satisfactory polyphenol fortification and acceptable sensory scores at specific addition rates (Preeti, & Poonia, 2021). However, limited studies have evaluated the potential of *Z. mauritiana* in extending the yoghurt shelf-life highlighting the novelty and relevance of this research area.

2.7 Safety and Regulatory Considerations

Although *Z. mauritiana* is widely consumed as a fruit, the use of concentrated extracts in food systems requires rigorous safety assessment. Toxicological studies, allergenicity evaluation, and determination of acceptable daily intake are essential and regulatory approval also requires standardization of extract composition and demonstration of consistent product quality. For industrial application, the plant extracts must comply with food safety regulations, including GRAS or Qualified Presumption of Safety (QPS) status. Compliance with these frameworks is essential for large scale production and use of these plant extracts. Moreover, in order to

demonstrate parity or superiority over chemical preservatives, integrated shelf-life techniques must be tested against relevant spoilage organisms (yeasts, molds, *Bacillus* spp., post-process pollutants) and pathogens under normal cold-chain storage conditions. According to Soutelino et al, (2024), reviews and empirical study reveal that plant extracts can extend the refrigerated shelf-life of dairy, but regulatory procedures and uniform safety testing are crucial stages prior to commercial implementation.

2.8 Research Gaps and Justification of the Study

Despite significant evidence of the antimicrobial properties of plant extracts, limited information exists regarding the application of *Z. mauritiana* extracts as natural preservatives in yoghurt systems. Furthermore, a few studies have evaluated their effectiveness against spoilage yeasts and molds under accelerated shelf-life conditions or compared their preservative performance with conventional preservatives such as potassium sorbate. This study therefore seeks to bridge this knowledge gap, by investigating the antimicrobial activity of *Z. mauritiana* extracts and evaluating their potential use as natural preservatives in yoghurt.

2.9 Chapter summary

The chapter reviewed the relevant literature on food preservation, with a focus on growing shift from the use of synthetic preservatives to natural preservatives, driven by customer demand for safer food. It goes on to discuss the potential of plant extracts, and examines studies that have been carried out, to determine the phytochemical, and antimicrobial properties of these extracts. In addition to this, the chapter examines studies on the antimicrobial and antioxidant properties of *Z. mauritiana* extracts, and establishes the foundation of this particular research. Furthermore, it analyses relevant literature on the application of plant extracts in yoghurt systems, and identifies research gaps regarding the optimization of plant extract concentration, mechanisms of action and shelf-life performance in yoghurt. However, it also emphasises the importance of safety and regulatory considerations for the use of plant extracts in food systems.

Chapter 3
Materials and methods

3.0 Introduction

This chapter outlines the materials, experimental procedures and analytical methods used in the evaluation of the antimicrobial properties of *Z. mauritiana* and their effectiveness in extending shelf-life of yoghurt under accelerated storage conditions. It integrates the microbiological, analytical and food processing techniques to assess both the antimicrobial activity of the plant extracts against selected spoilage microorganisms (yeasts and molds) and their practical application as natural preservatives in the yoghurt.

The methodology was designed in two phases. The first phase involved the preparation of aqueous and hydro-ethanolic extracts for the *Z. mauritiana* fruit, followed the determination of their antimicrobial efficacy, using standardized *in vitro* assays, including minimum inhibitory concentration (MIC) testing. The second phase centred on the incorporation of selected extract concentrations into yoghurt. The approach reproduced extended storage conditions over a shorter period by inducing controlled environmental stress factors such as elevated temperatures. In addition to this, the chapter outlines the statistical methods used to analyse the data, to ensure the validity of the obtained results.

3.1 Study area

The research study was conducted at the Bindura University of Science Education biological science laboratory and the food production site.

3.2 Experimental design

3.2.1 Preparation of extracts

Dried *Z. mauritiana* fruits were sampled from the university storage rooms and washed using running water to remove dirt and debris. The fruits were allowed to dry and then ground using a blender to obtain a fine powder. The powder was then autoclaved at 121 degrees for 15 minutes, in order to eliminate contaminating micro-organisms that could interfere with subsequent

antimicrobial assays and shelf-life analysis. Extraction was carried out using three solvent systems as follows:

i. Aqueous extraction: 50 grams of the powder were soaked in 500mls of distilled water
ii. 50% hydro-ethanolic extraction: 50 grams of the powder were soaked in 500mls of 50 % ethanol solution.
iii. 80 % hydro-ethanolic extraction: 50 grams of the powder were soaked in 500mls of 80% ethanol solution.

The mixtures were agitated and left to stand for 24hrs at room temperature. The mixtures were then filtered through Whatman paper. Each filtrate was poured into a sterile beaker and placed in the laminar flow cabinet, where the solvents were evaporated over a period of 5 days, and a thick paste of crude extract was obtained. The thick pastes were mixed with 1ml of the respective solvents used during the solvent extraction, in order to reduce viscosity and enable re-application of the extract.

3.2.2 Antimicrobial susceptibility testing

The yeasts and molds were isolated from a spoilt yoghurt sample and cultured on Potato Dextrose Agar (PDA), in an incubator at 25 ºC for a period of 5 days and this was followed by microbial suspension preparation, using saline solution. The microbial suspension was prepared by aseptically transferring isolated yeast and mold colonies into test tubes containing 5mL of sterile saline solution using an inoculating loop. The suspension was shaken thoroughly to obtain a uniform distribution of microbial cells, and the solutions were standardised to 0.5 McFarland turbidity by either adding more microbial isolates or sterile solution as required to ensure uniform microbial concentration. For antimicrobial assessment, agar well diffusion technique was employed. PDA was poured into petri dishes and left to solidify. Subsequently, the inoculum was poured over the agar and spread using a glass rod. The wells were then punched into the solidified agar, and filled with varying concentrations of the test extracts (100 %, 75 %, 50 % and 25 %). The plates were incubated at 25° C for 5 days, and the zones of inhibition were measured, using a ruler. All tests were done in triplicate, for reproducibility.

3.3 Minimum inhibitory Concentration

The MIC was determined using the broth microdilution method in 96-well plates. A stock solution of aqueous *Z. mauritiana* extract (400mg/mL) was prepared and subjected to two-fold serial dilution to obtain concentrations of 200,100,50,25,12.5, 6.25 and 3.125 mg/mL, and this was followed by inoculation with standardised microbial cultures. The plates were incubated at 25°C for 72hrs, and microbial growth was viewed visually. The MIC was recorded as the lowest concentration showing no visible growth. Replicate MIC values were obtained and the mean and modal values were calculated. Eighteen millilitres (18mLs) of the aqueous extract was incorporated into the yoghurt samples, based on the modal MIC value 200mg/mL.

3.4 Yoghurt Fortification

The selected extraction, of 200 mg/mL, was incorporated into yoghurt samples, each packed in 90ml samples. The yoghurt was divided into

i. Positive control – yoghurt with potassium sorbate (synthetic preservative)
ii. Negative control- yoghurt without any preservative
iii. Treatment- yoghurt with treatments from aqueous extracts. In order to comply with food safety guidelines, only aqueous extracts were added to the yoghurt

The fruit extracts were added to the yoghurt samples, as pastes, as obtained from the solvent extraction process. However, due to a lack of proper solvent evaporation facilities, only aqueous extracts were incorporated into the yoghurt samples.

3.5 Accelerated shelf-life testing

The yoghurt samples were subjected to accelerated shelf-life testing, by storing them at elevated temperatures, in order to simulate extended storage conditions. The samples were monitored over a period of 15 days, for lactic acid development, pH changes, physical and sensory characteristics and microbial growth. The observations were recorded at 3day intervals.

3.6.0 Biochemical Analysis

3.6.1 Titratable acidity

Titratable acidity was determined according to standard analytical procedures. Ten grams of yoghurt were weighed in a conical flask and diluted with 20mls of distilled water. Three drops of phenolphthalein indicator were added and the mixture was titrated against 0.1M sodium hydroxide (NaOH) until a faint pink endpoint persisted for 30 seconds. The volume of NaOH used was recorded and titratable acidity was calculated as percentage lactic acid using the standard conversion factor as follows:

Percentage lactic acid = (V×N×M / W), where

V= Volume of NaOH used (mL)
N= Normality of NaOH
M=Mol weight of Lactic acid
W= Weight of yoghurt sample

3.6.2 pH changes

The pH was measured using a bench-top pH meter. Results were expressed as mean + standard deviation.

3.7 Microbiological Analysis

Yeasts and mold counts were determined using the spread plate technique. Ten millilitres (10mL) of yogurt was aseptically transferred into 90mls of sterile saline solution. This was followed by preparation of serial 10-fold dilutions. One millilitre (1 mL) of the solutions were spread onto PDA plates and incubated at 25-28°C for 5 days. Plates containing 30-300 colonies were selected for enumeration and the CFU was determined using a colony counter. The yeast and mold count was calculated as colony-forming units per millilitre (CFU/ mL) and results were expressed as mean $\pm$ standard deviation for duplicates.

3.8 Sensory evaluation

Five sensory evaluators were randomly selected near the area of study. A 5-point hedonic scale was used to evaluate sensory properties in terms of taste, colour, texture and consistency. The values were recorded and expressed as mean $\pm$ standard deviation.

3.9 Data analysis

Data obtained from antimicrobial susceptibility testing, lactic acid development, pH analysis and yeast and mold counts and sensory evaluation were analysed using Microsoft Excel and R studio 4.6.0. Descriptive statistics including means and standard deviations for the antimicrobial susceptibility test (AST), MIC, lactic acid development and pH changes, were calculated from triplicate measurements. Differences in zones of inhibition size, lactic acid development, pH changes and sensory evaluation among treatments and extract concentrations were evaluated using two-way analysis of variance (ANOVA). Statistical significance was accepted at $p < 0.05$. Tukey's HSD post hoc analysis was also done to identify which specific group means differed from each other, for the AST.

Chapter 4
Results

4.1 Introduction

The chapter presents findings from the investigation of the antimicrobial efficacy of aqueous, 50% and 80 % hydro-ethanolic extracts of *Z. mauritiana* against yoghurt spoilage yeasts and molds. The chapter further presents the effects of the extracts on the yoghurt shelf-life under accelerated storage conditions (30-35°C). Parameters evaluated include zones of inhibition, minimum inhibitory concentration (MIC), pH changes, lactic acid development, yeast and mold counts, and comparative storage stability, in addition to sensory evaluation. Data were analysed using descriptive statistics and inferential statistical methods.

4.2 Antimicrobial activity (Zones of Inhibition)

Results show that all three treatments, showed antimicrobial activity against yeasts and molds, in all plates. The zones of inhibition increased with extract concentration, with the 80 % hydro-ethanolic extracts, producing the largest zones of inhibition at all levels tested (Figure 2).

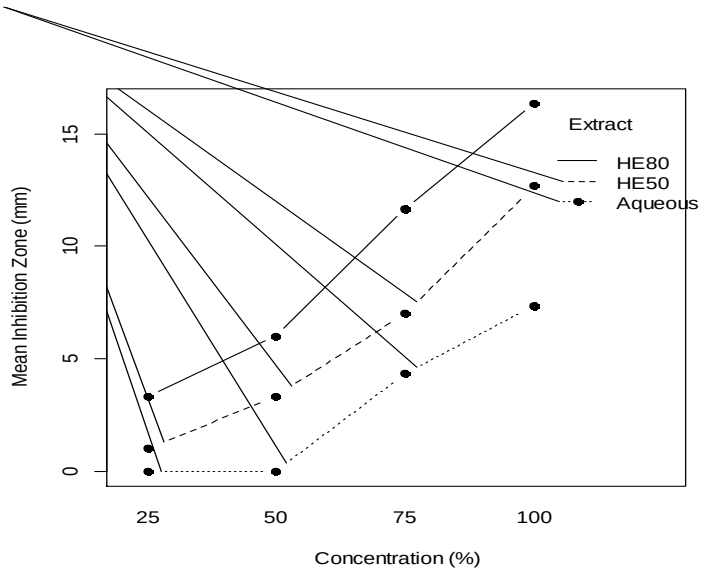

Figure 2 A graph illustrating the effect of extract type and concentration on yeast and mold growth.

Analysis of results shows a concentration-dependent response across all of the extracts. The aqueous extract exhibited limited antimicrobial activity, producing measurable inhibition only at 75% (4.33 $\pm$ 0.58 mm) and 100 % (7.33 $\pm$1.53 mm) concentrations, with no inhibition observed at 50% and 25%. In contrast, both hydroethanolic extracts demonstrated activity at all tested concentrations. The 50% hydroethanolic extract, displayed zones of inhibition ranging from 10.00$\pm$1.00 mm at 25% concentration to 12.67 $\pm$ 1.53 mm at 100% concentration. The 80 % hydroethanolic extract showed the highest overall activity, with mean inhibition zones increasing from 3.33 $\pm$ 0.58 mm at 25% to 16.33 $\pm$ 1.15 mm at 100%. The results suggest that antimicrobial efficacy was influenced by both the solvent type and extract concentration, with hydroethanolic extracts and higher concentrations displaying greater inhibition of yeasts and molds.

A two-way ANOVA with replication was carried out to determine the effects of the extract type and concentration on the zones of inhibition against yeasts and molds. The analysis revealed a statistically significant effect for extract type, ($F(2,24) = 103.47$, $p = 1.59\times10^{-12}$), indicating that the mean zone of inhibition differed significantly across all three extracts. The significant main effect of concentration (($F(3,24) = 173.79$, $p = 2.08 \times 10^{-16}$) indicated that higher extract concentrations produced significantly larger zones of inhibition and this trend was consistent across all extract types. Furthermore, the interaction effect between extract type and concentration was statistically significant ($p=0.0049$), suggesting that the effect of concentration was not uniform across the three extract types. In all cases, the calculated F values exceeded the critical F values, and $p< 0.05$, allowing rejection of the null hypotheses. The results statistically confirm that both the solvent used for extraction and the concentration of the extract significantly influences the antimicrobial efficacy against yeasts and molds. Tukey's HSD post hoc analysis revealed that the 80% hydroethanolic extract exhibited significantly greater antifungal activity than the 50% hydroethanolic and aqueous extracts ($p < 0.05$). Inhibition zones increased significantly with concentration, with the 100% concentration producing the largest zones of inhibition. No significant difference was observed between the 25% and 50% concentrations, indicating limited antifungal activity at lower extract concentrations.

4.3 MIC values

The minimum inhibitory concentration of the extract against yeasts and molds was determined. The results obtained indicate that across the three independent replicates, MIC values were 200mg/mL, 100mg/mL and 200mg/mL, respectively. The statistics indicated a modal MIC value of 200mg/mL with the mean 166.7$\pm$ 57.7 mg/mL. These results demonstrated that the MIC is relatively high. Putting into consideration, the need to maintain sensory acceptability, the extract was incorporated at a practical fortification level of 18 mL per 90mL yoghurt sample (20% v/v) for subsequent preservation trials.

Table 4. 1 MIC results

Modal MIC	Mean MIC		SD
200 mg/mLs	166.7 mg/mLs	$\pm$	57.7mg/mLs

4.4 Sensory evaluation

Sensory evaluation of the yoghurt samples containing natural preservative (NP), synthetic preservative (SP), and a control was conducted at day 0,6 and 12 using a 5 point hedonic scale (table). Mean scores for taste, texture, colour and consistency showed a time-dependent decline, with the rate of deterioration varying between formulations (Figure 3). At day 0, all samples exhibited high acceptability with scores $\geq$ 4.4. By day 6, SP maintained acceptable scores of $\geq$3.6, however in contrast the NP samples showed a substantial decrease in taste (2.6), texture (2.6) and consistency (2.0), although the colour remained high at 4.6. The control sample became unacceptable for taste and texture, recorded as Not Available (N/A), with consistency dropping to 1.8. At day 12, all samples were declared organoleptically unacceptable for taste and texture, with scores for colour and consistency falling to $\leq$ 2.0.

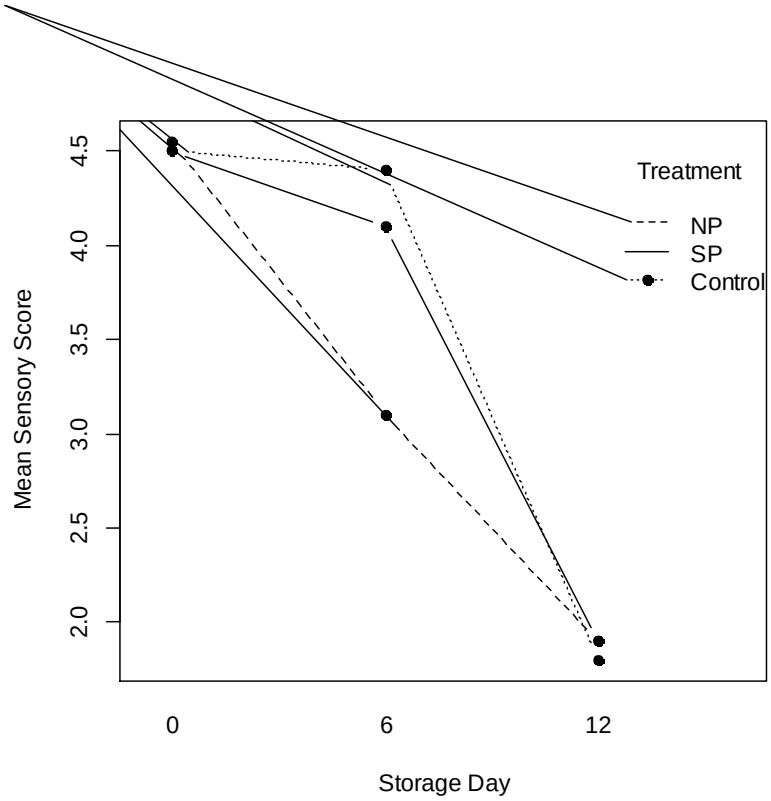

Figure 4 A graph illustrating the sensory evaluation scores during storage

4.5 Lactic Acid development and pH

The development of lactic acid in in the yoghurt samples was monitored during storage. At day 0, all three formulations recorded mean lactic acid concentrations of 0.90%, with no deviation (SD=0.00), indicating uniform starter culture activity. Throughout storage, lactic acid levels increased in all samples, but the rate of accumulation differed between all treatments (figure 4). The control samples exhibited the highest rate of acid development, increasing from 0.90% to 1.96 $\pm$0.004%, by day 12. The NP sample showed an intermediate increase to 1.83 $\pm$ 0.04%, while the SP sample demonstrated the most restricted lactic acid development, at only 1.34$\pm$ 0.06%. The standard deviations remained low across all treatments, indicating good reproducibility between replicates. The results suggest that the SP was more efficient in inhibiting post-acidification, as compared to the NP and control. Consequently, the natural preservative yielded better results in comparison with the control.

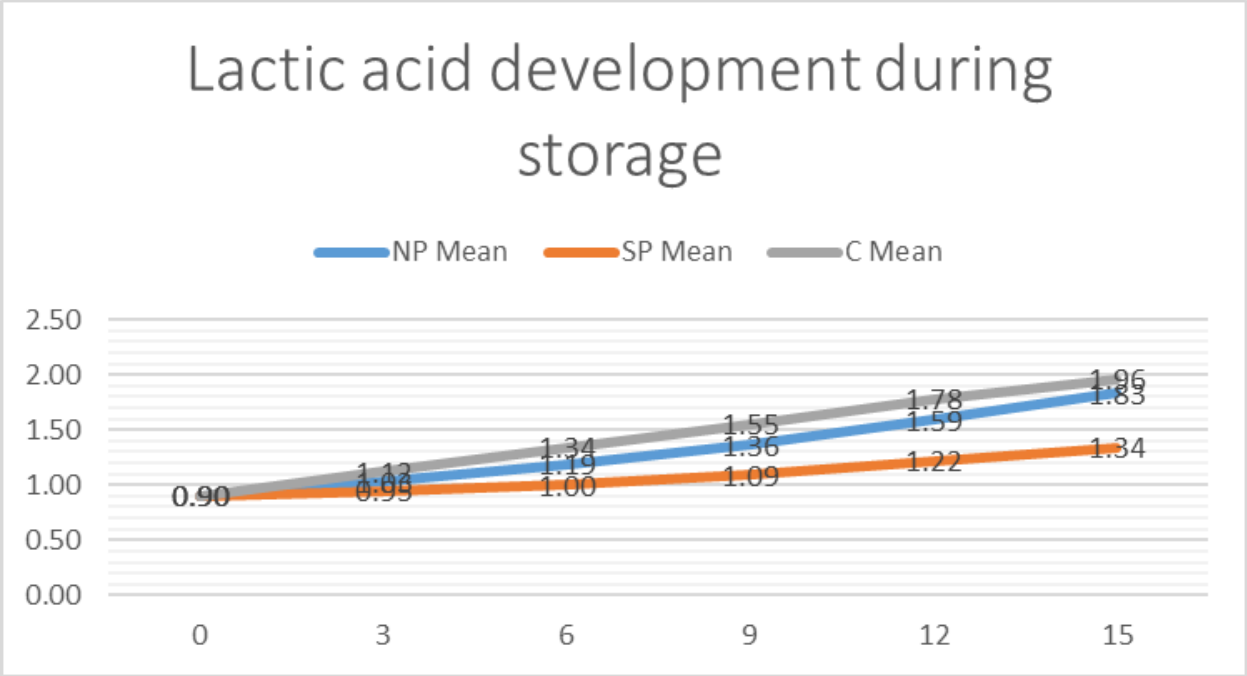

Figure 5 Graph illustrating lactic acid development during storage

4.6 pH Changes During Storage

The pH of the yoghurt samples was monitored during storage. At day 0, all three formulations exhibited a mean of 4.63 $\pm$ 0.00, showing a uniform acidification during the fermentation process. A progressive decrease in pH, however, was observed for all samples over the storage period (Figure 6), which was consistent with post acidification by lactic acid bacteria. The control samples showed the most rapid decline in pH, reaching a final mean of 3.47 $\pm$ 0.03, by day 15. The NP sample exhibited an intermediate rate of decrease to 3.53 $\pm$ 0.02, while the SP had the lowest rate of decrease, with a final value of 4 $\pm$ 0.05, by day 15. The standard deviations were low, indicating good reproducibility between replicates. The observed pH trends correspond inversely with the lactic acid development reported in the above section.

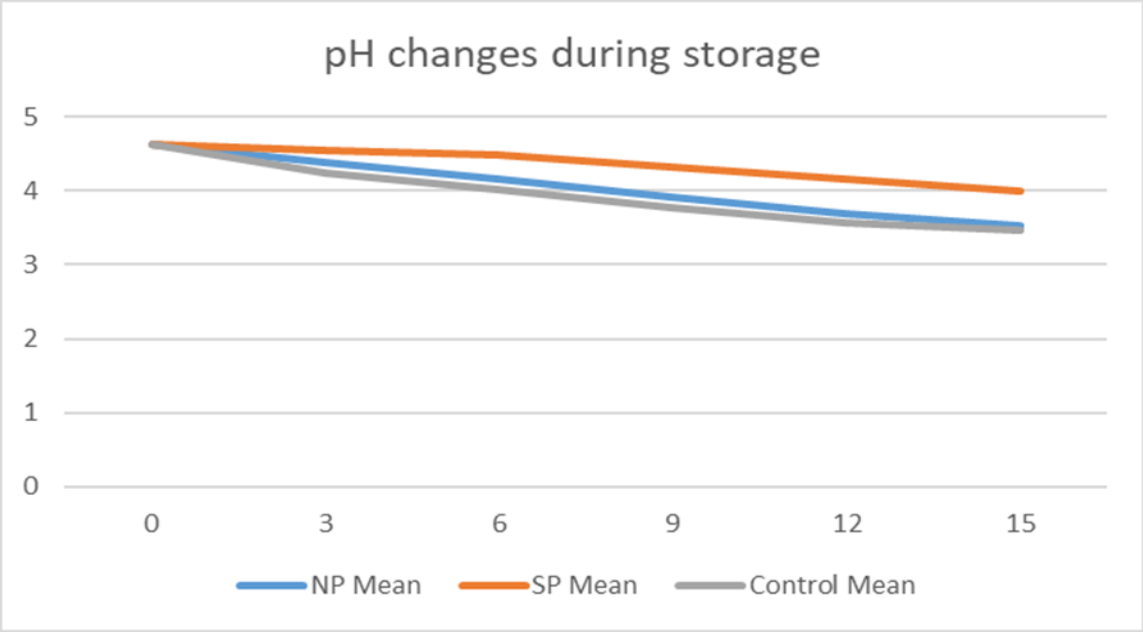

Figure 6 Graph illustrating pH changes during storage

Pearson correlation analysis was performed to examine the relationship between lactic acid percentage and pH. The results indicated a very strong statistically significant negative correlation between the two variables, $r(52) = -0.985$, $p < 0.001$, $n=54$. The coefficient determination of $R^2=0.971$, indicates that 97.1% 0f the variance in pH is explained by lactic acid concentration. This indicates an inverse relationship, that is: As lactic acid increases, pH decreases.

4.6 Yeast and mold count

Yeast and mold counts were enumerated for the yoghurt samples, on day 0 and day 15. Day 0 results were recorded as > 30CFU/mL for all samples (Table 4.3). In contrast after the 15 day storage period, the yeast and mold enumeration yielded different results. The control samples showed extensive fungal proliferation, with both replicates recorded as Too Numerous To Count (TNTC). The NP samples had mean counts of $2.0\times10^2 \pm 0.57\times10^2$ CFU/mL, corresponding to "moderate to high growth". The SP samples demonstrated the greatest inhibition, with a mean count of $1.05\times10^2 \pm 0.07\times10^2$ CFU/mL and observations of moderate growth. The results show that the synthetic preservative was the most effective at suppressing yeast and mold growth, while the NP provided intermediate protection, relative to the control.

Table 4. 2 Shows results from yeast and mold colony counts

Sample	Colony count	Observation
C1	TNTC	Dense fungal growth
C2	TNTC	Confluent growth
NP1	$1.6 * 10^2$	Moderate to high growth
NP2	$2.4 * 10^2$	High fungal load
SP 1	$1.1 * 10^2$	Moderate growth
SP 2	$1.0 *10^2$	Moderate growth

Chapter 5

Discussion, Summary, Recommendations and Conclusions

5.1 Discussion

The present study investigated the antimicrobial activity of aqueous, 50% and 80 % hydroethanolic extracts of *Z. mauritiana* against yoghurt spoilage yeasts and molds, and further evaluated the potential application of aqueous extracts in yoghurt preservation under accelerated shelf -life conditions.

The agar well diffusion results demonstrated that all extracts showed varying degrees of antimicrobial activity, although the effectiveness differed depending on the solvent type and extract concentration. The 80 % hydroethanolic extract consistently produced the largest zones of inhibition, followed by the 50 % hydroethanolic extract, whilst the lowest inhibitory activity was exhibited by the aqueous extract. The superior activity observed in the hydroethanolic extracts maybe attributed to the improved extraction efficiency of bioactive phytochemicals, in the presence of ethanol-water mixtures. Hydroethanolic solvents are known to have a wider range of antimicrobial compounds, including phenolics, flavonoids, tannins and saponins, compared to water alone. The higher inhibition zones observed with the 80 % hydroethanolic extraction, therefore suggest a greater concentration or availability of the active compounds, thus the extract efficacy followed the trend: 80 % hydroethanolic > 50% hydroethanolic >Aqueous

Similarly, a concentration-depended inhibition was observed, which followed the trend:
100% > 75% > 50 % > 25 %. The mean zones of inhibition increased with increasing extract concentration. At 100 % concentration, the 80 % hydroethanolic extract showed the highest inhibitory effect, with the mean inhibition values exceeding those of both the 50 % hydroethanolic and aqueous extracts. Conversely, lower concentrations such as 25%, exhibited minimal inhibition, with no inhibition being observed in the aqueous extract, at the same concentration. This indicates a concentration-dependent antimicrobial effect, with higher concentrations providing a greater suppression of fungal growth. The aqueous extract showed relatively weaker antimicrobial activity, especially at lower concentrations, and this suggests that some of the bioactive compounds

in *Z. mauritiana* are poorly soluble in water alone. Nevertheless, slight inhibition observed at higher aqueous concentrations suggests that water extraction still recovered some active compounds capable of inhibiting spoilage yeasts and molds. The superior efficacy of the hydroethanolic extracts is consistent with observations by Butt et al. (2021) and Javed et al. (2022), which demonstrated that ethanol-water solvent systems yield higher recovery of phenolic compounds and flavonoids from *Z. maurititana.*

The MIC results demonstrated measurable antifungal activity of the aqueous *Z. mauritiana* extract is against spoilage yeasts and molds. The modal value of 200 mg/mL and mean value of 166.7mg/mL $\pm$ 57.7 mg/mL indicates while the extract exhibits antifungal activity, the MIC required to completely inhibit microbial growth is relatively high. The antimicrobial effect may be attributed to phytochemicals such as flavonoids, phenolics and tannins that interfere with microbial cell membrane integrity and metabolic activity, as observed by Salamatou & Bayoï (2024).

The use of 18 mL extract per 90mL yoghurt sample (20% v/v) was selected as a practical fortification level for evaluating the preservative efficacy in the yoghurt. Although the modal MIC of the extract was 200mg/ML under lab conditions, a study by Wang et al. (2024), found that food matrices can reduce the bioavailability and antimicrobial activity of plant-derived compounds, due to interactions with proteins, lipids, carbohydrates and other food constituents. The study highlighted a need to use higher concentrations in food applications, than those indicated by laboratory MIC, and consequently an inclusion level of 20% (v/v) was considered appropriate for incorporation into the yoghurt samples, while maintaining acceptable sensory characteristics.

Sensory evaluation results indicated were generally well accepted on Day 1, with mean taste, texture and consistency scores of (4.6), (4.4), and (4.6) respectively. However, there was a sensory quality declined by Day 7, with taste, texture and consistency scores decreasing to (3.6). Some of the key changes noted were development of a bitter taste in the control samples, and a slightly bitter taste in the NP samples, which was attributed to the development of lactic acid. It was also noted that the samples with the natural preservative, were richer in colour than the other samples, a factor attributed to the colour of the extract paste.

The lactic acid content of the yoghurt samples, increased progressively throughout the storage period, though the rate of increase differed significantly between the different treatments and the control samples. Control samples exhibited the highest increase from 0.90 % to 1.96%, and in contrast, the synthetic preservative samples showed the least increase, from 0.90% to 1.40 %. The sample with natural preservative showed a moderate increase within the first 7 days, which increased rapidly till day 14, with the lactic acid being as high as 1.83%. The development of the lactic acid through fermentation, is responsible for the bitter taste noted in the sensory evaluation. Consequently, lactic acid production and pH change is inversely proportional, as indicated by the correlation coefficient, hence increase in lactic acid leads to a decrease in the pH. The observed pH readings reflect the relationship and correlation between the lactic acid and pH. The progressive increase in lactic acid was not only associated with continued activity of lactic acid bacteria, but also with the growth of yeasts and molds during storage. As spoilage microorganisms proliferate, they interact with the yoghurt microbiota and contribute to the biochemical changes that influence acidity and product stability. The control samples showed the highest lactic acid content likely due to the absence of inhibitory agents, allowing greater microbial activity and spoilage by yeasts and molds. The moderate acid development observed in the NP- treated samples, suggests that the extracts possess antifungal and antimicrobial properties, capable slowing fermentation and acid production. In contrast, the SP-treated samples recorded the lowest increase in lactic acid, indicating the more effective inhibition of spoilage micro-organisms and stabilization of the yoghurt during storage.

The yeast and mold counts increased progressively in all yoghurt samples during storage, indicating gradual microbial spoilage over time. The control samples recorded the highest CFU counts because of the absence of preservatives, which allowed unrestricted growth of spoilage microorganisms under accelerated storage conditions. In comparison, the NP-treated samples showed lower yeast and mold conditions, suggesting that the natural preservative possessed antifungal compounds capable of partially inhibiting microbial proliferation and slowing spoilage. The SP – treated samples exhibited the lowest CFU counts, throughout the storage duration, demonstrating a stronger microbial effectiveness. The reduced yeast and mold growth observed in both the NP and SP samples also correspond with slower lactic acid development and reduced pH decline, since microbial metabolism contributes to biochemical deterioration in yoghurt. These

findings demonstrate that both preservatives improved the microbiological stability and shelf life of yoghurt, though the synthetic preservative provided greater inhibitory activity against spoilage yeasts and molds, in comparison to the natural preservative.

The results also demonstrated that the NP treatment had a positive effect on the shelf life stability of the yoghurt samples by reducing the rate of microbial spoilage and slowing physicochemical deterioration during storage. The NP yoghurt samples showed lower yeast and mold counts as compared to the control treatments, indicating that the plant extract possessed antifungal activity capable of inhibiting yoghurt spoilage micro-organisms. The stability maybe attributed to the presence bioactive phytochemicals, such as phenolics, flavonoids, and tannins, in the extract, which are known to disrupt microbial cell membranes, and inhibit, microbial metabolism. These findings therefore support the potential use of *Z. mauritiana* extracts as a natural alternative to synthetic preservatives, for improving shelf life and while maintaining product quality and safety.

5.2 Summary

The study investigated the antimicrobial properties of aqueous, 80% and 50 hydroethanolic extracts of *Z. mauritiana* against spoilage yeasts and molds, associated with yoghurt. The plants extracts, were prepared using different solvent systems and evaluated using agar diffusion techniques. Zones of inhibition were measured in millimetres and statistically analysed using mean and standard deviation from triplicate experiments.

The results obtained, showed that all extracts showed some level of antifungal activity, and that the effectiveness of the extracts is highly dependent on the extract concentration. The inhibitory effects of the extracts differed, with the 80% hydroethanolic extracts showing the highest inhibitory activity. However, only the aqueous extracts were incorporated into yoghurt samples, primarily due to food safety, regulatory acceptance and GRAS considerations. The findings of the shelf-life analysis, indicate that there is potential for the use and application of the plant extracts as natural preservatives, though they were not on par with the synthetic preservatives.

5.3 Conclusions

This study successfully demonstrated the antimicrobial potential of *Z. mauritiana* extracts and their effectiveness in improving shelf life of yogurt under accelerated storage conditions. Different extraction methods and solvent systems were evaluated, and the extracts exhibited inhibitory activity against yeasts and molds, which are major spoilage microorganisms in yoghurt. The determination of the minimum inhibitory concentration (MIC), confirmed that the plant extracts possessed measurable antifungal properties, supporting their suitability as natural preservatives in yoghurt.

The fortification of yoghurt with the natural preservative (NP) resulted in improved microbial stability compared to untreated samples, as evidenced by reduced yeast and mold growth during storage. In addition, the NP treated yoghurt maintained more stable physicochemical properties, including pH and lactic acid content, indicating slower spoilage progression as compared to the control samples. Sensory evaluation further showed that the fortified yoghurt remained generally acceptable, with regard to taste, texture, consistency and colour, suggesting that the incorporation of the extract did not significantly compromise consumer acceptability.

Overall, the findings of this study demonstrate that *Z. mauritiana* extracts possess antifungal activity against yoghurt spoilage yeasts and molds and can contribute to the improved microbial stability of yoghurt during storage. Although the aqueous extract was less effective than potassium sorbate, it reduced microbial growth, slowed physicochemical deterioration and maintained acceptable sensory characteristics, and thus the research objectives were met.

According to the data obtained, the null hypothesis (H_o), was not statistically supported and was therefore rejected. The alternative hypothesis (H_1), was statistically supported was accepted. Therefore, *Ziziphus mauritiana* fruit extracts can be considered as a potential alternative preservative for yoghurt.

5.4 Recommendations

Based on the findings and limitations of the study, the following recommendations are proposed

for the future research.

1. There is need for optimisation of solvent extraction methods, so as to ensure that the extracts are safe for incorporation into food, and maximize the recovery of antimicrobial compounds, while maintaining food safety and cost-effectiveness. The findings of the AST, demonstrate that the hydroethanolic extracts possess stronger antimicrobial properties, than the aqueous extracts, and should be considered for further development as natural preservatives.
2. There is need for phytochemical characterization and identification of the specific bioactive actives in the extracts.
3. Research into synergistic approaches of the extracts with other methods of preservation, such as refrigeration.
4. There is need for real-time validation of the preservative, under normal storage conditions

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Appendices

Appendix 1: Zones of Inhibition

Extract	**Concentration (%)**	**Mean ± SD (mm)**
Aqueous	100	7.33 ± 1.53
Aqueous	75	4.33 ± 0.58
Aqueous	50	0.00 ± 0.00
Aqueous	25	0.00 ± 0.00
50% H E	100	12.67 ± 1.53
50%H E	75	7.00 ± 1.73
50% H E	50	3.33 ± 0.58
50% H E	25	1.00 ± 1.00
80% H E	100	16.33 ± 1.15
80% H E	75	11.67 ± 1.53
80% H E	50	6.00 ± 1.00
80% H E	25	3.33 ± 0.58

H E – hydroethanolic

Appendix 2 Summarizes ANOVA resuls

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	247.1667	2	123.5833	103.4651	1.59E-12	3.402826
Columns	622.75	3	207.5833	173.7907	2.08E-16	3.008787
Interaction	30.16667	6	5.027778	4.209302	0.004953	2.508189
Within	28.66667	24	1.194444			
Total	928.75	35				

Appendix 3: MIC values

Replicate	MIC Well	MIC Value (mg/mL)
1	P1	200
2	P2	100
3	P1	200

Appendix 4 Lactic acid development Mean and standard

Day	NP Mean	NP SD	SP Mean	SP SD	Control Mean	Control SD
0	0.90	0.00	0.90	0.00	0.90	0.00
3	1.03	0.04	0.95	0.01	1.12	0.03
6	1.19	0.05	1.00	0.02	1.34	0.04
9	1.36	0.04	1.09	0.01	1.55	0.05
12	1.59	0.03	1.22	0.04	1.78	0.04
15	1.83	0.04	1.34	0.06	1.96	0.04

Appendix 5 pH changes, Mean and Standard deviation

Day	NP Mean	NP SD	SP Mean	SP SD	Control Mean	Control SD
0	4.63	0.00	4.63	0.00	4.63	0.00
3	4.39	0.05	4.55	0.02	4.25	0.02
6	4.16	0.03	4.49	0.02	4.02	0.04
9	3.91	0.05	4.32	0.01	3.77	0.05
12	3.70	0.04	4.16	0.01	3.57	0.06
15	3.53	0.02	4.00	0.05	3.47	0.03

Appendix 6 Sensory evaluation scores

Day	Attribute	NP Mean Score	S P Mean Score	Control Mean Score
0	Taste	4.6	4.6	4.6
	Texture	4.4	4.4	4.4

	Colour	4.6	4.4	4.4
	Consistency	4.6	4.6	4.6
6	Taste	2.6	4.4	N/A
	Texture	2.6	4	N/A
	Colour	4.6	4.4	4.4
	Consistency	2	3.6	1.8
12	Taste	N/A	N/A	N/A
	Texture	N/A	N/A	N/A
	Colour	2	2	1.8
	Consistency	1.8	1.8	1.8

Appendix 7 Solvent extraction process



Appendix 8 Yoghurt samples





Appendix 9 Yeast and mold plates

