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DEPARTMENT OF BIOLOGICAL SCIENCES

Antimicrobial effects of *Adansonia digitata* leaf extracts against bacteria causing foodborne illnesses (*Escherichia coli* and *Salmonella*).

By

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

June 2024

APPROVAL FORM

The undersigned certify that they have read the dissertation titled **'Antimicrobial effects of *Adansonia digitata* leaf extracts against bacteria causing foodborne illnesses (*escherichia coli* and *salmonella*)'** and confirm that it is suitable for submission to the Biological Sciences Department, Faculty of Science and Engineering, for assessment.

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Signature of Department Chairperson:  Date: 17/10/24

DECLARATION

I, Makora William Munashe (B1953741) declare that this research herein is my own work and has not been plagiarized from another source(s) without acknowledgment of the concerned author(s) either electronically or otherwise.

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Supervisor

I, Nyembezi Mgocheki, declare that l have supervised this thesis and l am satisfied that it can be submitted to the Biological Sciences Department, Faculty of Science and Engineering, at Bindura Universty of Science Education.

Signature: Nyembezi Mgocheki Date: 07/06/2024

DEDICATION

I dedicate this project to my parents, Mr and Mrs Makora, for their unwavering presence and support.

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First and foremost, I want to express my gratitude to the Almighty Lord for guiding me in this research project. I want to express my gratitude to my family for supporting me both financially and emotionally during my academic career. Additionally, I would like to thank my supervisor, Dr N Mgocheki, for her constant support and mentorship during the research. My special thanks also go to the staff members of the Biological Sciences Laboratory who assisted me in carrying out this project. My appreciation also goes out to my friends who have been a constant source of motivation. Lastly, I would like to express my gratitude to everyone who has helped to bring this research project to a successful conclusion.

LIST OF ACRONYMS

ANOVA- Analysis of Variance

Cm- centimeter

EMB- Eosin Methylene Blue

MBC- Minimum Bacterial Concentration

mg- milligrams

MHA- Mueller Hinton Agar

MIC- Minimum Inhibitory Concentration

ml- milliliter

NGS- Next Generation Sequencing

NA- Nutrient Agar

PCR- Polymerase Chain Reaction

SPSS- Statistical Package for the Social Sciences

TNTC- Too Numerous To Count

TSB- Tryptic Soy Broth

TSA- Trypsin Soy Agar

XLD- Xylose-Lysine-Deoxycholate

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ABSTRACT

Foodborne illnesses are a major global public health concern, causing significant morbidity and mortality. These illnesses are caused by the consumption of contaminated food. This study investigated the antimicrobial activity of *Adansonia digitata* leaves against foodborne bacteria *Escherichia coli* and *Salmonella spp* using disk diffusion assay. It was hypothesized that *A. digitata* leaves have antimicrobial effects against bacteria associated with foodborne illnesses. Methanol, ethyl acetate, and aqueous solvents were used for the extraction of extracts from *A. digitata* leaves. *A. digitata* leaves were collected from suitable trees in Mutoko. Methanolic *A. digitata* leaf extracts demonstrated a significant inhibitory effect of what value? against both *Salmonella spp* and *E. coli* while ethyl acetate and aqueous *A. digitata* leaf extracts did not show any significant antimicrobial effects. This finding suggests that methanolic *A. digitata* leaf extracts may be a potential source of antimicrobial compounds against foodborne pathogens. The lack of antimicrobial effects of ethyl acetate and aqueous *A. digitata* leaf extracts indicates that different solvents may be required to extract antimicrobial compounds from *A. digitata* leaves. Further research could explore the use of different solvents and extraction conditions to optimize the yield of antimicrobial compounds from *A. digitata* leaves. The potential use of *A. digitata* leaf extracts for food safety applications and disease control could be significant.

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CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

Foodborne illnesses are a major global public health concern, causing significant morbidity and mortality. Contaminated food, particularly of animal origin, serves as a primary source for the transmission of pathogenic microorganisms to humans. Each year, millions of people worldwide suffer from foodborne infections, leading to a considerable economic burden on healthcare systems and society at large. Among the most common foodborne pathogens, *Escherichia coli* and Salmonella are responsible for a significant number of outbreaks and cases of food poisoning (Barakat, 2021).

Escherichia coli is a diverse group of bacteria commonly found in the intestines of humans and animals. It is among the most prevalent pathogenic agents of foodborne diseases, which are a critical health hazard with growing concern as shown by the escalating incidence of infectious diarrhoea from epidemiological data (Osservasalute, 2009). *E. coli* is one of the most important pathogenic agents causing food poisoning resulting in gastrointestinal infections in fowls and mammals, including humans (Barnes *et al.*, 2008). While most strains are harmless, certain pathogenic strains, such as *Escherichia coli O157:H7*, can cause severe gastrointestinal symptoms, including diarrhoea, abdominal cramps, and in some cases, life-threatening complications. According to the Centers for Disease Control and Prevention (CDC), *Escherichia coli 0157:H7*, one of the most common strains, causes an estimated 96,000 illnesses, 30,000 hospitalized and 2000 deaths in the sub-Sahara each year. Globally, the World Health Organization (WHO) estimates that there are approximately 1.8 million cases of foodborne illness caused by *E. coli* each year. The prevalence of *E. coli* in a specific population can be influenced by factors such as age, dietary habits, water quality, and socioeconomic status. *E. coli* is more commonly found in developing countries where food safety and sanitation standards may be less stringent. There are several ways that *E. coli* can be transmitted to humans. The most common route of transmission is through contaminated food and water. *E. coli* can contaminate food during production, processing, or preparation. For example, raw vegetables can become contaminated if they are

washed in water that is contaminated with animal feces and undercooked meat or poultry can contain *E. coli* if the animal was infected before slaughter. Drinking water can also become contaminated with *E. coli* if it comes into contact with animal or human waste. In addition to contaminated food and water, *E. coli* can also be transmitted through direct contact with animals or other people or through contact with contaminated surfaces (Barakat, 2021).

Salmonella, another prevalent foodborne pathogen, encompasses numerous serotypes that can cause salmonellosis, a gastrointestinal infection characterized by diarrhoea, fever, and abdominal pain. *Salmonella* is transmitted through contaminated food, water and animals. The most common route of transmission is through contaminated food. Food can become contaminated with salmonella at any point. Some sources of *Salmonella* include eggs if infected hens lay them, and undercooked poultry or meat can contain *Salmonella* if the animal was infected before slaughter. Other foods that can be contaminated with *Salmonella* include raw fruits and vegetables, unpasteurised dairy products and sprouts (Bayot & Bragg, 2022).

The baobab tree, *Adansonia digitata*, is a native of Africa, and its leaves have been used traditionally for a variety of purposes, including the preservation of food. It is a globally known high-valued multipurpose tree (Musyoki *et al*., 2022). *A. digitata* also named "Baobab", belongs to the *Malvaceae* family and is a majestic tree revered for its medicinal and nutritional values (Barakat, 2021). Recent studies have shown that the leaves of the baobab tree have antimicrobial properties, and could potentially be used to prevent foodborne illnesses caused by pathogenic microorganisms. *A. Digitata* also has antimicrobial, antiviral and anti-trypanosome activity (Kabore *et al*., 2011). This project will explore the potential of baobab leaves to inhibit the growth of food-borne pathogens

1.2 PROBLEM STATEMENT

Foodborne illnesses are a major public health issue, particularly in developing countries like Zimbabwe where access to clean water and refrigeration is limited. Conventional food preservation methods such as refrigeration and canning are often not available or are too expensive for many people in developing countries. The use of natural antimicrobial compounds from plants such as

baobab leaves could be a cost-effective and sustainable solution to the problem of food preservation in these areas. However, there is a lack of research on the efficacy and safety of using baobab leaves for food preservation. The main problem to be addressed in this project is the lack of information on the antimicrobial activity of baobab leaf extracts against common food-borne pathogens. In addition, there is a lack of understanding of the optimal conditions for using baobab leaf extracts for food preservation, and the potential impact of these extracts on the sensory properties of food products. There is also limited knowledge about the safety of consuming foods preserved with baobab leaf extracts and their use in disease control. Addressing these knowledge gaps is critical for ensuring the safe and effective use of baobab leaf extracts for food preservation.

1.3 JUSTIFICATION

The use of baobab leaf extracts for food preservation has several potential benefits, which justify further research on this topic. The antimicrobial activity of baobab leaf extracts could provide a natural and safe alternative to chemical preservatives, which may have negative health effects. Baobab leaves are a renewable and sustainable resource that is readily available in many parts of the world, particularly in Africa. Baobab leaves are relatively inexpensive, making them a cost-effective option for food preservation. In addition to the potential benefits of using baobab leaves for food preservation, there are several other factors that justify further research on this topic. First, the global demand for food is increasing, and there is a need for sustainable and cost-effective food preservation methods. Second, there is a growing interest in the use of natural products for food preservation, as consumers are increasingly concerned about the safety and environmental impact of chemical preservatives. Third, the use of baobab leaves for food preservation could have positive socioeconomic impacts, as it could provide a source of income for local communities.

1.4 Aim of the study

Evaluate the antimicrobial activity *of Adansonia digitata* leaf extracts against common food-borne bacteria.

1.5 Objectives

1. To isolate and identify bacteria associated with food-borne illnesses.
2. To evaluate the antimicrobial activity of *Adansonia digitata* leaves using ethyl acetate, methanol and aqueous extracts on bacteria associated with foodborne illnesses using the Kirby Bauer disk diffusion assay.
3. To identify the bioactive compounds present in *Adansonia digitata* leaves.

1.6 RESEARCH QUESTIONS

1. Which bacteria are associated with food-borne illnesses?

2. Does *Adansonia digitata* exhibit antimicrobial properties against bacteria associated with food-borne illnesses?

3. Which bioactive compounds are present in *Adansonia digitata* leaves?

1.7 HYPOTHESIS

H_1: *A. digitata* leaf extracts will have significant antimicrobial effects against common food-borne bacteria (*Salmonella* and *Escherichia coli).*

H_0: *A. digitata* leaf extracts do not have antimicrobial activity against common food-borne bacteria (*Escherichia coli* and *Salmonella).*

1.8 Limitations of the study

The baobab leaves were collected from trees from a very small geographical location in Mutoko and the exact age of the trees where not known. The composition of baobab leaves can vary depending on factors like the age of the leaves and the growing conditions, so this variability could affect the antimicrobial activity of the extracts. In addition, the isolated bacteria were obtained from feces and the small sample size limits the generalizability of the results.

1.9 Delimitations of the study

Alamycin discs were prepared to be used as positive controls because of the unavailability of commercial Alamycin disks. Nutrient agar was used for disk diffusion assay as an alternative to Muellur-Hinton agar because of the unavailability of the Muellur-Hinton media in the laboratory. Also an oven was used for the evaporation of the solvents from the extracts due to the non functioning of the rotary evaporator that was available and this created a potential danger on the degradation of the extracted compounds. The minimum inhibitory concentration (MIC) test, a quantitative assay required to determine the exact lowest concentration of *A. digitata* leaf extracts required to inhibit bacterial growth was not done due to resource constraints.

1.10 Definition of terms

Antimicrobial Effect is a term used to describe any substance that prevents and eliminates microbial development (Saga & Yamaguchi, 2009).

Antimicrobial Susceptibility Test is a procedure performed to measure the effectiveness of a compound against pathogens (Bayot & Bragg, 2022).

Foodborne illnesses are diseases that are caused by the consumption of contaminated food.

Adansonia digitata is a tree commonly known as the 'Tree of Life' that is native to regions of Africa, Australia and Madagascar, it's a tree that is used as a source of food, and as a potential antimicrobial, antioxidant and anti-inflammatory agent (Pamela *et al*., 2019).

CHAPTER 2: LITERATURE REVIEW

Worldwide, food-borne illnesses caused by bacteria such as *Salmonella spp* and *E. coli* pose a serious threat to public health. These bacteria place a significant burden on public health systems because they are frequently linked to foodborne illnesses. Particularly, *Salmonella* is a major cause of foodborne diseases; many *serovars*, including *Salmonella enterica*, have been linked to outbreaks (Gutierrez *et al.,* 2022; Laufer *et al.,* 2014; Cui *et al.,* 2017; Hossain *et al.,* 2019; Harvey *et al.,* 2020). *Salmonella* is common in food products, such as meat and poultry, which emphasizes how crucial it is to keep an eye on and manage these bacteria in the food supply chain (Gutierrez *et al.,* 2022; Laufer *et al.,* 2014; Hossain *et al.,* 2019; Harvey *et al.,* 2020). Similarly, *E. coli*, especially enterohemorrhagic strains, is recognized as a significant threat to public health due to its association with foodborne illnesses (Klass *et al.,* 2021; Afsal *et al.,* 2021). Pathogenic *E. coli* strains have been linked to outbreaks of foodborne diseases, emphasizing the need for stringent food safety measures to prevent contamination (Klass *et al.,* 2021; Afsal *et al.,* 2021). Both *Salmonella* and *E. coli* are highlighted in the literature as primary bacterial causes of foodborne illnesses, with *Salmonella* being a leading cause of bacterial foodborne-related illnesses globally (Laufer *et al.,* 2014; Cui *et al.,* 2017; Harvey *et al.,* 2020). The transmission of these pathogens through food products, including poultry, beef, and other animal-derived products, underscores the importance of implementing control strategies to reduce the risk of foodborne infections (Laufer *et al.,* 2014; Harvey *et al.,* 2020).

2.1 The impact of foodborne illnesses in Zimbabwe

Pathogens causing foodborne infections have a major effect on public health, as they can result in a number of serious conditions such reactive arthritis, mental impairment, intestinal inflammation, chronic renal diseases, blindness, and even death (Yasmin *et al.,* 2016). 90% of foodborne illnesses, hospitalizations, and deaths in the United States that are linked to known pathogens are domestically acquired and involve seven main pathogens: Campylobacter, Clostridium perfringens, *Escherichia coli O157*, *Listeria monocytogenes*, norovirus, and *Toxoplasma gondii*

(Scallan *et al.,* 2015). *Salmonella enteritidis*, *Escherichia coli O157:H7*, and *Listeria monocytogenes* have been found to be the most common foodborne pathogenic bacteria among the many causes of foodborne diseases (Jiang *et al.,* 2016). Approximately 60% of foodborne diseases that result in hospitalization and deaths are caused by bacterial pathogens, which also account for roughly two-thirds of the estimated number of foodborne pathogen-related deaths (Koohmaraie *et al.,* 2005). In Zimbabwe alone foodborne infections are thought to be responsible for thousands of illnesses, hundreds of hospital admissions, and fatalities per year. (Kasowski *et al.,* 2002). To be more precise, bacterial *enteric* pathogens are responsible for millions of foodborne sickness episodes, hundreds of thousands of hospital admissions, and thousands of fatalities annually worldwide *(*Hansen *et al.,* 2023). *Listeria monocytogenes*, a facultative intracellular pathogen, is highlighted as a significant contributor to foodborne illnesses, being the third leading cause of death from such illnesses in the United States, with a considerable number of cases leading to fatalities annually (Heredia & García, 2018). The pathogen continues to cause outbreaks of foodborne illnesses with high mortality rates ranging from 20% to 30% (Cauteren *et al.,* 2017). The key to reducing foodborne pathogen-related infections, illnesses, and mortality is early detection and control of these pathogens (Davis *et al.,* 2019).

2.2 Bacteria associated with foodborne illnesses

2.2.1 Salmonella

A major pathogen that contributes significantly to the worldwide illness burden is *Salmonella*. Research has indicated that bloodstream infections in African nations are primarily caused by *Salmonella enterica*. (Reddy *et al*., 2010). *Salmonella* are commonly found in chicken, however they can also be found in other animals (Hanning *et al*., 2009). Based on genetic distinctions, *Salmonella* is divided into two species: *Salmonella enterica* and *Salmonella bongori* (Eng *et al*., 2015). Every year, *Salmonella* infections cause a great deal of hospitalizations and substantial medical expenses worldwide (Li *et al*., 2023). Studies have investigated the transmission routes of *Salmonella*, including through wild birds and reptiles (Machado *et al*., 2017). Developing successful therapies requires an understanding of how *Salmonella* interacts with the host's immune system, notably through mechanisms like autophagy. (Wu *et al*., 2020; Hurley *et al.,* 2014). There have been initiatives to identify and stop the spread of *Salmonella* in several contexts, including food items like eggs and chicken excrement (Gast *et al*., 2021). Additionally, an attempt is being

made to better understand the traits and behaviours of *Salmonella* strains by genomic study (Kagambèga *et al.*, 2021).

2.2.2 Escherichia coli

Escherichia coli is a multipurpose bacterium that has been thoroughly investigated in several fields. Understanding harmful strains of *Escherichia coli* in various contexts, including as human health, animal reservoirs, food safety, and environmental survival, has been the focus of research. (Croxen *et al*, 2013; Jang *et al.*, 2017; Lagerstrom & Hadly, 2021). Research has demonstrated that drug-resistant strains of *Escherichia coli*, such as the one that produces ESBL, are common in healthy persons, underscoring the significance of monitoring and managing antibiotic resistance (Bezabih *et al.*, 2020). Additionally, studies on the pathogenicity, genetic diversity, and antibiotic resistance of *E. coli* in wild animals have illuminated the ecology and dynamics of this bacterium's transmission (Lagerstrom & Hadly, 2021). The pathotypes of *E. coli* that have been found include enteropathogenic, enterohermorrhagic, and enteotoxigenic. The clinical relevance of *E. coli* includes its ability to cause a variety of illnesses, including diarrhoea, meningitis, and urinary tract infections (Bichon *et al.,* 2018; Silberger *et al*., 2017). Furthermore, studies on the function of *E. coli* in foodborne illness and its capacity to build biofilms have been conducted, highlighting the significance of control strategies and quick detection techniques (Zhou *et al*., 2022).

2.3 Methods of detection and identification of foodborne pathogens

Foodborne pathogens can be found and identified using a variety of techniques. These include the application of nucleic acid-based techniques (Polymerase Chain Reactions, or PCR), immunological assays, culture-based techniques, and Next Generation Sequencing (NGS) techniques. All these strategies, however, will not be successful unless the proper aseptic sampling and sample storage procedures are followed. The target microbial groups, the type of food being tested, and the microbial detection techniques all influence the sampling strategy. For instance, every procedure involving the isolation and use of microbial cultures requires that sample collection and analysis adhere to standard protocols approved and created by official bodies like the FDA, FSIS/USDA, ISO, and AOAC (Da Silva, 2018).

2.4 The baobab tree

The baobab tree *(Adansonia digitata*) is a significant tree species native to Africa, particularly valued for its various uses and ecological importance. The French traveller and botanist Michael Adanson (1727–1806) is credited with writing the first botanical description of the entire species, which is why the species is known by its scientific name, *Adansonia* (Baum, 1995). *Digitata* refers to the digits of the hand, as the baobab has compound leaves with normally five (but up to seven) leaflets, akin to a hand (Diop., 2011). According to taxonomy, *A. digitata* is a member of the Bombacoideae subfamily of the Malvaceae family (Schumann *et al*., 2012; Singh *et al*., 2013; Pettigrew *et al.,* 2012). This famous tree, valued for its longevity and unique appearance, is essential to many ecosystems, but it is particularly important to sub-Saharan African dryland farming systems (Meinhold & Darr, 2020). The baobab tree is an essential part of many agroforestry systems in Africa and is valued for its ethnobotanical relevance (Assogbadjo *et al*., 2021; Chládová *et al.,* 2019). Research has brought to light the genetic diversity and organization of baobab populations across several regions, providing insights into the management and conservation of this species (Sanchez *et al*., 2009). The distinctive leaf form of the baobab tree has been connected to its resistance to drought, highlighting its adaptation to harsh conditions (Li *et al.,* 2017). Additionally, studies on the nutritional makeup of baobab fruit pulp have shown both its high nutritional content and its potential medical benefits (Singh *et al*., 2013; Assogbadjo *et al*., 2008).

Figure 2.1: Baobab (*Adansonia digitata*) tree.

Figure 2.2: leaves of *Adansonia digitata*

2.5 Taxonomical Classification of *Adansonia Digitata*

Kingdom:	Plantae
Division:	Embryophyta
Class:	Magnoliophyta
Sub class:	Magnoliopsida
Order:	Malvales
Family:	Malvaceae
Genus:	Adansonia

Species: digitata

2.6 *A. digitata* Leaves: Composition and Bioactive Compounds

A. digitata leaves contain a multitude of health benefits due to their substantial content of bioactive compounds. Research has indicated that baobab leaves are a good source of antioxidants, vital minerals, and bioactive substances such as flavonoids, phenolic compounds, and polyphenols (Habte *et al.,* 2021). The antioxidant qualities of *A. digitata* leaves are a result of these bioactive compounds, which can aid in the fight against oxidative stress and lower the risk of chronic illnesses (Otong & Musa, 2019; Braca *et al.,* 2018). *A. digitata* leaves are also known to have hypoglycemic qualities, which makes them useful for controlling postprandial glycemia (Rita *et al.,* 2022). The nutritional value of *A. digitata* leaves is further enhanced by their amino acid composition. Lysine has been identified as the limiting amino acid in *A. digitata* leaf products, highlighting the importance of incorporating these leaves into diets to ensure a balanced amino acid profile (Chadare *et al.,* 2008). Studies on the phytochemical profile of *A. digitata* leaves have shown the presence of hydroxycinnamic acid glycosides, iridoid glycosides, and phenylethanoid glycosides, all of which are linked to the health-promoting qualities of the leaves (Li *et al.,* 2017). *A. digitata* leaf composition can change depending on things like storage conditions and drying techniques. Research indicates that baobab leaves that are shade-dried may hold onto more nutrients than sun-dried leaves, highlighting the significance of processing methods in maintaining the nutritional value of *A. digitata* leaves. (Amadou *et al.,* 2020*). A. digitata's* leaves are rich in phytochemicals, which are the cause of the plant's many health benefits, including its antibacterial and anti-inflammatory properties (Afolayan & Akindahunsi, 2018). These compounds possess the potential for antimicrobial properties.

2.7 Existing research

A few studies have examined the antibacterial properties of baobab leaves in vitro against microorganisms that cause foodborne disease. *A. digitata* leaf extracts showed antibacterial

efficacy against a range of bacteria and fungi, according to (Onyango *et al*., 2012) who investigated *A. digitata* leaf extracts for antimicrobial activity against bacterial and fungal pathogens in vitro. *A. digitata* leaf extract was discovered to have potent antibacterial activity against *Bacillus subtilis* and *E. coli* in another investigation. The extract was also found to have anti-inflammatory properties. A promising source of bioactive compounds with potential uses in food is the baobab tree's leaves (Grieve, 2015).

2.7.1 Limitations of the existing research

There are some potential limitations of the existing research on the antimicrobial properties of baobab leaves and their active compounds. Many of the studies have been conducted in vitro (in a laboratory setting), and more research is needed to determine if these effects occur in vivo (in a living organism). The concentrations of the active compounds used in many of the studies are higher than what would be found in baobab leaves or other natural sources. Furthermore, the antimicrobial effects of the active compounds may vary depending on factors such as the species of bacteria being studied and the specific conditions of the experiment.

2.8 Potential Commercial Applications

There are numerous potential commercial uses of *A. digitata* leaves as a food preservative. *A. digitata* leaves possess antibacterial activity, making them a possible natural food preservative (Ukaegbu *et al*., 2021). The meat business is one prospective market for *A. digitata* leaves as a food preservative because of their antibacterial and antioxidant qualities, which may help lower the chance of infection and spoiling in meat products. The leaves could also be utilized to create novel packaging materials that are better able to maintain the safety and quality of meat products.. Another potential application is in the dairy industry, the high levels of antioxidants in *A. digitata* leaves could help to extend the shelf life of dairy products by preventing the oxidation of fats and

other components. This could lead to cost savings for dairy producers and consumers, as well as a reduction in food waste. Furthermore, *A. digitata* leaves could potentially be used in the production of fermented foods, such as yogurt or sauerkraut. The antimicrobial properties of the leaves could help to control the growth of undesirable microorganisms during fermentation, leading to a safer and more consistent product. The leaves could also be used to impart a unique flavor or color to fermented foods.

2.9 The social and Economic impacts

The use of *A. digitata* leaf extracts in food products could have several social impacts, both positive and negative. In places where the trees are growing, employing baobab leaf extracts in food products may open up new business prospects for the surrounding populations (Muthai *et al*., 2017). This could include opportunities for employment, entrepreneurship, and improved standards of living (Kaimba *et al*., 2020). Additionally, it could help to preserve traditional knowledge about the uses of baobab trees. On the other hand, there is a risk that increased demand for baobab leaves could lead to over-exploitation of the trees and damage to the environment. Social unrest may also arise if some communities or groups believe that they are not benefiting from the use of *A. digitata* leaves in food products. Baobab farming in traditional agroforestry systems can improve livelihoods and address food security (Meinhold & Darr., 2020).

CHAPTER 3: MATERIALS AND METHODS

3 Materials and Methods

The project was carried out at the Bindura University of Science Education (BUSE), Astra campus Biological Sciences Laboratory (1041 Masembura Road off, Trojan Road, Bindura GPS coordinates 17.3167° S, 31.3228° E).

3.1 Sample collection

3.1.1 Ground beef and animal feces sample collection

Ground beef and human feces were used as sources of test organisms. Ground beef was collected from a butchery in Bindura Town and the human feces were collected from a toilet chamber at the University. The samples were collected and handled according to the regulations set by the Biosafety Board of Zimbabwe

3.2 Bacterial Culture

A small amount of the human feces sample was collected using a sterile swab and the swab was inoculated into a tube of Trypsin Soy Broth to dilute the sample and make it easier to work with. A sterile pipette was used to transfer a small amount of the diluted sample to a sterile petri dish containing Trypsin Soy Agar. A sterile swab was used to spread the sample on the surface of the agar. The petri dish was incubated at 37°C for 24 hours. The dish was examined for the growth of different colonies.

3.3 Isolation and Identification of Test Organism

3.3.1 Salmonella

Different colonies from the TSA were inoculated and streaked on Xylose-Lysine-Deoxycholate Agar (XLD). The dishes were incubated at 37°C for 24 hours. The Petri dishes were examined to check for the presence of black colonies.

3.3.2 Escherichia coli

Colonies suspected to be *E. coli* were inoculated and streaked on EMB Agar. The Petri dishes were incubated at 37°C for 24 hours. The Petri dishes were examined to check for the presence of *E. coli* colonies.

3.4 Gram Staining

To create a crystal violet stock solution, 20 g of crystal violet was mixed with 100 ml of ethanol. An oxalate stock solution was prepared by adding 1 g of ammonium oxalate to 100 ml of water. One ml of the crystal violet stock solution, 10 ml of water, and 40 ml of the oxalate stock solution were combined to create the working solution. A dropper was used to store the mixture.

Gram Iodine Solution:

Three hundred ml of water was mixed with 1 g of iodine, 2 g of potassium iodide, and 3 g of sodium bicarbonate.

Gram Decolorizer Solution:

A mixture of equal volumes of 95% ethanol and acetone was made.

Gram Safranin Solution:

A stock solution was prepared by dissolving 2.5 g of Safranin O in 100 ml of 95% ethanol. One part of the stock solution was diluted with five parts of water to create the working solution.

Gram Staining Method

An inoculating loop was heated on the Bunsen burner and a drop of water was transferred onto a slide. An inoculating loop was used to transfer the inoculum to the glass slide. A thin film was formed by the circular dissemination of the culture. The glass slide was gently flamed and air-dried. Five drops of Crystal Violet were added to the glass slide for 60 seconds and rinsed off with water. The iodine solution was poured off to cover the fixed culture for 30 seconds and the excess was rinsed off with water. The decolorizer, Methanol was added to let the solution trickle down the slide and after 5 seconds, it was rinsed off with water. Five drops of Safranin solution were added as a counterstain for 20 seconds. The excess solution was washed with water after it was poured off. The excess water was removed with blotting paper. Using an oil immersion, the slide was examined under a light microscope at magnifications of X4, X10, X40, and X100. Gram-positive and Gram-negative bacteria were differentiated.

3.5 Preparation and extraction of baobab leaves

3.5.1 Collection of baobab leaves

The baobab leaves were obtained from a suitable baobab tree that had fresh and healthy leaves. The leaves were cut from a tree using a clean sterile and sharp knife and then were transported to the laboratory in a clean and dry container. The average height of baobab tree species in Zimbabwe is 17 to 25 meters.

3.5.2 Preparation of baobab leaves

The collected leaves were washed thoroughly with clean water to remove any contaminants. The leaves were shed and allowed to dry entirely in a well-ventilated space . The dried leaves were then ground into a fine powder using a mortar and pestle. The maceration extraction method was used whereby 20g of crushed leaves were soaked in three separate solvents which were aqueous,

ethyl acetate, and methanol by continuous mixing and agitating until the biomass was dissolved for 24 hours in the dark. The resulting solutions were filtered using Whatman filter paper number one. Then the filtrates were placed in an oven for the evaporation of the solvents to obtain Baobab leaf extracts at 40°C.

The percentage yield of each extract was calculated by:

$$\% \text{ Yield} = \frac{Amount\ of\ extract\ recovered}{Initial\ weight} \times 100$$

For antimicrobial susceptibility testing, the different concentrations made from each solvent extract were as follows: 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml.

Figure 3.1: *A. digitata* powdered leaves

Figure 3.2: *A. digitata* Aqueous, methanol and ethyl acetate solvents for extraction

3.6 Phytochemical Analysis

The phytochemical analysis was done to check the presence of tannins, alkaloids, saponins, flavonoids, and phenols.

3.6.1 Flavonoids

To 2ml of the extract, 1ml of 2M Sodium hydroxide was added. Yellow colour denotes the presence of flavonoids.

3.6.2 Alkaloids

A few drops of Mayer's reagent and 2 drops of concentrated hydrochloric acid were added to 10ml of the extract; the appearance of a green color indicated the presence of alkaloids.

3.6.3 Tannins

Two ml of 5% ferric chloride was added to 1 ml of the extract, the appearance of a greenish-black color indicates the presence of tannins.

3.6.4 Saponins

Five ml of the extract was mixed with 5 ml of distilled water and agitated in a graduated cylinder. The formation of frothing denotes the presence of saponins.

3.6.5 Phenols

To 5mls of the extract, 5 drops of 10% ferric chloride were added. A bright yellow colour solution was formed which then changed to a dark green solution to show the presence of phenols.

3.7 Antimicrobial Susceptibility Test

The antimicrobial susceptibility testing of *A. digitata* leaf extracts on *Escherichia coli* and *Salmonella* bacteria was done using the Kirby Bauer disk diffusion protocol. The 24-hour bacterial culture was used on Nutrient Agar. Whatman Filter paper number 1 was used to create disks for the diffusion assay, the Whatman filter papers were punched with a puncher, and discs of 5mm in size were created. Concentrations of 100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml of *A. digitata* leaf extracts were prepared using methanol, ethyl acetate, and aqueous extracts for the antimicrobial susceptibility tests. Whatman filter paper discs were impregnated with appropriate concentrations of Baobab leaf extracts for 30 minutes and then air dried. The dried discs were placed on Nutrient Agar containing the inoculum. Alamycin discs were prepared and used as positive controls.

3.7.1 Preparation of a standardized inoculum

A 0.5McFarland standard of 1% $BaCl_2$. $2H_2O$ and 1% H_2SO_4 were used for standardizing the concentration of the 24-hour inoculum. The 0.5McFarland working standard was prepared by adding 0.5mls of 1% $BaCl_2$. $2H_2O$ and 99.5mls of 1% H_2SO_4. 0.85g of Sodium Chloride was weighed and 100ml of water was added to make 0.85% saline water. The saline water was sterilized in an autoclave for 15 minutes at 15psi before using it to adjust the turbidity of the inoculum to be that of the 0.5 McFarland standard.

3.7.2 The Kirby- Bauer disc diffusion assay

Nutrient Agar was used as the medium and the inoculum from 24-hour bacterial cultures in Trypsin Soy broth was pipetted onto NA and spread on the agar using a swab. Whatman filter paper discs impregnated with *A. digitata* leaf methanolic, ethyl acetate, and aqueous extracts were placed on NA agar containing the inoculum. NA plates were incubated at 37^{o}C for 24 hours and then observations and measurements of zones of inhibition using a measuring ruler were done. Alamycin disks were used as positive controls in each petri plate and the negative controls were the solvents used for each extraction.

3.8 Identification of Bioactive Compounds

Fourier Transform Infrared (FT-IR) spectrometer was used for the identification of the bioactive compounds and functional groups in *A. digitata* ethyl acetate, aqueous and methanolic leaf extracts. The obtained wavelengths were compared to an infrared table chart to determine specific functional groups present.

3.9 Statistical Analysis

The data was subjected to a Statistical Package for the Social Sciences (SPSS) IBM 20, a statistical software for analysis. The data obtained was subjected to a normality test and One-way ANOVA to determine the levels of difference among *A. digitata* leaf ethyl acetate, methanol, and aqueous extracts as antimicrobials on bacteria associated with foodborne illnesses.

CHAPTER 4: RESULTS

4.1 Colony Morphology

The microbes associated with foodborne illnesses that were isolated from the test samples were *E coli* and *Salmonella*. The bacterial isolates from Trypsin Soy Agar exhibited different morphologies (Table 4.1).

Table 4.1: Colony Morphology for Bacteria Isolated from Tryptic Soy Agar

Colony Morphology	Colony 1	Colony 2	Colony 3	Colony 4
Colour	Brown	White	Yellow	Pink
Opacity	Transparent clear	Transparent clear	Transparent clear	Transparent clear
Margin	Undulate	Entire	Entire	Undulate
Form	Irregular	Irregular	Circular	Irregular
Elevation	Umbonate	Crateriform	Convex	Umbonate
Size	Punctiform	Punctiform	Punctiform	Punctiform
Surface	Rough	Smooth	Smooth	Rough

4.2 Bacteria in XLD Agar

The bacteria with brown colonies from the TS Agar turned black in XLD Agar to show the presence of *Salmonella spp.*(Appendix A)

4.3 Bacteria in Eosin Methylene Blue (EMB) Agar

The bacteria with dark pink colonies from TS Agar turned into blue-black colonies with green metallic sheen in EMB Agar to show the presence of *Escherichia coli*.(AppendixB)

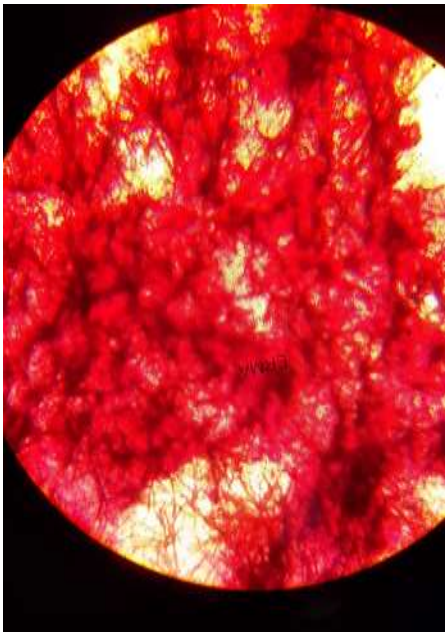

Figure 4.2: Gram-negative bacteria

4.4 Yields of *A. digitata* leaf extracts

Initially, 20g of *A.digitata* leaves were macerated in methanol, ethyl acetate, and aqueous solvents and after solvent extraction, the percentage yield obtained from each solvent were calculated and obtained as 20.5%, 16%, and 13% respectively (Appendix C).

4.5 Phytochemical Confirmatory Tests of *A.digitata* Leaf Extracts

The phytochemical screening of *A. digitata* leaf extracts revealed the presence and absence of secondary metabolites in ethyl acetate, methanol, and aqueous extracts. The presence of phytochemicals in *A. digitata* leaf extracts was denoted by a specific colour.

Table 4.2: Phytochemicals present in *A. digitata* leaf extracts

Phytochemicals	Type of Extract		
	Ethyl acetate	Methanol	Aqueous
Alkaloids	+	+	-
Flavonoids	+	+	-
Phenols	-	+	+
Saponins	+	-	+
Tannins	-	+	+

KEY: + Present, - Absent

(a) (b) (c) (d)

Figure4.3: (a) Tannins, (b) Flavonoids, (c) Phenols, (d) Saponins test in (M) methanol, (E) ethyl acetate and (A) aqueous extracts**.**

(e)

Figure 4.4: Alkaloids test in (M) methanol, (E) ethyl acetate, and (A) aqueous extracts.

4.6 Antimicrobial Susceptibility Test of *A. digitata*

The Kirby Bauer disk diffusion assay was used for the antimicrobial susceptibility test of *A. digitata* leaf extracts obtained from methanol, ethyl acetate, and aqueous solvents against *E coli* and *Salmonella.* Zones of inhibition were created on methanolic leaf extracts against *E coli* and *Salmonella* while ethyl acetate and aqueous extracts showed insignificant effects against both *E coli* and *Salmonella*.

A. digitata methanolic extracts inhibited bacterial growth for *E.coli* and *Salmonella* at 100, 50, 25, and 12.5mg/ml. No significant differences were noted between zones of inhibition for *E. coli* and *Salmonella spp* at all concentrations. At 100mg/ml, *A. digitata* had a similar inhibitory effect as the positive control, ie, no significant differences were noted. At 100mg/ml, the highest antimicrobial activity was detected against *Salmonella. A. digitata* ethyl acetate and aqueous extracts had no antimicrobial activity against both *E.coli* and *Salmonella* (Appendix D and Appendix E).

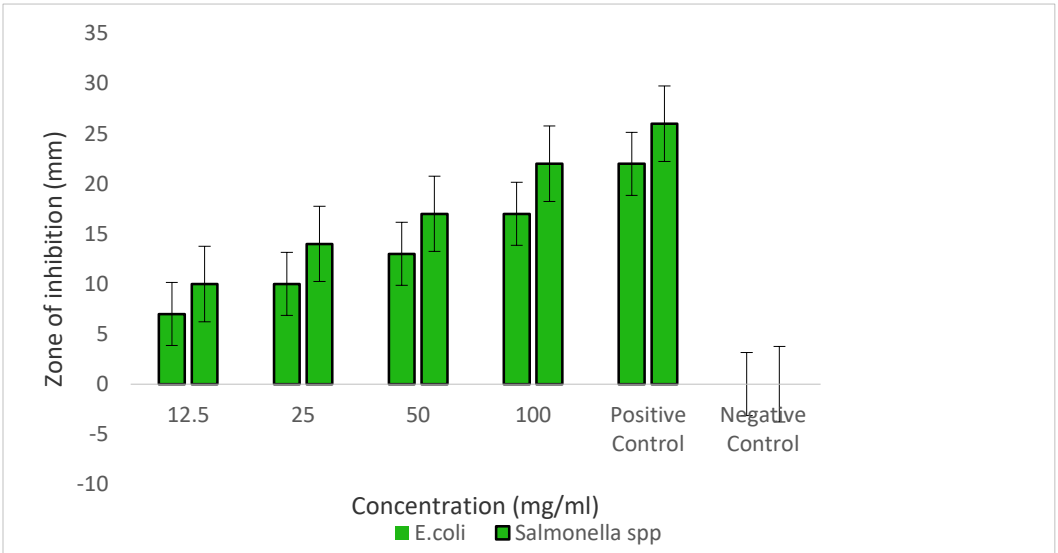

Figure 4.5: Mean Zones of Inhibition for *A. digitata* methanolic extracts.

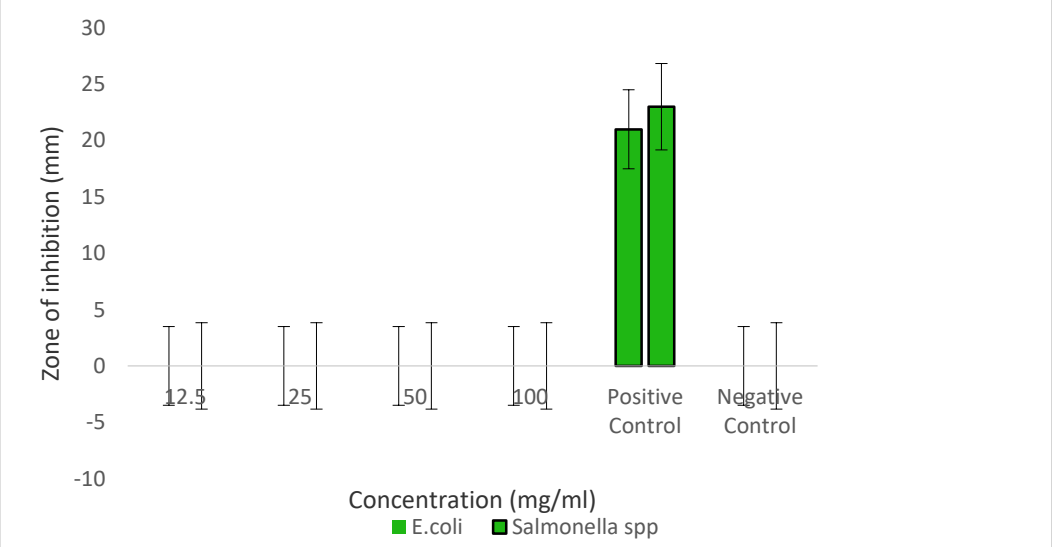

Figure 4.6: Mean Zones of Inhibition of *A. digitata* ethyl acetate extracts

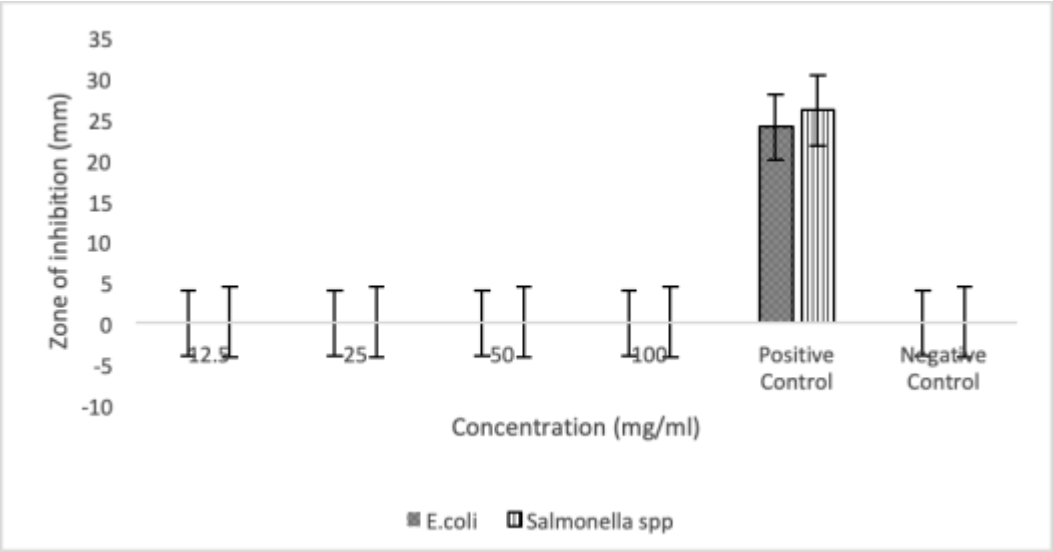

Figure 4.7: Mean Zones of Inhibition of *A. digitata* Aqueous extracts

4.7 FT-IR Spectra Analysis

FT-IR spectra analysis for *A. digitata* aqueous extracts showed the presence of alkanes, allenes, carbon dioxides, halo compounds, nitro compounds, and isothiocyanate functional groups. *A. digitata* ethyl acetate extracts demonstrated the presence of alkenes, aliphatic primary amine, aldehydes, isothiocyanate, carboxylic acids, phenols, amines, tertiary alcohols, primary alcohols, and halo compounds. While *A. digitata* methanolic extracts revealed the presence of alkanes, carboxylic acids, ketones, sulfonate, amines, anhydride, and halo compounds (Appendix H).

CHAPTER 5: DISCUSSION

5.1 Bacteria Associated with foodborne illnesses

The purpose of the study was to determine the antimicrobial activity of *Adansonia digitata* leaf extracts against bacteria associated with foodborne illnesses. The results confirm that *A. digitata* leaf extracts have significant antimicrobial effects against the isolated bacteria, *E coli* and *Salmonella spp.*

5.2 Antimicrobial Susceptibility of *A. digitata*

In this study, the *A. digitata* methanolic leaf extracts had significant antimicrobial effects against *E. coli* and *Salmonella*, the isolated bacteria whilst ethyl acetate and the aqueous extract showed no antimicrobial activity. The observed differences in antimicrobial activity between *A. digitata* leaf extracts could be due to several factors, methanol is a polar solvent and is more effective at extracting bioactive compounds with antimicrobial properties such as phenolic compounds, tannins, and alkaloids. So this shows that methanol is more effective at extracting antimicrobial compounds from *A. digitata* leaves than ethyl acetate and water. The ethyl acetate extracts could have been affected by the extraction conditions which resulted in insignificant antimicrobial activity. The effectiveness of the results in this study could have been affected by the use of the Nutrient Agar in the disk diffusion assay because nutrient agar contains a variety of nutrients that can mask the growth-inhibitory effects of the extracts. A better medium for a disk diffusion assay would be Mueller-Hinton agar, which has minimal nutrients and is optimized for the growth of bacteria as this will allow for a more accurate assessment of the extract's ability to inhibit the growth of bacteria.

The highest percentage yield obtained in this study was of *A. digitata* methanolic extract, followed by ethyl acetate and the least was aqueous extract. The highest yield obtained could have been attributed to the ability of methanol to dissolve polar and semi-polar compounds including many bioactive compounds from the plant. Ethyl acetate yields could have been affected by the fact that ethyl acetate may be more selective in extracting non-polar compounds from the *A. digitata* leaf. Water, a polar solvent had the least yield because of its ability to only dissolve polar compounds from the plant. The findings were similar to that of (Bayot & Bragg, 2022).

5.3 Phytochemicals in *A. digitata* leaf extracts

The study revealed the presence of phytochemicals such as alkaloids, flavonoids, phenols, saponins, and tannins in *A. digitata* leaf extracts. The study also for the first time, revealed that *A. digitata* leaf extracts have phytochemical components that act as protective barriers against bacteria causing foodborne illnesses. However, further research is required to understand the mechanisms underlying these effects. In this study, FT-IR analysis of *A. digitata* leaf extracts revealed the presence of several functional groups such as alcohol, carbon dioxide, alkanes and amines.

CHAPTER 6: CONCLUSION

6.1 CONCLUSION

In conclusion, the study has shown that *Adansonia digitata* leaf extracts have antimicrobial effects against *E.coli* and *Salmonella,* the bacteria associated with foodborne illnesses. However, there were only two bacteria species used in this study and yet literature has many bacteria species associated with foodborne illnesses. The methanolic extracts of *A .digitata* leaves were found to be more effective in inhibiting bacterial growth compared to the ethyl acetate and aqueous extract. Findings suggest that methanol may be able to extract bioactive compounds from *A.digitata* leaves. *A. digitata* revealed its bactericidal effect against bacteria associated with foodborne illnesses. This can be used to develop natural remedies and reduce the reliance on antibiotics. These findings suggest that *A. digitata* leaf extracts could have a potential use in food safety applications and disease control.

6.2 RECOMMENDATIONS

- There is a need to determine whether the antimicrobial effects of other tree parts, such as barks, fruit seeds, and fruit pulp, are the same as that of *A. digitata* leaves.
- All other parts of *A. digitata* could be combined to see if their combined effect is the same as that of the individual.
- There is a need to use Analytical Profile Index (API) kits as they are highly specific, highly sensitive, and reasonably priced for identifying a wide range of bacteria linked to foodborne illnesses.

- For better results, the antimicrobial activity of *A. digitata* might be assessed further using different solvent extraction techniques.
- The precise lowest concentration of *A. digitata* leaf extracts required to inhibit bacterial growth can be found quantitatively using the minimum inhibitory concentration (MIC) test.
- The study was done in vitro so there is a need for further in vivo studies to determine if these effects occur in vivo.
- There is a need to determine the original concentrations of the active compounds found in the leaves.
- There is a need to understand the precise mechanisms by which the active compounds carry out their antimicrobial effects.

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APPENDICES

Appendix A *Salmonella spp*

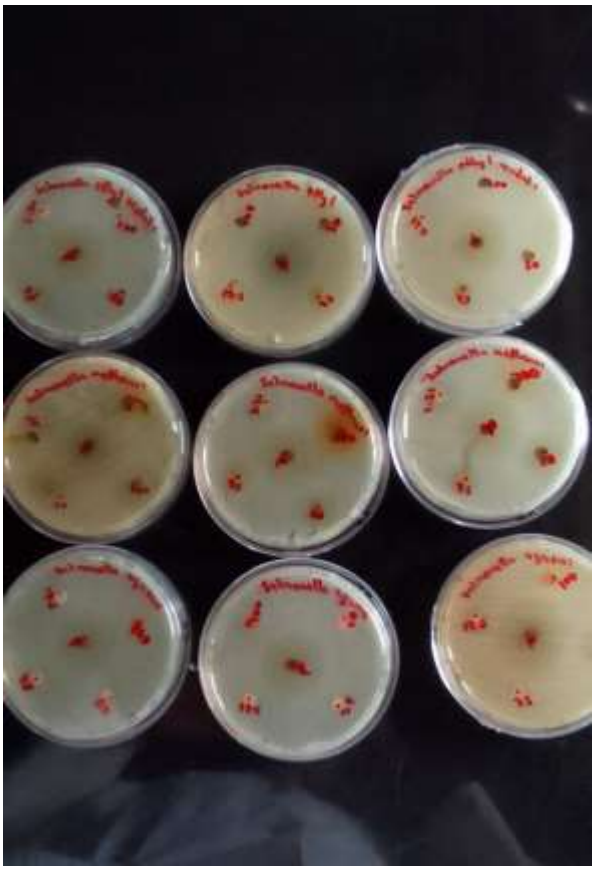
Appendix B *E coli* in EMB Agar

Appendix C Yield of Extracts

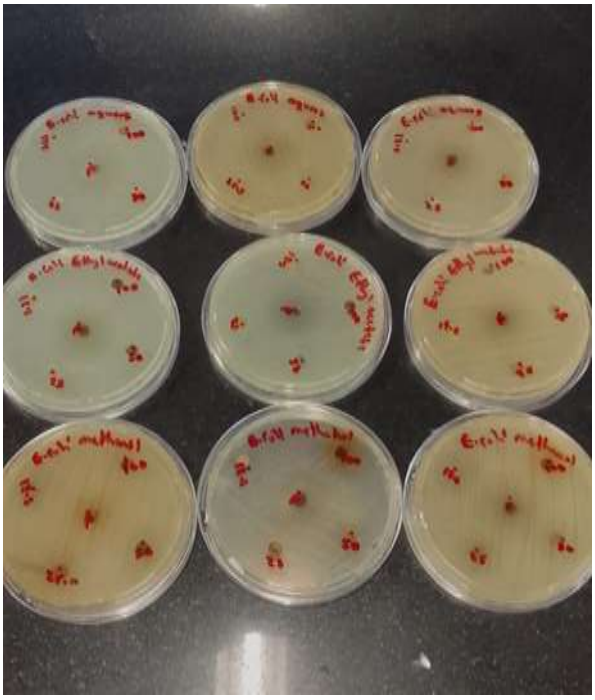
$$\% \text{ Yield} = \frac{Amount\ of\ extract\ recovered}{Initial\ weight} \times 100$$

1. Methanol extract: $\frac{4.10}{20} \times 100 = 20.5\%$
2. Ethyl acetate extract: $\frac{3.2}{20} \times 100 = 16\%$
3. Aqueous extract: $\frac{2.6}{20} \times 100 = 13\%$

Appendix D Zones of Inhibition of Methanol, ethyl acetate, and aqueous extracts against *Salmonella spp*

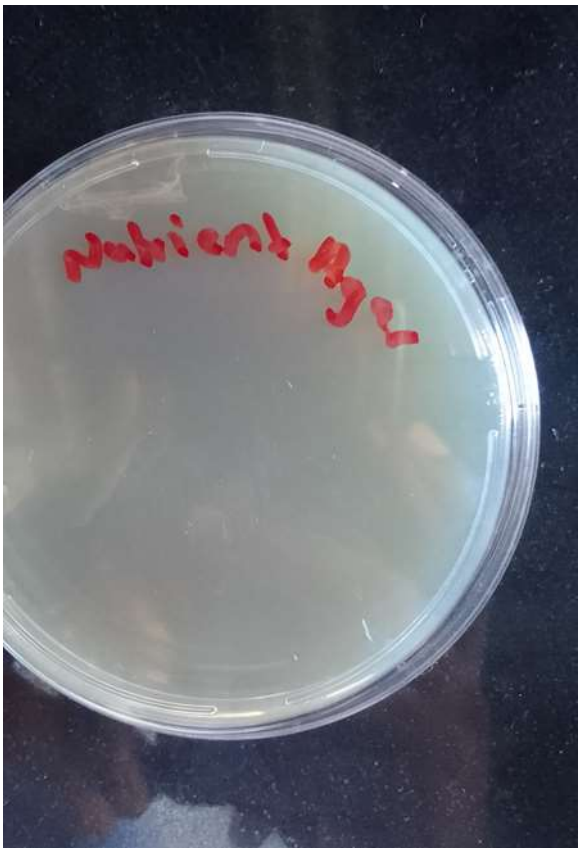


Appendix E Zones of Inhibition of Methanol, ethyl acetate, and aqueous extracts against *E coli*



Appendix F Negative Control (disks impregnated with methanol, ethyl acetate and aqueous)

Appendix G Sterile Nutrient Agar



Appendix H FT-IR spectra for *A. digitata* leaf extracts