Bindura University of Science Education

FACULTY OF SCIENCE

DEPARTMENT OF BIOLOGICAL SCIENCES

To investigate the antimicrobial activity of *Datura* plant leaf extracts against *Candida albicans* and *Staphylococcus aureus*, pathogens responsible for skin infections.

BY

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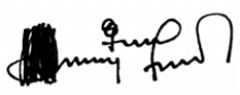
A research project submitted in partial fulfilment of the requirements for the Bachelor of Science Honours Degree in Biological Sciences.

June 2026

Approval Form

The undersigned certify that they have read the dissertation titled 'To investigate the antimicrobial activity of *Datura* plant leaf extracts against *Candida albicans* and *Staphylococcus aureus*, pathogens responsible for skin infections' and confirm that it is suitable for submission to the Biological Sciences Department, Faculty of Science and Engineering, for assessment.

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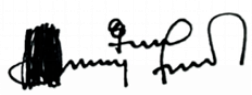
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I, Dr Claudious Gufe, declare that I have supervised this thesis and I am satisfied that it can be submitted to the Biological Sciences Department, Faculty of Science and Engineering, at Bindura University of Science Education.

Signature:  Date: 19/06/2026

Dedication

This project is dedicated to my family, whose unwavering love and support have been my greatest source of strength throughout this journey. Your sacrifices and encouragement made this possible, and every achievement here belongs to you as it does to me.

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First, I would like to thank God Almighty for his protection and guidance throughout the whole process. Secondly, I want to give my gratitude to my mother and her relatives for their financial support in this project. I would also like to express my heartfelt gratitude to the senior laboratory technicians, Mr Mphiri, Mr Hatidani, and Dr Chibukira, for their invaluable technical support, guidance on laboratory procedures, and their generosity in sharing their knowledge. Special appreciation goes to the chief technician, Mr Arshley, for his constant support and coordination and for ensuring that all necessary laboratory resources were available.

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LIST OF ACRONYMS

PDA – Potato Dextrose Agar

SDA – Sabouraud Dextrose Agar

AMR – Antimicrobial Resistance

MRSA – Methicillin-Resistant *Staphylococcus aureus*

MIC – Minimum Inhibition Concentration

MBI – Minimum Bacterial Inhibition

MFC – Minimum Fungicidal Concentration

CFU – Colony Forming Units

HCl – Hydrochloric acid

TTC – 2,3,5-Triphenyltetrazolium chloride

NaOH – Sodium hydroxide

$BaCl_2$ – Barium chloride

$2H_2O$ – Two molecules of water (water of crystallization; e.g., dihydrate)

H_2SO_4 – Sulfuric acid

µm – Micrometer

mL – Milliliter

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Abstract

Skin infections caused by microbial pathogens remain a significant public health concern, particularly with the increasing emergence of antimicrobial resistance. This study focused on investigating the antimicrobial activity of *Datura stramonium* leaf extracts against *Candida albicans* and *Staphylococcus aureus*, which are common causative agents of skin infections. Crude extracts of *Datura* leaves were prepared using three different solvents, which are absolute alcohol, chloroform, and distilled water, to assess the influence of solvent polarity on phytochemical extraction and antimicrobial efficacy. Revived cultures of ATCC 25923 for *S. aureus* and ATCC 10231 for *C. albicans* are strains that were used in this project. Firstly, they were identified by their morphological features after Gram staining and lactophenol cotton blue staining. The antimicrobial activity of the extracts was evaluated using standard microbiological techniques including agar well diffusion method and microdilution method, and their effectiveness was determined by measuring zones of inhibition. First concentrations were prepared, which are 100µg/ml, 300µg/ml and 600µg/ml, and used to evaluate the antimicrobial effect of the extracts against these microbial strains. Furthermore, the MIC and MBC/MFC were determined by using a 24-well plate diffusion to establish the lowest concentrations required to inhibit and kill the test organisms. Muller-Hinton agar and PDA were the culture media that were used in all these processes. The findings of this study demonstrated that *Datura stramonium* leaf extracts exhibit antimicrobial activity against both *Candida albicans* and *Staphylococcus aureus*, with variations observed depending on the solvent used. Organic solvent extracts, particularly absolute alcohol and chloroform, showed higher antimicrobial activity compared to aqueous extracts. These results suggest that solvent type may significantly influence the extraction of bioactive compounds and their antimicrobial effectiveness. The data collected was analyzed using one-way ANOVA, and bar charts were also used to interpret the data. Due to differences in zones of inhibition of plant extracts from different solvents, there was a significant difference in using solvents highlighted to extract phytochemicals against *S. ureus* and *C. albicans.* This study suggested that the alkaloid content in this plant is high which makes it more poisonous; on the other hand, absolute alcohol and chloroform can both extract this phytochemical. This might be the reason why the extract from these solvents has a greater zone of inhibition than distilled water. This simply highlights the type of compounds being extracted, and the choice of solvent are two things that should be taken into consideration for phytochemical analysis.

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CHAPTER 1
INTRODUCTION

1.1 Background of the Study

Skin infections are one of the most common problems that affect countless people each year, and their effects are quite detrimental to morbidity rates, costs of treatment, and overall quality of life. Due to the penetration of pathogens through breaks caused by wounds, burns, insect stings, or underlying medical conditions, infections like bacterial, fungal, or viral can occur. In developing countries, there are many socio-economic factors that play a role in this, for example poor sanitation, overcrowding, malnutrition, excess moisture, and limited access to health care facilities, which predispose individual to higher risk of infection and make it more difficulty to prevet, diagnose and to treat infectious diseases effectively (Wanga & Nyamboki, 2023).

In addition to this, there is also varying severity in skin infections, which range from conditions that can be quite severe, such as cellulitis, abscess, and necrotizing fasciitis, to conditions that are milder in nature and include impetigo, folliculitis, and fungus infections. There could be pain and other symptoms, and in cases where the infections are severe, there could even be life-threatening results (Richard et al., 2022).

Candida albicans and *Staphylococcus aureus* are microbes that have been a threat to animals and humans in causing skin infections and a threat to human health. A typical part of the human microbiota, *Candida albicans* is an adaptive fungal infection that can turn harmful in certain situations. It is responsible for a range of infections, from superficial conditions like cutaneous candidiasis to invasive systemic infections (Bongomin et al., 2017); (Pappas et al., 2015).

The capacity of *Candida albicans* to build biofilms, generate hydrolytic enzymes, and change morphologically from yeast to filamentous forms is largely responsible for its pathogenicity.

According to (Silva et al., 2012), biofilm formation is associated with increased difficulty in treating infections and a higher tendency for reinfection, as it promotes resistance to antifungal agents and immunological response of the host.

Staphylococcus aureus is a Gram-positive bacterium found on the surface of the skin and nasal cavity of healthy persons. When it enters the body through skin cracks and causes infections including abscesses, cellulitis, boils, impetigo, and wound infections, it can

become pathogenic. In severe cases, it can lead to invasive diseases such as bacteraemia and endocarditis (Cheung et al., 2021a).

The emergence of antibiotic-resistant forms of *S. aureus*, especially MRSA, has made treating infections difficult. Due in large part to the abuse and overuse of antimicrobial medications, antimicrobial resistance (AMR) is increasingly becoming a danger to world health. This has led to increased treatment failure, higher healthcare costs, and the urgent need for new antimicrobial agents (Guo et al., 2020).

Medicinal plants have long been used as alternative sources of treatment, particularly in rural and resource-limited settings where access to conventional medicine is limited. These plants have a variety of bioactive substances with antibacterial, anti-inflammatory, and wound-healing qualities, including alkaloid compounds, flavonoids, tannins, phenolics, and saponins. The continued reliance on herbal medicine highlights the importance of scientifically validating these plants to ensure their safety and efficacy (Yuan et al., 2016).

One plant of particular significance is the *Datura* species, which are members of the Solanaceae family. *Datura*, often referred to as devil's trumpet or thorn apple, is found throughout tropical and subtropical countries and has long been used to cure a variety of ailments, such as pain, inflammation, and skin infections (Nasir et al., 2020).

Datura active compounds like alkaloids can reduce swelling, ease pain, and stop bacteria from growing. *Datura* is extremely toxic, though, and misuse can result in fatal poisoning. Nevertheless, controlled experiments have shown that *Datura* extracts have encouraging antibacterial action against bacteria and fungi (Joy Nkeiruka et al., 2021).

The leaves of *Datura* plants have been commonly used as topical treatments or in herbal medications for the treatment of skin infections in alternative medicine. This means that there are chemical compounds within the plant that can be used to protect against pathogens.

Extraction of plant bioactive compounds depends on the solvent used, as different solvents extract different classes of phytochemicals based on their polarity. Flavonoids and phenolic substances can be effectively extracted using alcoholic solvents; chloroform extracts non-polar compounds such as alkaloids, and aqueous solvents extract tannins and glycosides. Using multiple solvents increases the likelihood of obtaining a wide range of bioactive compounds with antimicrobial activity (Solomon et al., 2017).

Standard microbiological procedures such as disc diffusion, agar well diffusion, and broth dilution are primarily used to assess the antibacterial activity of plant extracts. These methods allow researchers to observe the efficiency of extracts against certain bacterial strains.

Furthermore, determining the MIC and MBC/MFC provides important information regarding the efficacy and therapeutic potential of the extracts

Disc diffusion method, Agar well diffusion method and Broth Dilution method are some common standard methods employed in microbiology for studying the antibacterial effect of plant extracts. In addition, finding out the MIC and MBC/MFC is significant since it helps in understanding about the effectiveness of these extracts (Balouiri et al., 2016). The main objective aims to examine the antibacterial action of *Datura* leaf extracts against *S. aureus* and *C. albicans*. This experiment entails the extraction of active ingredients with the use of such solvents as absolute alcohol, chloroform, and distilled water, followed by evaluating the antimicrobial action of those using standard laboratory techniques. Also, the experiment will attempt to establish the MIC and MBC/MFC values for evaluation of the effectiveness of the extract.

Importance of this study lies in its potential to improve the effectiveness of traditional methods used in medicine, as well as in development of new drugs. In cases when financial possibilities are limited, herbal treatment can be viewed as a replacement of traditional treatment. Also, the findings of this study may help to develop medications for skin disorders.

Datura leaves' study constitutes a very important bridge connecting traditional practices with modern science because it allows one to base further studies on searching for, characterizing, and testing the safety and efficacy of different biologically active substances.

1.2 Problem Statement

Skin infections caused by *Candida albicans* and *Staphylococcus aureus* are widespread and represent a significant health burden, particularly in rural communities where access to modern healthcare is limited and in immunocompromised and hospitalised patients. Even though there is access to conventional drugs for these opportunistic skin pathogens, the growing problem of antimicrobial resistance has reduced the effectiveness of conventional drugs, making treatment more difficult and costly (Lee Ventola, 2015). This resistance limits the efficacy of popular topical and systemic antibiotics and antifungals. So, there is a need for urgent finding new effective and affordable substitute therapeutics to manage these drug-resistant pathogens. Many traditional medical systems use botanical therapies for skin conditions because plants have historically been a rich source of antibacterial chemicals. Traditional medicine continues to be the predominant form of healthcare in many Zimbabwean rural communities. *Datura spp* have long been used in folk medicine to treat

boils, wounds, skin irritations, and other skin conditions. The antibacterial effectiveness of *Datura* plant extracts, especially against *S. aureus* and *Candida albicans* in connection to skin infections, has, however, received little attention.

The existing research work is sporadic in nature, and at times it lacks mechanisms, phytochemical analysis, or standardization procedures. The antibacterial properties of the leaves of *Datura* should be studied in detail in view of this lack of information.

1.3 Justification

First of all, the research presents an opportunity to evaluate the effectiveness of a plant that has been used through the ages traditionally on a scientific basis. The study will reveal how well *Datura* leaf extracts work against *Candida albicans* and *Staphylococcus aureus*. It is particularly important because in the countryside people can have no access to regular medications and use traditional ones instead (Nyagumbo et al., 2025).

Secondly, the study makes a contribution towards the worldwide campaign against antibiotics resistance due to the search for plant-based options. *Datura*'s antimicrobial action can be confirmed, which may open up new avenues for medication development. Researchers have been known to find a multitude of physiologically active chemicals in nature. Lastly, the findings from this paper might help produce affordable topical preparations such as creams. Such preparations can reduce dependency on artificial medications and contribute to medicine in the long run.

This research also closes the gap between the past experience and modern times. The research ensures that the valuable ethnobotanical information is not lost but rather further developed through the rigorous testing of traditions. The significance of the research increases on both the local and international levels through this connection.

1.3 Objectives of the Study

1.3.1 Main Objective

To investigate the antimicrobial activity of *Datura* plant leaf extracts against *Candida albicans* and *Staphylococcus aureus*, pathogens responsible for skin infections.

1.3.2 Specific Objectives

1. To determine the physicochemical composition of crude extracts of *Datura* leaves using suitable solvents (absolute alcohol, chloroform, and distilled water).
2. To evaluate the antimicrobial activity of the extracts against *Candida albicans* and *Staphylococcus aureus* using standard microbiological assays.

3. To determine the minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) of the extracts.

1.4 Research Questions

- Do *Datura* leaf extracts exhibit inhibitory effects against *Candida albicans* and *Staphylococcus aureus*?
- What concentrations of the extracts are required to inhibit or kill these pathogens?

1.5 Hypothesis

1st Null Hypothesis (H_0)

There are no significant antimicrobial effect of the *Datura* leaf extracts against *Candida albicans* and *Staphylococcus aureus*.

1st Alternative Hypothesis (H_1)

There is significant antimicrobial activity of *Datura* leaf extracts against *Candida albicans* and *Staphylococcus aureus*.

2nd Null Hypothesis

There is no significant difference in using different solvents for plant extract when evaluating the antimicrobial effects of *Datura stramonium* leaf extract against *Candida albicans* and *Staphylococcus aureus.*

2nd Alternative Hypothesis

There is a significant difference in using different solvents for plant extract when evaluating the antimicrobial effects of *Datura stramonium* leaf extract against *Candida albicans* and *Staphylococcus aureus.*

1.6 Significance of the Study

This study's prospective contributions to science, healthcare, and society make it significant. Due to antimicrobial resistance and limited availability of effective medications in rural communities, skin infections caused by *Candida albicans* and *Staphylococcus aureus* are frequent and frequently challenging to treat (Davis et al., 2015a). This study offers a chance to verify conventional methods and investigate novel therapeutic approaches by examining the antibacterial qualities of *Datura* leaves.

From a scientific perspective, the study will give information empirically on the antibacterial potential of *Datura* leaf extracts. This is important as although there have been studies which show the medicinal value of the plant, there has been very little research done on its ability to fight certain types of infection, such as *Staphylococcus aureus* and *Candida albicans* (Kacholi, 2024). The findings may contribute to the growing body of knowledge on medicinal plants and their potential role in battling infections.

In terms of medicine, the research may offer cheap and readily available means for treating skin infections in distant areas. Antifungal and antibacterial treatments are not always available and affordable, especially in underdeveloped areas. The verification of the antibacterial effect of *Datura* leaves could facilitate the creation of inexpensive and culturally acceptable topical treatments, like ointments.

In terms of pharmacology, it may also help create new pharmaceuticals. Natural sources of medicines are known to be a good source of bioactive substances, which *Datura* might contain (Mukherjee Singh & Rajeep Singh, 2013a). Particularly, identifying and characterizing these molecules may open the door to novel treatments.

The study bridges the gap between traditional knowledge and contemporary science from a socio-cultural perspective. Indigenous methods frequently provide insightful information about managing illness, but they remain anecdotal and possibly dangerous in the absence of scientific confirmation. The study guarantees the preservation of traditional knowledge while advancing safety and efficacy by putting *Datura* through a rigorous scientific examination. This increases
the research's significance on a local and international level.

1.7 Scope of the Study

The objective of study is to evaluate the antimicrobial activity of the Datura leaf extract against Staphylococcus aureus and Candida albicans. The methods involved in the testing process include agar well diffusion method and broth dilution method in determining zone of inhibition, MIC (minimum inhibitory concentration) and MBC/MFC (Minimum Bactericidal/ Fungicidal Concentration). This study is confined to in-vitro study and does not involve any clinical trial in human subjects nor any test on human toxicity.

1.8 Limitations of the Study

First, the research has been conducted in a lab setting only, where the complicated reactions in vivo might not have been captured. Second, the toxicity of the compounds extracted from *Datura* will pose problems for the usage of the alkaloids without additional purification. (KORKMAZ et al., 2019). Third, the resource constraint could lead to the limitation of the solvent options and the extraction process, hence impacting on the comprehensiveness of the results. Finally, the research lacks the focus on the stability of the ointments in question.

1.9 Definition of terms

- *Datura stramonium*: Is a poisonous medicinal plant belonging to the family Solanaceae (Rasila Devi et al., 2011).
- Antifungal susceptibility: Is a method used in laboratories to assess how sensitive or resistant fungal organisms are to antifungal medications (Aneke et al., 2018).
- Antibacterial susceptibility: Is a laboratory technique that assesses how well antibiotics work against particular bacterial infections (Cunliffe et al., 2021).
- Boils: Also called furuncles, boils are painful, pus-filled skin illnesses that form in oil glands or hair follicles (Silverberg, 2021).
- Cellulitis: Is a bacterial infection that affects the deeper layers of the skin and underlying tissues (Silverberg, 2021).
- Maceration: An extraction technique in which plant material is soaked in a suitable solvent for a period of time to allow the soluble bioactive compounds to dissolve into the solvent(Bryda & Stadnytska, 2021).

CHAPTER 2
Literature Review

2.1 History of Datura stramonium

The genus *Datura* has thousands of years of ethnobotanical history. *Datura stramonium* is a member of the Solanaceae family and is frequently referred to as Jimson weed or thorn apple. Historical records indicate that *Datura* species were used in ancient civilizations such as India, China, and the Americas for medicinal, spiritual, and ritualistic purposes (Särkinen et al., 2018). Asthma, wounds, ulcers, and skin disorders were mostly all treated with datura in Ayurvedic medicine. Native American tribes used the plant for its psychoactive and analgesic properties, particularly in ceremonial rites (Sun et al., 2024).

The genus *Datura* is thought to be named after the Sanskrit word "Dhatura," because of its early mention in the Indian traditional medicinal system. After colonial explorations, there was an increase in the European documentation of the plant during the 16th and 17th centuries. *Datura stramonium* was scientifically documented by Carl Linnaeus in Species Plantarum in 1753. Despite being notorious for its poisonous alkaloids, traditional herbalists have been using the antimicrobial and anti-inflammatory qualities of the plant in skin infection treatments. (Kaur, 2020).

Datura are well naturalized and used as medicinal herbs in Africa, including Zimbabwe. Zimbabwean herbalists have used crushed leaves for topical application in treating boils, fungi, and inflammations (Muvengwi & Maroyi, 2025).Despite its toxicity, controlled usage

in ethnomedicine has persisted, prompting scientific investigations into its antimicrobial potential.

2.2 Features and Historical Classification

Large, ovate leaves with uneven lobes, trumpet-shaped white or purple flowers, and spiny capsule fruits with lots of seeds are the characteristics of the annual herbaceous plant *Datura stramonium*. The plant thrives on disturbed soils, along roadsides, and on agricultural land, and it has a powerful smell (Ünal et al., 2025).

Taxonomically, *Datura* belongs to:

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Solanales
- Family: Solanaceae
- Genus: *Datura*
- Specie: *stramonium*

The historical classification of the plant species *Datura* was that of the Solanaceae family along with other alkaloid-containing species like *Atropa belladonna*. The early classification of species was done based on flower structure, fruit spines, and seeds. (Särkinen et al., 2018). Modern phylogenetic studies using molecular markers have confirmed the distinctiveness of *Datura* species while maintaining their placement within *Solanaceae*.

The plant's classification as a harmful or medicinal plant has been impacted by its bioactive components, especially atropine, scopolamine, and hyoscyamine. Because of these characteristics, it is both potentially dangerous and helpful pharmacologically.

2.3 Discovery and Scientific Recognition

Despite the plant being in use since ancient times, it was not until the 19th century that its chemical components were scientifically discovered. The first extraction of atropine from solanaceous plants by German scientists was conducted at the beginning of the 19th century (Carpa et al., 2017). Later studies found scopolamine and hyoscyamine to be among the primary alkaloids accountable for the medicinal properties of the plant. In the 20th century, the antibacterial and antifungal properties of *Datura* leaves were recognized due to scientific experiments conducted in the lab (S et al., 2008a). Studies have now been carried out for the efficacy of crude extract preparation with solvents like ethanol, methanol, and water. They

showed efficacy against bacteria and fungi proving its traditional use as an anti-skin infection drug (Mukherjee Singh & Rajeep Singh, 2013b).

2.4 Phytochemistry of *Datura stramonium*

Datura stramonium is characterized by the presence of a variety of bioactive chemical compounds, particularly alkaloids, which contribute to its pharmacological and toxicological properties. Tropane alkaloids are the most bioactive compounds found in this plant and are mainly concentrated in the leaves, seeds, and roots of the plant and are responsible for many of its medicinal effects, including anticholinergic, antispasmodic, and sedative activities, according to (Joy Nkeiruka et al,. 2021).

In addition to tropane alkaloids, *Datura stramonium* also contains other phytochemical constituents such as flavonoids, tannins, phenolic compounds, saponins, and glycosides, which contribute to its antioxidant and antimicrobial properties (Satina & Blakeslee, 1941). In many traditional medical systems, these phytochemicals from the plant are used to treat infections, pain, and asthma. *Datura stramonium* has therapeutic promise, but when administered incorrectly, its high amounts of toxic alkaloids make it a very hazardous plant. Therefore, pharmaceutical research and the safe use of plant-based medications depend on an understanding of its phytochemical makeup.

2.5 Introduction to *S. aureus* and *Candida albicans*

Candida albicans and *Staphylococcus aureus* are both major opportunistic pathogens which infect human beings in different ways. *Staphylococcus aureus* is a gram-positive bacterium which is usually found in the skin and nose of healthy individuals, but once they enter the body via wounds or medical devices, they cause infection. This infection ranges from mild skin and soft tissue infections to serious conditions such as endocarditis, pneumonia, and blood stream infections (Tong et al., 2025). Along the same lines, *Candida albicans* is a fungus which usually lives in the mouth, digestive system, and urogenital system as part of the normal flora of the human body. However, in certain situations, especially when the body's immunity is low or there is an imbalance of the microflora, this type of fungus may lead to candidiasis infections, such as oral thrush and vaginal candidiasis. (Chen et al., 2020). These diseases affect more immunocompromised people including those suffering from HIV/AIDS, diabetes, or even under antibiotic treatment for prolonged periods of time. The bacteria *S. aureus* and *Candida albicans* remain an important issue in both communities and hospitals due to their ability to cause both mild and life-threatening diseases.

2.5.1 *Staphylococcus aureus* and Skin Infections

Gram-positive *S. aureus* bacteria normally inhabit human skin and mucosa. It is referred to as an opportunist organism that causes disease such as infection of the soft tissues and skin. Some of the mild infections include impetigo and boils while some serious cases of the infections include cellulitis (Touaitia et al., 2025a). There are many virulent features in *S. aureus* which make the bacterium colonize the skin causing diseases. The feature that makes *Staphylococcus aureus* bind to the host's body parts is an exceptional feature of the bacterium. The bacteria produce a substance known as adhesins which are proteins on the surface enabling the bacteria to stick to skin cells and injured tissues. Clumping factor and fibronectin-binding proteins are types of adhesins (Touaitia et al., 2025). *S. aureus* will multiply on attachment, producing enzymes and toxins that lead to tissue injury.

This bacterium contains virulent genes that helps it to cause skin infection. This organism will produce several toxins including alpha toxin and Panton-Valentine Leukocidin. Host cell injury caused by these toxins leads to infection. The enzymes such as lipase, hyaluronidase, and coagulase destroy host tissue structures (Howden et al., 2023). Bacterial communities that produce an outer layer enhancing their resistance to antimicrobial drugs and the body's defence mechanisms are called biofilms. Biofilm production enables *S. aureus* to survive on skin and medical devices, causing repeated infections (Pinho et al., 2025).

Further, there is a significant risk posed by MRSA in terms of public health because the *Staphylococcus aureus* bacteria have developed a form of treatment resistance. Resistance can lead to recurrence of infections on the skin (Lewis et al., 2018). The necessity for substitute antibacterial agents, including plant extracts, is highlighted by the increasing resistance.

2.5.2 *Candida albicans* and Skin Infections

Another common opportunistic fungal pathogen in the typical human microbiome is *C. albicans*. Under normal circumstances, it lives on the skin, mouth cavity, gastrointestinal system, and genital mucosa without infecting them. However, when host immunity or skin integrity is weakened, *Candida albicans* can cause infections ranging from superficial skin infections to systemic illnesses (Xiao et al., 2018).

One of the most significant virulence features of *Candida albicans* is its capacity for morphological changes. The pathogen is also more virulent because it can be present in the yeast, pseudohyphal, and hyphal stages. The tissue invasion becomes much easier with the

conversion from the yeast to the hyphal stage, where they have the capacity to invade and damage host tissues (Thomas-Rüddel et al., 2022).

The organism produces adhesins, making the organism able to stick to the skin surface as well as the mucosal membrane. Once the organism has successfully stuck, *Candida albicans* will invade the host tissues and produce hydrolytic enzymes responsible for causing the infections and damaging tissues, including lipase, phospholipase, and protease. The production of biofilms is among the major reasons *Candida albicans* infects the skin tissues. Biofilms will allow the organism to reside in the host body to develop chronic diseases (Moncho et al., 2024). Furthermore, *Candida albicans* is known for its adaptability to different environments such as changes in temperature and pH. Adaptation is an important factor that contributes to virulence and survival skills. *Candida albicans* can cause infections due to its immunity against the immune response of the host body.

2.6 Mechanisms of Antimicrobial Action

The existence of different bioactive phytochemicals, including alkaloids (particularly tropane alkaloids like atropine and scopolamine), flavonoids, tannins, saponins, and phenolic compounds, is largely responsible for the antimicrobial activity against bacterial and fungal pathogens. In both bacterial and fungal cells, these phytochemicals interfere with vital cellular functions through a variety of strategies (Balouiri et al., 2016b).

The breakdown of the integrity of the microbial cell wall and membrane is one of the main mechanisms. When substances like phenolics and saponins interact with membrane lipids, they increase permeability and cause essential intracellular components to flow out, which eventually results in cell death. This disruption interferes with ergosterol-containing membranes in *Candida albicans*, causing fungal cell lysis, whereas it disrupts peptidoglycan stability and osmotic balance in *Staphylococcus aureus* (Mphahlele et al., 2020).

These compounds can halt the growth and proliferation of microorganisms through blocking crucial enzymes involved in transcription and replication. Furthermore, it has been demonstrated that flavonoids and tannins affect metabolic pathways, block microbial enzymes, and create complexes with proteins, all of which worsen cellular function (Das et al., 2010).

Furthermore, reactive oxygen species (ROS), which harm biological components like lipids, proteins, and DNA, can be produced by several phytochemicals found in *Datura* extracts. The bactericidal and fungicidal actions often seen at higher extract concentrations are caused

by this oxidative damage, which is in line with the discovery of MBC and MFC in antimicrobial investigations (S et al., 2008).

The kind of solvent employed during extraction may also affect the efficacy of these antimicrobial processes because various solvents extract different types of phytochemicals. For instance, compared to aqueous extracts, organic solvents like ethanol and chloroform are more effective at extracting non-polar and moderately polar chemicals like flavonoids and alkaloids, which frequently show better antibacterial activity. Sometimes, the idea that the choice of solvent has a major impact on the antibacterial effectiveness of *Datura stramonium* leaf extracts is supported by this variance (Bongomin et al., 2017).

2.7 Global Impact of Candida albicans and Staphylococcus aureus

Candida albicans is frequently results in vaginal candidiasis, mouth thrush, and skin infections, especially in immunocompromised people. Due to growing antifungal resistance, candidiasis is a significant global health concern. *Candida albicans* can cause infections ranging from minor to serious systemic disorders when the host's immune system is compromised by diseases including HIV/AIDS, cancer treatment, diabetes, or long-term antibiotic usage. Oral candidiasis, vulvovaginal candidiasis, and invasive candidiasis are among the most common clinical signs. Worldwide, certain fatality rates are associated with Candidemia, or *Candida* bloodstream infection, particularly in hospitalized and immunocompromised patients. In addition, the emergence of antifungal resistance, especially to commonly used drugs such as fluconazole, has made treatment more challenging and costly (Mhondoro et al., 2019).

As HIV/AIDS is widespread among people in Sub-Saharan Africa, this part of the world suffers from a high rate of *Candida albicans* infections. According to a literature review of studies performed in Africa, the prevalence of oral candidiasis among HIV-positive patients is between 7.6% and 75.3% and involves the presence of *Candida albicans* (Bongomin et al., 2022). Also, studies have shown that up to 50 percent of people who are HIV positive in Africa may suffer from oropharyngeal candidiasis somewhere along the course of their condition. This shows the importance of *Candida albicans* as a causative agent for opportunistic infections in immunocompromised individuals (Bähler et al., 2025).

These pathogens increase the global burden of diseases considerably, particularly in hospitals. The search for other methods to fight these infections, including the development of plant-based antimicrobial compounds, has been expedited by bacterial resistance to antibacterials. *Staphylococcus aureus* has also emerged as a significant global pathogen as one of the

common sources of bacterial infections in the community and in the hospital environment. It is found in the skin and nostrils of healthy people but causes diseases once it gets access to the body via cuts or invasive instruments. According to (Cheung et al., 2021b), the infections caused by *S. aureus* could be as minor as skin infections such as boils and abscesses, but sometimes may prove fatal in form of pneumonia, endocarditis, sepsis and toxic shock syndrome. The increasing concern in today's world about staphilococcus infections is that there have been many cases of the development of resistant *S. aureus* bacteria, especially Methicillin-resistant *Staphylococcus aureus* (MRSA). This bacterium is difficult to treat due to its resistance to various commonly prescribed medications (Tong et al., 2025).

2.8 Regional Impact

Infection rates, absence of health services, and diagnostics laboratories make the problem of pathogens even more urgent in countries such as Zimbabwe and Sub-Saharan Africa. Skin infections that can be attributed to the bacteria *Staphylococcus aureus* and *Candida albicans* are widespread in Zimbabwe, particularly in rural areas without medical services (Pfavayi et al., 2021). HIV prevalence, inadequate sanitation, and overcrowding all raise a person's vulnerability to opportunistic illnesses.

Fungal infections, such as candidiasis, are a significant public health concern in Zimbabwe, according (Gharehbolagh et al., 2020). The number of people who suffer from fungal infections in the country amounts to more than two million, which translates to approximately 15% of the population. Some of the common fungal infections include oral candidiasis, oesophageal candidiasis, and recurrent vulvovaginal candidiasis. The fact that the immuno-compromised individuals are prone to fungal infections means that the presence of HIV infection in Zimbabwe exacerbates the situation. As indicated in some of the clinical studies carried out in Zimbabwe, oral candidiasis is one of the most common lesions among HIV infected individuals (Mahdi & Muhsen, 2025). These infections can cause severe discomfort, difficulty swallowing, malnutrition, and reduced quality of life for affected patients.

Treatment outcomes have been exacerbated by the growth of antibiotic resistance in Southern Africa (Osaigbovo et al., 2024). In this regard, many Zimbabweans seek medical attention in the use of ethnomedicine such as the use of herbs like the leaves of *Datura*. For the treatment process to be successful, ethnomedicine has to be used together with scientific confirmation.

2.9 Methods of Detection and Diagnosis

Accurate identification allows healthcare professionals to select appropriate antimicrobial therapy and prevent the spread of infection, particularly in hospital environments. Diagnosis

usually involves a combination of clinical examination and laboratory methods such as microscopy, culture, biochemical tests, serological tests, and molecular techniques. The detection of *Staphylococcus aureus* commonly begins with the collection of appropriate clinical specimens depending on the site of infection. These may include blood, wound swabs, sputum, pus, nasal swabs, or urine. According to (Moncho et al, 2024), Gram staining method offers a quick initial indication of the presence of *staphylococcal* bacteria. Culture methods are used to identify further after microscopic examination. Mannitol and other special media are used to inoculate the specimen. The high amount of sodium chloride in mannitol allows *S. aureus* to ferment it to produce yellow colonies due to the formation of acids by *staphylococci* (Tam & Torres, 2019).

Biochemical tests are used to verify the identification of *Staphylococcus aureus* after culture. One of the most commonly used tests is the catalase test, which differentiates staphylococci from streptococci by detecting the catalase enzyme that breaks down hydrogen peroxide into water and oxygen, producing bubbles. Another important confirmatory test is the coagulase test. *S. aureus* produces the enzyme coagulase, which causes clotting of plasma, distinguishing it from other coagulase-negative staphylococcal species (Mauchaza et al., 2016). Additionally, tests for antibiotic susceptibility, such as the disk diffusion (Kirby–Bauer) method, are used to find new resistant strains of bacteria and evaluate whether antibiotics are effective against the isolated bacteria.

The diagnosis of *S. aureus* infections has been significantly enhanced by contemporary molecular approaches. Polymerase Chain Reaction (PCR) can be used to detect specific genes associated with the bacterium, including the *mecA* gene responsible for methicillin resistance. PCR-based diagnostic methods provide faster and more sensitive results than conventional culture methods and are particularly useful in detecting infections early and guiding appropriate treatment (Tong, Davis, et al., 2015).

Detection and diagnosis of *Candida albicans* infections also involve several laboratory techniques. Clinical samples such as oral swabs, vaginal swabs, blood, urine, or tissue biopsies are collected depending on the suspected site of infection. *C. albicans* appears as oval budding yeast cells under a microscope and may form real hyphae or pseudohyphae, which are distinctive characteristics that aid in identification (Richardson & Moyes, 2015). *Candida albicans* can be definitively identified through the use of culture techniques. Sabouraud dextrose agar, a specific fungal growth medium that encourages the growth of yeasts and molds while suppressing the majority of bacteria, is typically used to cultivate clinical specimens. *C. albicans* usually forms smooth, creamy, white colonies on the agar

surface following incubation. The germ tube test, which is frequently used for quick detection of *Candida albicans*, is another crucial diagnostic technique. In this test, yeast cells are treated for two to three hours at 37°C in either human or animal serum. If germ tubes, which are tube-like outgrowths from yeast cells, are observed under the microscope, the organism is likely *C. albicans* (Walker et al., 2006b).

Colony colour can also be utilized to distinguish between various species of *Candida* using chromogenic media like CHROMagar. For example, *C. albicans* typically develops green colonies on this medium when mixed infections are present, making identification easy. In addition, biochemical tests that assess carbohydrate assimilation and fermentation patterns can be used to confirm the species identity (Mirza et al., 2018). Molecular methods such as PCR and DNA sequencing are increasingly used in modern laboratories to detect *Candida* species with greater accuracy and speed, especially in cases of invasive candidiasis where early diagnosis is critical.

2.10 Laboratory Tests

Laboratory tests are vital in the correct detection of *S. aureus* and *C. albicans* since they are responsible for causing opportunistic infections in humans. The detection of *S. aureus* starts with obtaining samples from the patient in form of wounds, blood, sputum, or nasal swabs. Mannitol salt agar is very effective since it consists of high levels of salt which inhibit the growth of all bacteria except *staphylococci*. The *S. aureus* species ferment mannitol to produce yellow colonies (Tong, Schaumburg, et al., 2015). In addition to this, *S. aureus* is observed through the use of Gram staining technique under the microscope, whereby it appears in clumps of Gram-positive cocci resembling grapes. Other methods of identification involve the use of biochemical tests like the coagulase and catalase tests. The coagulase test distinguishes *S. aureus* from other species of *staphylococcus* since it produces an enzyme called coagulase that causes clotting of plasma (Pfaller et al., 2007).

As in the case with the above method, various techniques used for microbiological studies including microscopy, culture and biochemical tests are used in the lab diagnosis of *Candida albicans*. Various clinical specimens like oral, vaginal swab, urine or blood is subjected to microscopic examination by KOH preparation or Gram stain. Microscopically, *Candida albicans* appears as ovoid budding yeast cells capable of producing true or pseudo hyphae (AL-kahfaji, 2022). Cultural techniques may be conducted on Sabouraud dextrose agar since it supports the growth of fungi while preventing any bacterial contamination. In such media,

C. albicans forms cream-colored, smooth colonies. Another confirmation test that is very common in identifying *C. albicans* is the germ tube test, where yeast cells are incubated in the serum at 37°C to form germ tubes within a few hours. Germ tubes are one of the characteristics of *Candida albicans* (Walker et al., 2006a). Furthermore, *Candida* strains can be identified on the basis of color using chromogenic agar. These tests performed in laboratories play an important role in making an exact diagnosis and help medical practitioners determine the most suitable antifungal/antibacterial treatment for their patients.

2.11 Management practices and therapeutic strategies

Effective management and therapeutic strategies for infections caused by *Staphylococcus aureus* and *Candida albicans* are important in reducing morbidity, mortality, and the spread of antimicrobial resistance. *S. aureus* infections are usually treated with antibiotic medication, proper wound care, and infection control methods. For mild skin and soft tissue infections, drainage of abscesses and treatment with antibiotics such as cephalosporins or penicillinase-resistant penicillins may be helpful. However, intravenous antibiotics and careful clinical monitoring are necessary for more serious infections such pneumonia, endocarditis, or bacteremia. Due to the bacteria's resistance to many common drugs, treating MRSA has grown more difficult. In such cases, alternative antibiotics such as vancomycin, linezolid, or daptomycin are commonly used depending on the severity and location of infection (Davis et al., 2015b). In addition to drug therapy, infection prevention strategies such as proper hygiene, sterilization of medical equipment, and screening of hospital patients help reduce the transmission of *S. aureus* in healthcare settings.

The treatment of *Candida albicans* infection mainly involves the use of antifungals and treating risk factors that make one prone to getting an infection. Superficial Candida infections can be managed using antifungal drugs like nystatin and clotrimazole. Systemic antifungal therapy is indicated when there is a systemic fungal infection like fluconazole and itraconazole (Satapathy et al., 2024). Antifungal drugs taken intravenously include drugs like amphotericin B or echinocandins that need to be administered immediately because candidiasis is a dangerous disease which can affect the internal organs or blood.

Boosting the immune system, controlling diseases like diabetes and HIV, and reducing unnecessary consumption of antibiotics, which can disturb the balance of normal microbiology and cause fungal infections, are some other ways of managing the condition.

2.12 Problems Associated with Treatments

One of the biggest obstacles to treating bacterial and fungal illnesses is still antibiotic resistance. Similarly, *Candida albicans* antifungal resistance complicates treatment strategies (McCormick & Ghannoum, 2023). There are a number of challenges that usually arise during the treatment of infections caused by *Staphylococcus aureus* and *Candida albicans*. The emergence of MRSA is as a result of the presence of various strains of *Staphylococcus aureus* that are resistant to commonly prescribed antibiotics like penicillin and methicillin. The treatment of infection caused by such bacteria has proven to be difficult and expensive owing to resistance posed by these MRSA strains to many β-lactam antibiotics. Stronger antibiotics like vancomycin and linezolid have to be prescribed (Idrees et al., 2021). Respectively, as highlighted by (Otto, 2018), another problem involves the capability of *S. aureus* to produce biofilms on devices used in medicine, such as implants, joint prostheses, catheters, and the like. As the biofilm protects bacteria from the activity of the immune system as well as drugs, infections tend to last for an extended period of time and sometimes even necessitate the removal of the device. The ongoing spread of resistant bacteria is caused by both the misuse and overprescription of antibiotics as well as the inappropriate application of infection control strategies.

Infection treatment with *Candida albicans* poses another set of problems, especially in patients with suppressed immunity. Antifungal drug resistance is one of the main concerns in such infections. Drug-resistant strains of *C. albicans* cause ineffective treatments and prolonged infections, especially in cases when antifungal medication is prescribed for an extensive period of time (Pappas et al., 2015). Furthermore, since the signs and symptoms of candidiasis may be vague and can appear similar to those caused by bacteria, an early diagnosis becomes difficult. Complications and even death are more likely when diagnosis and treatment are delayed. Another issue related to candidiasis is that the *Candida albicans* species has the ability to form biofilms on devices such as catheters, making it harder for antifungals to work (Silva et al., 2017).

In addition, underlying conditions such as diabetes, HIV/AIDS, or prolonged antibiotic therapy may lead to immune deficiency and thus make an individual more susceptible to infections. This indicates that improved diagnosis, prudent use of antibiotics, and more effective infection control methods are necessary for successful treatment of infections caused by *Staphylococcus aureus* and *Candida albicans*.

2.13 Antimicrobial Resistance

Resistance of micro-organisms like bacteria and fungi against antimicrobial treatment which will normally inhibit or destroy such microbes is known as AMR and is an important global health issue. The management of infection has been made harder because pathogens like *Candida albicans* and *Staphylococcus aureus* have developed ways of developing resistance. (Lee Ventola, 2015). For instance, *S. aureus* has acquired resistance by developing processes such as biofilm formation that make the organism resistant to antibiotics, enzymatic degradation of the drugs, and mutations in penicillin binding proteins (such as MRSA) (Heinzinger et al., 2023). Similarly, *C. albicans* exhibits resistance through efflux pumps, biofilm formation, and alteration of drug targets, particularly against azole antifungals (Pristov & Ghannoum, 2019).

In view of increasing antimicrobial resistance, it has become imperative to find new drug candidates. Plants like *Datura* genus are rich in biologically active chemical compounds such as alkaloids, flavonoids, tannins, and saponins, which have been found to be antimicrobial in nature (Soni et al., 2012). These phytochemicals can disrupt microbial cell walls, interfere with enzyme activity, and inhibit nucleic acid synthesis, thereby offering potential solutions to combat resistant pathogens.

2.14 Factors Influencing Antimicrobial Activity

Several variables, including the solvent used, the method of extraction, the type of plants, the location where they were collected from, the techniques used for analysis, and the varieties of bacteria tested affect the antibacterial properties of plant extracts. The efficacy and reproducibility of antibacterial drugs derived from plants are significantly influenced by these parameters. The extraction and activity of active compounds are highly dependent on technical and environmental factors (Bryda & Stadnytska, 2021).

2.14.1 Solvent and Extraction Method

The extraction procedure and the solvent used have a significant impact on the antibacterial activity of plant extracts. Different solvents extract different types of phytochemicals based on their polarity and chemical characteristics (Cvetanović, 2019). Non-polar solvents such as chloroform and hexane will dissolve non-polar compounds such as terpenoids and alkaloids, while polar solvents including water, methanol, and ethanol will be useful for dissolving polar compounds such as phenolics, tannins, and flavonoids (Carvalhaes et al., 2022). Ethanol extracts usually have greater antimicrobial effect compared to water extracts since ethanol is able to dissolve polar and non-polar compounds, and thus allows the extraction of

various bio-active components (Parekh J & Chanda S, 2007). Similarly, (Płotka-Wasylka et al., 2017) found that chloroform and ethanol extracts have high antibacterial efficacy against bacterial and fungal infections. These data emphasize the significance of solvent selection in antibacterial research.

Infusion, Soxhlet extraction, cold extraction, and maceration are typical extraction techniques. Maceration is commonly utilized due to its simplicity and ability to maintain heat-sensitive substances. Soxhlet extraction, on the other hand, allows for continuous extraction and may produce larger levels of bioactive chemicals (Dai & Mumper, 2010). However, the high temperatures required for Soxhlet extraction may damage some heat-sensitive chemicals.

Temperature, plant material pretreatment, and the length of the extraction process are other variables that impact extraction efficiency. Plant materials' surface area and extraction efficiency are increased by drying and grinding them. Improved solvent penetration and phytochemical extraction are made possible by fine powder (Azmir et al., 2013).

Solvent concentration also affects extraction and antibacterial activity. Larger solvent volumes increase extraction efficiency even if they may dilute active substances. Therefore, creating efficient antibacterial plant extracts requires improving extraction conditions. In this work, pure alcohol, chloroform, and distilled water were used as solvents to assess the antibacterial activity of *Datura* leaf extracts against *C. albicans* and *S. aureus*.

2.14.2 Plant Species and Geographic Origin

Plant species and geographic origin have a considerable impact on antibacterial efficacy because phytochemical composition varies. Many plant species produce unique secondary metabolites that have antibacterial qualities. These secondary metabolites include phenols, terpenoids, alkaloids, flavonoids, and tannins (Doughari & Saa-Aondo, 2021).

The content and kind of these chemicals vary according to plant species and environmental conditions. Climate, soil type, altitude, and rainfall are among the environmental elements that influence plant metabolism and bioactive chemical production. Plants subjected to environmental stress frequently develop more secondary metabolites as a form of protection against diseases and herbivores (S et al., 2008).

Antimicrobial activity is also influenced by seasonal variations. Some plants produce more bioactive chemicals during specific seasons or growth stages. For example, (Altameme et al., 2015) discovered that the phytochemical makeup and antibacterial effectiveness of medicinal

plants were significantly impacted by seasonal change. In antimicrobial studies, harvesting time is therefore crucial.

The phytochemical content may be influenced by environmental factors among plants grown in different areas. Several pieces of research indicate that variation in soil nutrients, temperature, and rainfall affects the antibacterial potential of *Datura* plants grown in different geographical locations (Semenya & Maroyi, 2018). The other factor influencing the antibacterial effect is the growth phase of the plant. Fresh leaves contain more active substances compared to older ones. The age of the plant should be taken into account during experimentation.

2.14.3 Microbial Strain Variability

Microbial strain diversity has a significant impact on the antibacterial efficacy of plant extracts, according to (Pfaller & Diekema, 2007). Microbes of the same species often exhibit substantial differences in their genetic make-up; as such, there is an effect on their sensitivity to antibiotics. This variability is brought about by mutation, development of genes of resistance and physiological adjustments to enable growth in tough environments. Therefore, drugs that work well with one strain do not work as effectively with another strain of the same species (Álvarez-Martínez et al., 2021).

Antibiotic resistance is a well-known characteristic of *S. aureus*. Because MRSA strains have altered their penicillin-binding proteins, they are less vulnerable to a number of antibiotics (Wang et al., 2023). It is also possible that such resistance mechanisms can influence sensitivity to the antibacterial properties of plant compounds. Some *Staphylococcus aureus* strains form biofilms that enhance resistance against antibiotics by restricting their access (DeLeo & Chambers, 2009). Due to their ability to adhere to body tissue and medical devices and hence pose difficulties in treatment, biofilm formation is important in skin infections.

While some strains have developed mechanisms for gaining resistance through efflux pumps that pump out antifungal agents from the cell, others have evolved strategies for changing the membrane composition so that there is reduced permeability to these drugs. Additionally, *Candida albicans* forms biofilms on body tissues, thus developing resistance to antifungals and increased resistance to plant extracts (Calderone & Fonzi, 2001). These mechanisms of resistance may affect the ability of antifungal activity of the plant extracts such as *Datura* leaf extract. The growth phase of the microorganism is yet another important aspect that affects the microbial variability. This is because there is an increase in the resistance of antimicrobials because of the synthesis of stress proteins by the bacteria (Garvey & Rowan,

2023). It therefore goes to show that the point at which the microorganisms are in the process of developing will play a very significant role in determining antimicrobial results.

An essential element in assessing the effectiveness of antibiotics includes the quantity of the inoculum. As there is competition for resources among the bacteria, larger quantities will result in weaker antimicrobial actions (Mudenda, 2024). Environment-related variables such as temperature, pH, and nutrients also impact bacterial susceptibility to antimicrobials. Since they are subjected to antimicrobial agents during their clinical treatment, clinical isolates are more resistant than laboratory pathogens. When testing the effectiveness of antimicrobial agents, it is advised to use both kinds. Determining the antibiotic efficacy of the extracts from *Datura* leaves in combating *Staphylococcus aureus* and *Candida albicans* associated with skin ailments necessitates a grasp of strain variability.

2.14.4 Assay Methods

Different test protocols differ in terms of sensitivity, precision, and consistency, which could affect how results are perceived. Broth dilution, disk diffusion, agar well diffusion, and microdilution are common antimicrobial testing methods. Each method offers benefits and drawbacks, depending on the plant extract and microorganisms being examined (Balouiri et al., 2016b).

Because of its ease of use and low cost, the agar well diffusion method is commonly employed to assess antibacterial activity of plant extracts. In this procedure, wells are formed in agar plates inoculated with bacteria, and plant extracts are put into them. However, the efficacy of this approach is dependent on chemicals' capacity to permeate through the agar media. Compounds with weak diffusion characteristics may have smaller zones of inhibition, even if they have substantial antibacterial activity. Therefore, diffusion characteristics of plant extracts should be addressed while interpreting data.

Disk Diffusion Test is also another antimicrobial testing procedure. This procedure uses disks that contain plant extract and are placed on top of plates containing bacteria. The antibacterial activity is evident from zones created around the disks. However, while this method is easy to use and common in practice, it depends on diffusion properties and hence may not reflect the true bactericidal activity of nondiffusing drugs (Hossain, 2024).

The disk diffusion method is still applicable in assessing the antibacterial activity of plants. However, it is assumed that the broth dilution method, which includes macro- and micro-dilutions, is more precise in establishing the minimum inhibitory concentration (MIC). This involves testing the bacteria in different concentrations of the plant extracts in liquid medium,

after which growth of the microbes is observed. The MIC is the lowest concentration where growth is inhibited (Ford et al., 2021). This method generates quantifiable data and enables for the comparison of antibacterial efficacy across different extracts.

After determining the MIC, samples from wells that show no growth are subcultured on new media. The lowest concentration at which microbial growth is inhibited is MBC or MFC (Urbán & Stone, 2019). There are many parameters that influence the outcome of an antimicrobial test including inoculum size, temperature, pH, and growth medium among others. There are some conditions that have to be set to ensure reproducibility. To maintain consistency in different laboratories, there are recommendations from the CLSI for antimicrobial susceptibility tests (Carvalhaes et al., 2022).

Moreover, the effectiveness of the bacteria could be affected by the growth medium used. Although selective media would inhibit growth and influence its diffusion properties, nutrient media would encourage bacterial growth but prevent their activity.

2.15 Extraction Solvents and Their Advantages

Plant extractants are an important procedure in phytochemical research because it enables researchers to isolate bioactive components from plant matter. The selection of the solvent is important in deciding how successful this procedure will be. Various solvents are selected depending on several factors such as their polarity, toxicity and their solubility properties in relation to (Dhawan & Gupta, 2016). Some commonly used solvents include water, ethanol and methanol, chloroform, acetone, hexane, and ethyl acetate, all these have their specific benefits depending upon the nature of the compounds to be extracted from plant material.

Water is perhaps the most commonly used traditional solvent for the extraction of phytochemicals. Water is quite polar in nature and is very useful for extracting those compounds that have a good affinity for water like sugars, glycosides, tannins, proteins, and some alkaloids. Water has certain benefits like its availability, safety, and affordability but some researches have shown that it is not suitable for extracting non-polar compounds and is prone to microbial infections while extraction (Kumar et al., 2023).

Moreover, solvents like alcohol, including ethanol and methanol, are some of the most common solvents used in phytochemistry research owing to their capacity to dissolve a broad spectrum of materials. Ethanol is one of the good solvents since it is fairly safe for human ingestion and is capable of extracting phenolics, flavonoids, tannins, and alkaloids effectively (Dai & Mumper, 2010). Moreover, methanol, being more polar, is highly effective for

extracting low molecular weight phenolic compounds and often results in higher extraction yields. However, its toxicity limits its use in products intended for human use.

Chloroform is a nonpolar solvent mostly used for extracting lipophilic compounds such as lipids, terpenoids, steroids, and certain alkaloids. Its selectivity for non-polar substances makes it valuable in fractionation processes. However, chloroform is toxic and poses environmental and health risks, requiring careful handling and limiting its application mainly to laboratory research.

Based on acetone, it is a moderately polar solvent that can extract both polar and non-polar compounds, including flavonoids, phenolics, and some essential oils. Its key advantages include high volatility, which allows easy removal after extraction, and a strong ability to penetrate plant tissues, thereby improving extraction efficiency. It also reduces the likelihood of thermal degradation of sensitive compounds while extracting (Dai & Mumper, 2010).

Hexane is a nonpolar solvent that can be used to extract hydrophobic substances including fats, oils, waxes, and pigments such as chlorophyll. Moreover, it has a low boiling point and hence allows easy evaporation. But it is very flammable and cannot be used for polar plant chemicals extraction.

Ethyl acetate is an intermediate polarity solvent that is effective in extracting semi-polar compounds such as flavonoids, tannins, and some alkaloids. It provides a balance between polar and non-polar solvent properties, making it particularly useful in fractionation and purification processes. Compared to chloroform, it is less toxic and has good extraction efficiency for a wide range of phytochemicals because it balances between polar and nonpolar compounds.

CHAPTER 3

Materials and Methods

3.1 Study Design

The antibacterial efficacy of Datura leaf extracts against *Candida albicans* and *Staphylococcus aureus* was examined using a laboratory-based experimental methodology. Standard microbiological and phytochemical methods were used to evaluate the physicochemical composition, antibacterial activity, MIC, and MBC/MFC. Botanists at the University of Zimbabwe's Department of Ecology initially recognized the plants that were gathered.

3.2 Biosafety Protocols

All laboratory procedures were performed in a biosafety level 2 setting. Before being disposed of, the cultures and extracts were also autoclaved. All necessary aseptic conditions were provided such as the use of laminar flow, disinfection of the working environment using

70% alcohol and wearing of protective gear to reduce contamination and the spread of the pathogens. Moreover, most the processes were performed in a specialized closed room to reduce the risk of pathogens escaping from the lab.

3.3 Study Area

Depending on the availability of resources, this project was carried out in a number of locations. The Zinyama village in Chiweshe, Mashonaland Central Province, is where the Datura plants were gathered. This study was conducted in a microbiology laboratory under aseptic conditions, where all microbial and phytochemical analyses were performed following standard laboratory protocols. Additionally, phytochemical extraction and analysis, and antifungal susceptibility test were conducted at the University of Zimbabwe's Department of Biological Sciences.

3.4 Preparation of microbial inoculum

Pure cultures of *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231 were subcultured on nutrient agar and Sabouraud dextrose agar, respectively, to revive them from preservation. Fungal cultures grow well on SDA or PDA agar at 25-30ºC, and bacterial cultures also grow well on nutrient agar at 37ºC.

3.5 Observing morphological features of the inoculum

After reviving the cultures, morphological features were identified, such as colony colour and appearance on agar plates. For *Staphylococcus aureus*, a Gram stain procedure was done to observe some of the morphological features under the microscope at low magnification and high magnification. For *Candida albicans*, cotton blue staining was done to observe some of the morphological features on the microscope as well.

3.5.1 Cotton blue staining

An inoculating loop was used to mount a sample on a clean, sterilized glass slide. After applying a small drop of cotton blue, the slide was covered with a cover slip. The slide was viewed at both low power (100X) and high power (400X) using a microscope.

3.5.2 Gram staining

A sterile loop was used to collect a little bit of bacterial culture from a drop of distilled water on a clean slide, which was then combined with the water to create a thin smear. The smear was set to air dry until it was totally dry. The attachment of the bacterial colony on the slide’s

surface was done by running it under the flame for two or three times while dry. After the application of crystal violet on the slide and leaving it for a minute, the wash with distilled water was carried out. After flooding the smear with Gram's iodine solution and letting it sit for a minute or so, distilled water was used to rinse it. Few drops of 75% alcohol were added for 10–20 seconds, and the specimen was rinsed immediately with distilled water. After flooding the smear with safranin for 30 to 60 seconds, distilled water was used to rinse it. The slides were allowed to air-dry before observation under a light microscope at 100X low power and 400X high power after applying a drop of immersion oil.

3.6 Biochemical tests for bacteria

3.6.1 Oxidase test

One or two drops of the oxidase reagent (tetramethyl-p-phenylenediamine) were added to a sterile petri dish covered with filter paper. Next, a colony of *S. aureus* was selected using a sterile loop and applied to the paper that had been soaked in reagent. It took ten to thirty seconds to see the colour shift.

3.6.2 Methyl Red Test

MR-VP broth was prepared, aseptically inoculated with *S. aureus*, and incubated for 24 hours at 37ºC. The liquid was shaken to check for color changes after three to five drops of methyl red indicator were added.

3.6.3 Catalase test

A drop of 3% hydrogen peroxide was placed on a clear glass slide. A sterile loop was then used to pick a colony from the culture and mix it into the hydrogen peroxide drop. Immediately, the reaction was observed.

3.6.4 Indole test

Tryptone broth was prepared as directed by the manufacturer and supplemented with *S. aureus*. After a 24-hour incubation period at 37ºC, Kovac's reagent was carefully added down the tube's side. After allowing the reagent to accumulate in a layer, the surface was examined.

3.6.5 Citrate utilization test

A sterile loop was used to inoculate *S. aureus* on a Simmons citrate agar slant. Before the color shift was noticed, the slant was incubated at 37ºC for 24 hours.

3.7 Preparation of plant extract

A pestle and a motor were used to grind the dried *Datura* leaves into a fine powder. Absolute alcohol, chloroform, and distilled water were used as solvents throughout the extraction process. 20 g of powdered plant material was soaked in 200 mL of each solvent (1g:10ml), and the combination was then cold macerated for 48–72 hours with periodic shaking. The mixtures were filtered through Whatman filter paper, and the filtrates were concentrated into crude extracts using a rotary evaporator. The extracts were then stored at 4°C until they were required.

3.8 Phytochemical analysis

To determine bioactive substances such as alkaloids, flavonoids, saponins, and glycosides, preliminary phytochemical screening of the crude extracts was done using conventional qualitative techniques. A number of phytochemicals are known to affect the antibacterial activity of plant extracts.



Figure 1 Reagents that were used for phytochemical analysis

3.8.1 Testing for Glycosides

Acid Hydrolysis test

The method used was a hydrolysis test where 5ml of 1 M HCl was used to hydrolyse 2ml of the extract in a water bath for 20-30 min. Then 1m NaOH was added to neutralize the mixture before the addition of a few drops of phenolphthalein to record the colour change.

3.8.2 Testing for Alkaloids

Mayer's Test

Alkaloids in the plant extract were identified using Mayer's reagent. A test tube was filled with two millilitres of the extract and a few drops of Mayer's reagent. At last, the colour shift became noticeable.

3.8.3 Testing for Flavonoids

Lead Acetate Test

A few drops of lead acetate were added to 2ml of the plant extract, and the colour change was recorded.

3.8.4 Testing for Saponins

The extract's saponin content was tested using only distilled water. 1 ml of the plant extract and 5 ml of distilled water were mixed in a test tube. To see how the foam formed, the liquid was shaken for thirty seconds.

3.9 Phytochemical Extraction Using Soxhlet Apparatus

Absolute alcohol, chloroform, and distilled water were the solvents used to soak the powdered plant material for 48 hours with occasional shaking in a sterile container. The ratio of the mixture was 1 g: 10 ml. To separate the liquid extract from the solid residue, the solutions were first filtered through a fine sieve, and then filter paper was employed. The Soxhlet technique or rotary evaporator were used to concentrate the extracts through gentle heating. Then the mixtures were put in a glass petri dish to complete the vaporization of the solvent, and the extracts were collected into an amber glass bottle. In addition, the glass petri dishes were weighed before and after in order to calculate the total plant percentage extract. The values of percentage yield extract after extraction are shown in Table 3.1.

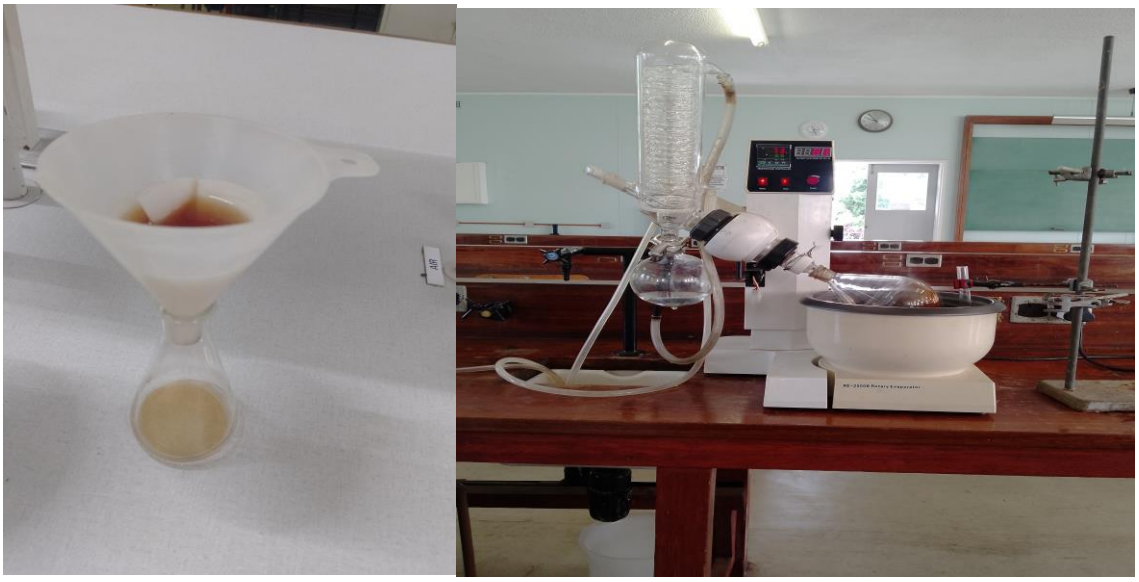


Figure 2 Filtration process(left) and Soxhlet method(right)



Figure 3 Plant extracts after rotary evaporation

3.10 Agar well Diffusion

S. aureus and *C. albicans* cultures that had been revived were subjected to agar well diffusion using the *D. stramonium* extract.

3.10.1 Preparation of *S. aureus* and *C. albicans* standard concentration

To guarantee consistent microbial density, the inocula were first standardized using the McFarland standard (0.5), which translates to roughly 1.5×10^2 CFU/mL. 0.05 mL of 1.175% $BaCl_2 \cdot 2H_2O$ and 9.95 mL of 1% H_2SO_4 in distilled water were combined to create a 0.5 McFarland standard. The turbidity was set at 0.5 McFarland (OD^2_{20} = 0.08–0.10). The colonies were suspended in a test tube filled with saline using a sterile swab. The microbial concentration was measured using a turbidimeter to see if it matched 0.5 McFarland, and if so, it was adjusted to 1.5 x 10^2 CFU/ml. For both strains, the same procedure was used.

3.10.2 Media Preparation

Mueller-Hinton Agar and Sabouraud Dextrose 4% Agar were used to prepare 5 plates. The manufacturer highlighted that 6.5 g of SDA must completely dissolved by gently heating 100 ml of distilled water until it boiled. After adding 4 g of chloramphenicol, the mixture was autoclaved for 15 minutes at 121 °C to sanitize it. Additionally, 3.9 g was gently heated until fully dissolved in 100 mL of purified water. To minimize plate contamination, the media were autoclaved, allowed to cool to 45ºC, and then plated in a film hood. Before employing the spread plate method to inoculate the plates with standard cultures of *Candida albicans* and *S. aureus*, the plates were allowed to solidify. 2 agar plates of PDA and 2 plates of Mueller-Hinton agar were used for disc diffusion, and the remaining plates were kept in a cold room.

3.10.3 Extract Concentrations Preparation

9 sterile test tubes were prepared and labelled as 100µg/ml, 300µg/ml, and 600µg/ml for *D. stramonium* extract of different solvents: absolute alcohol, chloroform, and distilled water. Each extract from these solvents was diluted accordingly.

3.10.4 Agar Well Diffusion Method

The antibacterial activity of the extracts was tested using the agar well diffusion method. We added standardized microbiological solutions to sterile agar plates. Using a sterile cork borer, we bored wells about 5 mm in diameter into the agar. Then, we added a measured amount of the extract from each concentration to each well. Standard antibiotics, miconazole and streptomycin, along with solvents, were included in both positive and negative control wells. These antibiotics were used to prepare the controls at a concentration of 100 µg/ml. We measured the zones of inhibition in millimeters after incubating the plates at 37°C for 24 hours for *S. aureus* and at 25–30ºC for 48–72 hours for *Candida albicans*.



Figure 4 Antibiotics that were used in agar well diffusion method

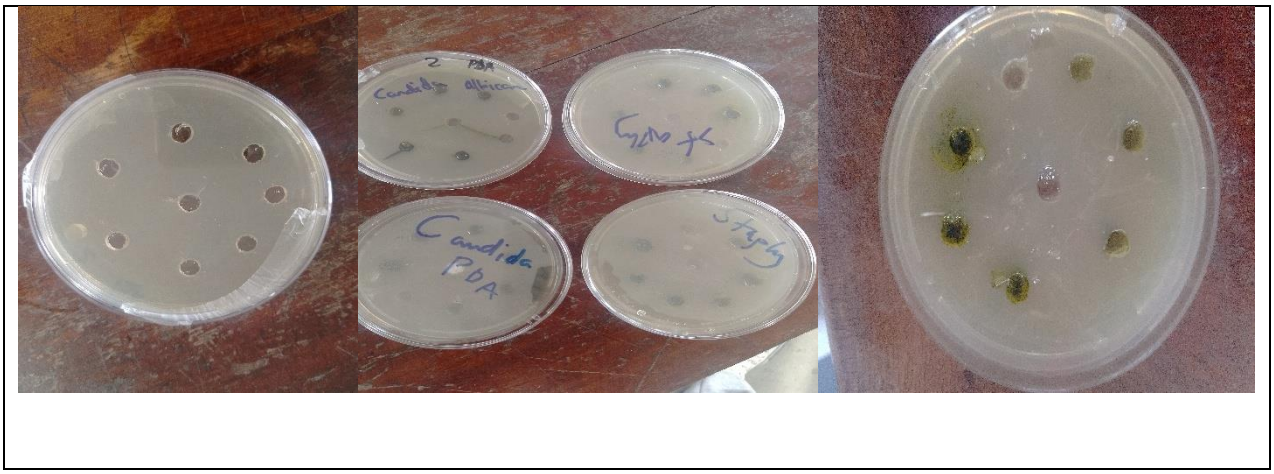


Figure 5 Agar Well Diffusion Method

3.11 24-well plate microdilution method

The lowest inhibitory concentration for both *S. aureus* and *Candida albicans* was found using the 24-well plate broth microdilution method. Sterile plates were first labelled according to the extract concentrations and controls. Muller-Hinton broth was used for *S. aureus,* and SDA was used for *C. albicans*. 900µl of sterile broth specific for the culture was dispensed into each well. Stock solutions for the extracts were prepared at a concentration of 100µg/ml, and 100µl of these extracts were added to the first wells. For every extract, two-fold serial dilutions were carried out by moving 500µl from the first well into the subsequent broth-containing well and thoroughly mixing. To get declining concentrations of 50, 25, 12.5, 6.25, and 3.125 µg/ml, the procedure was repeated across the row.

After preparing the 24-well plates with the extracts, standard microbial cultures were prepared by adjusting fresh cultures of *S. aureus and C. albicans* to a 0.5 McFarland standard using the same procedures highlighted in chapter 3.9.1. Thereafter, 100µl of the standard

culture was suspended in each well being tested. Controls were also prepared, including a positive control containing broth, microorganism, and standard antibiotic and antifungal agent; a growth control containing broth and microorganism only; and a negative control containing broth only. The plates were gently mixed and incubated at 37ºC for *S. aureus* for 24 hours and at 25-30ºC for *C. albicans* for 24-48 hours. After incubation, 20µl of 2% colour indicator Triphenyl Tetrazolium Chloride (TTC) was added to each well, and incubated for 2 hours to allow colour development. Colourless wells indicated inhibition.

Samples from the wells that were not showing colour changes were aseptically subcultured onto fresh specific media. The plates were incubated again under appropriate conditions, to identify MBC and MFC of these extracts.

3.12 Data analysis

The data collected from all these experiments were analysed by using bar charts and One Way ANOVA. For One Way ANOVA, it was used to check if there was significance differences in using different solvent for phytochemical extraction using zones of inhibition results. The Windows 10 Excel application software was also used to create the charts and to calculate the ANOVA.

CHAPTER 4

Research Findings

*4.1 Phytochemical analysis findings*Table 1 the total percentage yield of the extract from different solvents used calculated by Yield (%) = (extract dry weight / powdered leaf weight) * 100

Solvent used	**Petri dish weight(g)**	**Petri dish weight + extract(g)**	**Percentage Yield (%)**
Absolute alcohol	48.9	50.1	6
Chloroform	48.5	50.2	8.5
Distilled water	48.3	49.0	3.5

4.1.1 For Glycosides

In the acid hydrolysis test, a brick-red precipitate confirms the presence of glycosides, as observed on fig 6.



Figure 6 Biochemical test findings which are glycosides, flavonoids, alkaloids, and saponins

4.1.2 For Flavonoids

For flavonoids, the lead acetate test was used; it produces a yellow precipitate in their presence. In this experiment, a yellow precipitate was observed after heating the mixture in a water bath.

4.1.3 For alkaloids

Mayer's reagent was employed for the alkaloids test; a creamy-white precipitate was the result. The presence of flavonoids in the plant extract was suggested by this creamy white color.

4.1.4 For Saponins

For saponins, the foam test was used to detect their presence in a sample. In this experiment, a foam was produced after shaking the mixture for 30 seconds. The foam lasts for more than 30 minutes.

Table 2 Semi-quantitative phytochemical analysis

Phytochemical	**Distilled water**	**Absolute Alcohol**	**Chloroform**
Saponins	High	Moderate	Low
Alkaloids	Moderate	High	High
Flavonoids	Low	High	Low
Glycosides	High	Moderate	Low

4.3 Biochemical test findings

Based on the findings below, it is clear that *S. aureus* showed negative results for the oxidase test, indole test and citrate utilization test. There was no colour change for the oxidase test, no ring shape formed on the indole test and very little growth with no colour change for citrate utilization test.

Table 3 Biochemical methods used and the observations for *S. aureus*

Method used	**Observation**	**Negative / Positive**
Oxidase test	No colour change	-
Methyl Red test	Red colour	+

Catalase test	Rapid bubbling	+
Indole test	No red ring formed	-
Citrate Utilization Test	Very little growth, no colour change	-

4.2 Morphological features of inoculum

Here is a table showing the morphological features of each strain and how they appeared on agar plates after incubation. Gram staining was also used to identify the purple cocci clusters of *S. aureus* under a microscope at 1000X magnification. Whereas lactophenol cotton blue staining was used to identify blue yeast-like cells with pseudohyphae, as illustrated in Table 4.3.

Table 4 Morphological features of *S. aureus* and *C. albicans*

Feature	***S. aureus***	***C. albicans***
Colony colour	Whitish	Cream white
Colony appearance	Smooth, circular, shiny	Smooth, creamy, pasty
Texture	Buttery	Soft yeast-like
Gram-staining	Purple cocci clusters	Not required
Lactophenol cotton blue staining	Not required	Blue yeast-like cells with pseudohyphae

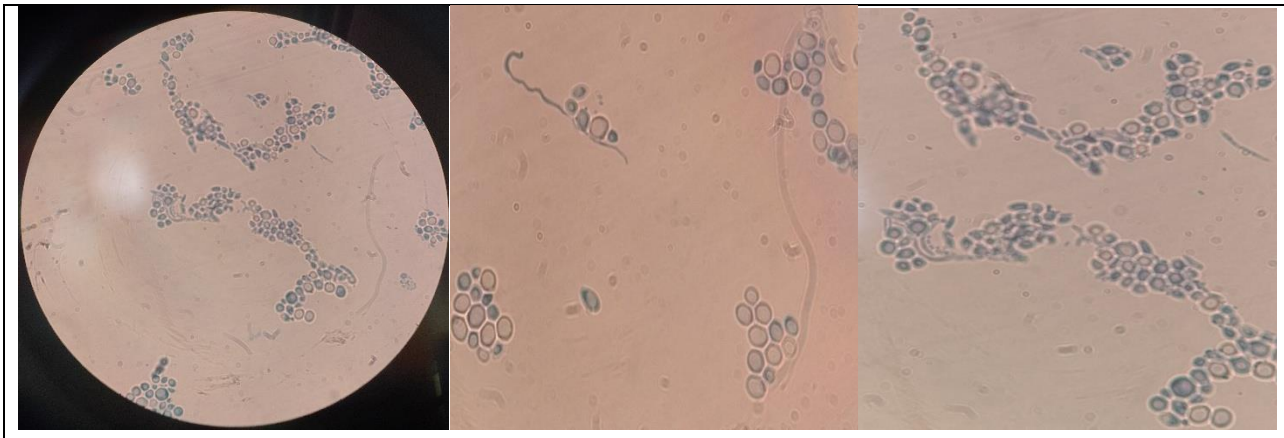


Figure 7 Lactophenol Cotton blue staining

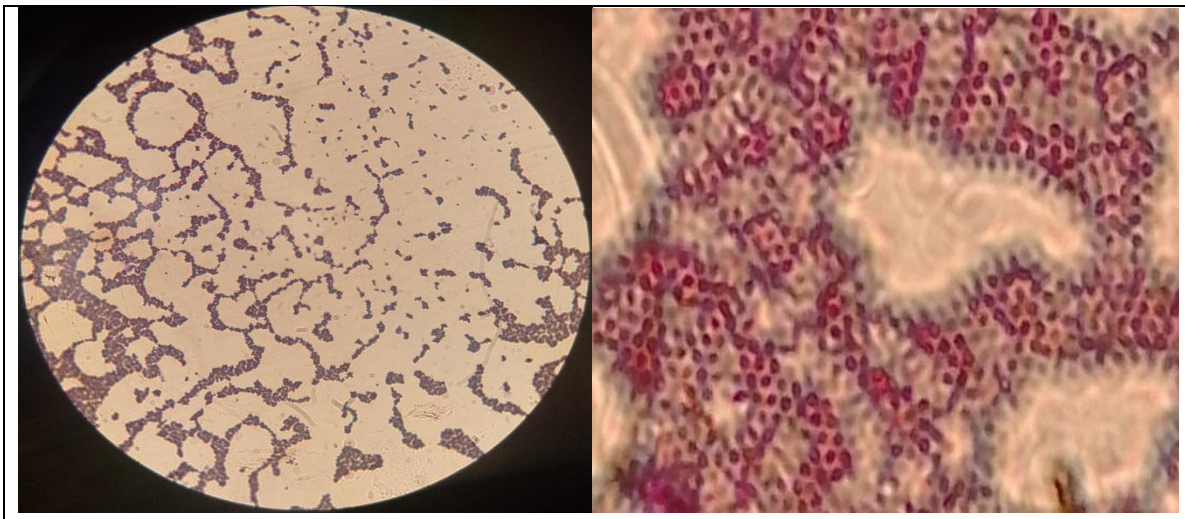


Figure 8 Gram-staining for *S. aureus*

4.4 Zone of inhibitions

As shown in Table 4.4 and Table 4.5, chloroform has a slightly larger zone of inhibition, followed by absolute alcohol, and finally distilled water with the least zone of inhibition and their means are higlihted in table 10 and table 13. As illustrated below, the difference in the zone of inhibition is not that big, however analysis of variance will actually highlight if there is a big difference scientifically. Additionally, the extract's efficacy against these strains varies slightly. In other words, *Datura* plant extract is showing more effectiveness against bacteria than fungi.

Table 5 Zone of inhibition of *C. albicans* using extracts from different solvents after the agar well diffusion method.

Concentrations	**Zone of Inhibition (mm)**		
	Distilled Water	**Absolute Alcohol**	**Chloroform**
100µg/ml	6	12	9
300µg/ml	7	14	11
600µg/ml	9	17	14

Table 6 Zone of inhibition of *S. aureus* using extracts from different solvents after agar well diffusion.

Concentrations	**Zone of Inhibition (mm)**		
	Distilled Water	**Absolute Alcohol**	**Chloroform**
100µg/ml	7	11	11
300µg/ml	9	17	13
600µg/ml	12	21	16

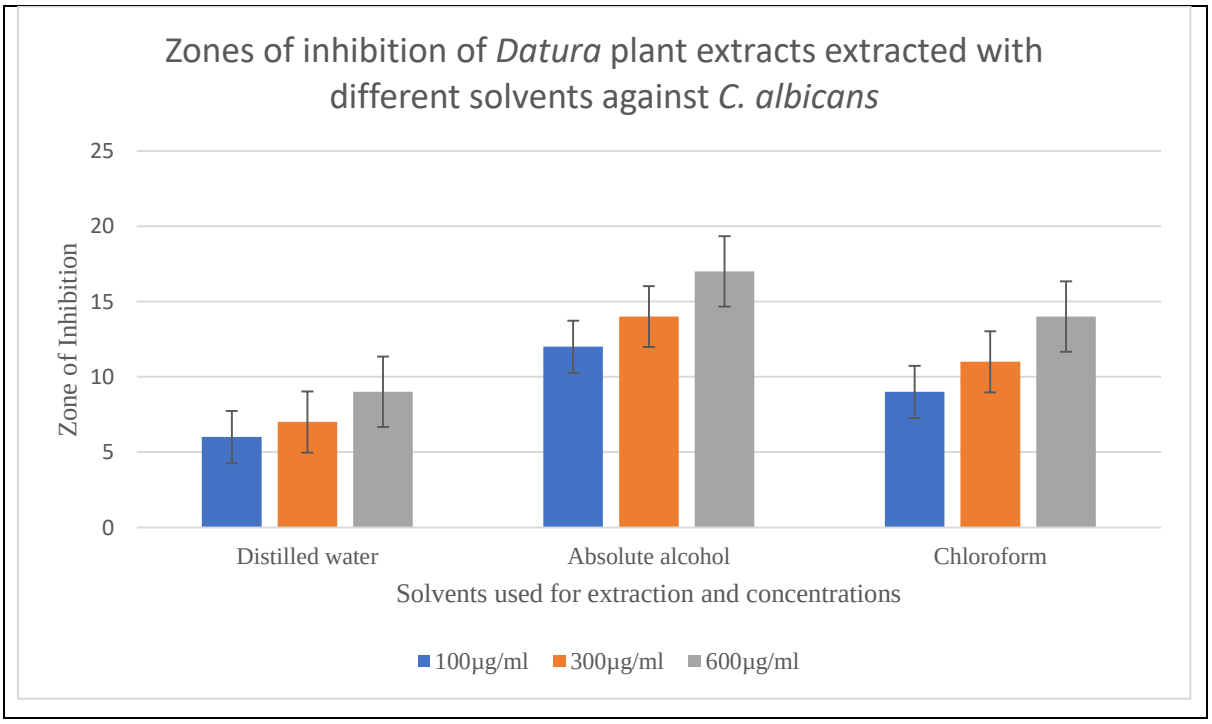


Figure 9 A comparative chart of the zone of inhibition of *C. albicans* using extracts from different solvents.

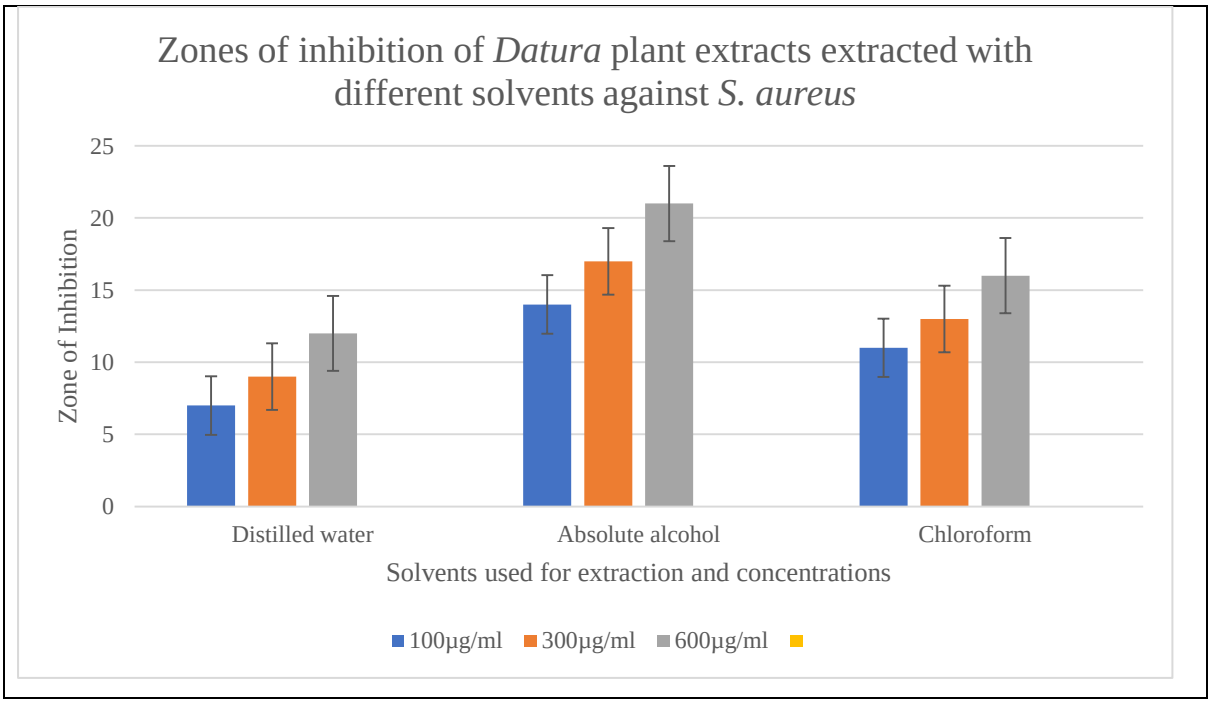


Figure 10 A comparative chart of the zone of inhibition using extracts from different solvents against *S. aureus*.

4.4.1 MIC of both *S. aureus* and *C. albicans*

Table 7 MIC of *D. stramonium* leaf extracts using different solvents against *S. aureus* and *C. albicans.*

Solvent Used	***S. aureus***	***C. albicans***
Chloroform	12.5µg/ml	25µg/ml
Absolute Alcohol	6.25µg/ml	12.5µg/ml
Distilled Water	25µg/ml	50µg/ml

4.4.2 MBC and MFC of both *S. aureus* and *C. albicans*

Table 8 MBC and MFC of *S. aureus* and *C. albicans*.

Solvent used	**MBC**	**MFC**
Chloroform	25µg/ml	50µg/ml
Absolute alcohol	12.5µg/ml	25µg/ml

Distilled water	50µg/ml	100µg/ml

4.4.3 MBC/MIC and MFC/MIC

Table 9 MBC/MIC and MFC/MIC of the extracts against *S. aureus* and *C. albicans* and how the data was interpreted.

Solvent used	**MBC/MIC**	**If ≤ 4 = Bactericidal** **If >4 = Bacteriostatic**	**MFC/MIC**	**If ≤ 4 = Fungicidal** **If > 4 = Fungistatic**
Chloroform	2	Bactericidal	2	Fungicidal
Absolute alcohol	2	Bactericidal	2	Fungicidal
Distilled water	2	Bactericidal	2	Fungicidal

4.4.4 Analysis of Variance

4.4.4.1 ANOVA for *Candida albicans*

Table 10 Variance of plant extracts against *C. albicans.*

This table below shows the means and variances of antifungal activity of extracts from different solvents represented as column 1 for distilled water, colmn 2 for absolute alcohol and column 3 for chloroform.

Anova: Single Factor

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	22	7.333333	2.333333
Column 2	3	43	14.33333	6.333333
Column 3	3	34	11.33333	6.333333

Key

Column 1 – Distilled water

Column 2 – Absolute Alcohol

Column 3 – Chloroform

Table 11 Analysis of Variance of extracts against *C. albicans.*

This table highlights ANOVA for antifungal susceptibility test for extracts from different solvents and the p-value is 0.024003 which is below 0.5.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	74	2	37	7.4	0.024003	5.143253
Within Groups	30	6	5			
Total	104	8				

Table 12 Post Hoc values of relationships on effectiveness of extract solvents against *C. albicans* susceptibility test.

As shown in the table below, HSD is 5.60 and the relationship of distilled water and absolute alcohol as solvents differ significantly because the mean different is greater than HSD. The remaining relationships they do not differ significantly according to this Post Hoc test.

		Mean Difference	**HSD=5.60**
Distilled	Absolute	7	Differ significantly
Absolute	Chloroform	3	Not differ
Distilled	Chloroform	4	Not differ

4.4.4.2 ANOVA for *S. aureus*

Table 13 Analysis of Variance of the extracts against S. aureus. This table below shows the average means and variances for antibacterial suscepitibility test of extracts using different solvent represented as column 1 for distilled water, column 2 for absolute alcohol and column 3 for chloroform aginst *S. aureus*.

Anova: Single Factor				
SUMMARY				

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	28	9.333333	6.333333
Column 2	3	52	17.33333	12.33333
Column 3	3	40	13.33333	6.333333

Key

Column 1 – Distilled water

Column 2 – Absolute Alcohol

Column 3 – Chloroform

Table 14 Analysis of variance of the zones of inhibition of extracts against *S. aureus*.

This table below highlights the calculated p-value of 0.040165 which is less than 0.05 using the results observed from antibacterial susceptibility test of *S. aureus* and *D. stramonium* extracts from three different solvents.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	96	2	48	5.76	0.040165	5.143253
Within Groups	50	6	8.333333			
Total	146	8				

Table 15 Post Hoc values of relationships of extract solvents used for phytochemical extracts of *S. aureus* susceptibility test.

This table shows the relationships of pair-wising of different solvents used for antibacterial susceptibility test. The HSD value is 7.23 and the relationship between distilled water and absolute alcohol has a mean difference of 8 which is greater than the HSD. This show that the relationship differ sigficantly while the remaining relationships do not differ significantly as highlighted in the table.

		Mean Difference	**HSD=7.23**
Distilled	Absolute	8	Differ significantly

Absolute	Chloroform	4	Not differ significantly
Distilled	Chloroform	4	Not differ significantly

CHAPTER 5
DISCUSSION

5.1 Phytochemistry of *D. stramonium*

From the results obtained, it can be noted that the *Datura stramonium* plant contains the phytochemical compounds that have been tested, and they include flavonoids, alkaloids, glycosides, and terpenoids. The results provided ample evidence regarding the phytochemistry of this plant, similar to (Gaire & Subedi, 2013) found. But then again, the solvents used also influence the quantity of the extract and the type of phytochemicals that will be extracted.

The findings suggest that the extract from distilled water had high content of saponins and glycosides, moderately high content of alkaloids and low content of flavonoids. This is because distilled water is an excellent solvent in the extraction of polar phytochemicals like saponins and glycosides since it is very polar solvent. The polar molecules of phytochemicals can dissolve in distilled water easily and therefore high concentration of saponins and glycosides. Some flavonoids, on the other hand, cannot dissolve in distilled water easily.

Furthermore, alkaloids and flavonoids were found in significant concentrations in the 100% alcohol extract, whereas saponins and glycosides were found in moderate amounts. This suggests that absolute alcohol is a more adaptable solvent since it can extract both polar and somewhat nonpolar substances. The higher levels of alkaloids and flavonoids observed in this extract may be attributed to the nature of ethanol being more effective at dissolving phytochemical compounds compared to water as well as its ability to penetrate the walls of the plant cells better.

The chloroform extract had alkaloids, but lacked glycosides, flavonoids, and saponins. This is due to the fact that chloroform mainly removes non-polar molecules because it is a non-polar solvent. Saponins, flavonoids, and glycosides are mostly polar compounds and do not dissolve well in chloroform, which explains their absence. The presence of high alkaloid

content in the chloroform extract suggests that some alkaloids in *Datura stramonium* have non-polar characteristics and are therefore soluble in chloroform.

Overall, the results show that solvent polarity had an effect on phytochemical extraction. Polar solvents such as distilled water extracted more polar compounds, absolute alcohol extracted a wide range of compounds, and chloroform extracted fewer compounds due to its non-polar nature. This explains why the absolute alcohol extract contained the highest diversity of phytochemicals, followed by distilled water, while the chloroform extract contained the least.

5.2 Antifungal Activity of Plant Extracts Against *C. albicans*

The results of this investigation showed that the plant extracts have antifungal activity against *C. albicans*, as evidenced by the zones of inhibition formed using the agar well diffusion method. The zone of inhibition clearly grew as the extract content rose, according to the results. At 100 µg/mL, the absolute alcohol extract had the largest zone of inhibition (12 mm), followed by chloroform (9 mm), and the distilled water extract had the smallest inhibition zone (6 mm). The same applies at 300 µg/mL, absolute alcohol extract again had the largest inhibition zone of 14 mm, followed by chloroform with 11 mm and distilled water with 7 mm. At the highest concentration of 600 µg/ml, absolute alcohol extract produced the largest inhibition zone of 17 mm, followed by chloroform with 14 mm and distilled water with 9 mm.

From this observation, it is clear that the bioactive substances involved in the inhibition of the fungi were more powerful at higher concentrations. The higher concentration may have facilitated the diffusion of the bioactive substances into the agar medium and led to growth inhibition. Studies conducted previously have shown that plant extracts possess higher antibacterial activity at higher concentrations (Cowan, 1999).

The ability of absolute alcohol extracts to extract a variety of bioactive compounds, including non-polar and moderately polar compounds like alkaloids, terpenoids, and phenolic compounds with strong antimicrobial effects, may be the reason for the higher antifungal activity seen in these extracts when compared to distilled water and chloroform. Organic solvents, such as absolute alcohol, have been shown to be more successful at extracting antimicrobial chemicals from plant materials than aqueous solvents (Mosić et al., 2021). As a result, the higher antifungal activity observed in absolute alcohol extracts in this investigation could be related to the presence of these bioactive chemicals.

This moderate antifungal activity recorded in 100% chloroform extracts might have resulted from the ability of the solvent to extract non-polar molecules. The chloroform extracts usually have alkaloids which are known to possess antifungal activity and toxicity as well. They may hinder the growth of fungi through the process of inhibition of the fungal cell membranes or enzyme action or even nucleic acids synthesis.

On the other hand, when comparing to alcoholic extracts, chloroform extracts show moderate antibacterial activity as earlier documented in literature. This could be because of the poor antifungal activity in distilled water extracts. The aqueous solvent extracts mainly polar molecules such as sugars, proteins, and glycosides which might not possess much of antibacterial activity. In addition, some antibacterial agents are unstable in aqueous solutions hence the low activity. Similar observations were made in researches done where aqueous extracts showed less antibacterial activity than organic solvent extracts (Doughari, 2009).

The ability to inhibit *Candida albicans* exhibited in this study suggests that there are active components in the extract of the plant that can inhibit the growth of *Candida albicans*. One thing that needs to be taken into consideration is that since the plant can inhibit *Candida albicans* which is responsible for skin infections, the plant can be medically exploited. Furthermore, the antifungal activity identified may be due to the synergistic action of several phytochemicals included in the plant extract. Plant bioactive compounds may work together to boost antifungal action. Synergistic interactions amongst phytochemicals have been shown to enhance antimicrobial activity when compared to individual compounds (Doughari, 2009).

As shown in the statistical data presented in Table 4.10, p-value = 0.024003 < 0.05, so H_0 can be rejected while accepting H_1. Consequently, it could be seen that there is a significant difference when using various solvents for the plant extract in measuring the antimicrobial activity of *D. stramonium* leaf extract on *C. albicans*. Hence, the extraction of the phytochemicals is largely dependent on the type of solvent used. Furthermore, it could also be deduced that the *Datura* plant extract shows a significant impact on *C. albicans*' growth.

The results presented in Table 4.8 show that all the extracts, regardless of the solvent used (chloroform, absolute alcohol, and distilled water), produced an MBC/MIC ratio of 2 against *C. albicans*. As the number is less than or equal to 4 (≤ 4), it implies that all of these extracts possess a fungicidal property since they are able to kill bacteria rather than simply inhibiting their growth. This approach simply highlights that this plant contains strong antifungal properties that actually destroy the existence of this particular strain. So, this plant can be exploited in the future for the development of a strong, effective agent for the treatment of infections caused by this particular strain.

5.3 Antibacterial Activity of Plant Extracts Against *S. aureus*

The plant extracts showed antibacterial action against *S. aureus* after agar well diffusion. The inhibition zones increased with increasing concentration in all solvents examined, as previously demonstrated for *Candida albicans* inhibition. Higher concentrations may cause more diffusion of antimicrobial substances into the agar medium, resulting in bigger zones of inhibition. These findings are consistent with prior research, which found that increasing extract concentration contributed to greater antibacterial activity.

The higher antibacterial activity noted in the chloroform extract may be attributed to the fact that the bioactive compounds like alkaloids, flavonoids, and phenolics present in chloroform extracts are known for their antibacterial activities. These chemical compounds can inhibit the growth of bacteria by interfering with cell membranes, enzymes, or protein synthesis. It is common practice in previous studies that the chloroform extracts often have high antibacterial activity because of antimicrobial compounds (Masoumeh Mirtaghi et al., 2014).

The strong antibacterial activity observed in alcohol extracts may be attributed to the presence of flavonoids and tannins extracted with alcohol. These chemicals have been shown to impede bacterial growth by destroying cell walls and interfering with metabolic activities. Alcohol extracts have been shown to have broad range on antibacterial action (Rasul, 2018).

Furthermore, the low antibacterial activity found in distilled water extracts could be due to poor antimicrobial ingredient extraction. Water extracts primarily comprise polar molecules, which may lack significant antibacterial effects. Similar results have been found in earlier studies, with organic solvent extracts exhibiting greater antibacterial activity than aqueous extracts. The antibacterial activity observed against *S. aureus* is significant because this bacterium is a common cause of skin infections, wound infections, and other opportunistic infections. The plant may be a possible source of antibacterial agents based on the plant extracts' capacity to inhibit *S. aureus*.

In addition to the solvents used in phytochemical extraction, Table 4.13, the p-value after analysis of variance is 0.040165, which is less than 0.05, so H_0 is rejected, and H1 is accepted, which states that there is a significant difference in using different solvents for plant extract when evaluating the antimicrobial effects of *D. stramonium* leaf extract against *S. aureus*. This only shows that the choice of solvent for phytochemical extraction plays a bigger role in the extract's efficacy.

5.4 Post Hoc Values of extract solvents against *S. aureus* and *C. albicans*

Based on the data that was found, the relationship between distilled water and absolute alcohol differs significantly, meaning these solvents have a great difference when it comes to phytochemical extraction from *Datura* plant. The Post Hoc value that was calculated was greater than the HSD value, which means the relationship differs significantly. The remaining relationships of solvents were not significantly different on pair-wise comparison. The difference is there but not significantly since the values were below the HSD on Turkey's Post Hoc Test. The relationship between chloroform and distilled water differs much more than the relationship between chloroform and absolute water.

These relationships greatly support the effects of the type of solvent used to extract a type of bioactive compound. In this scenario, absolute alcohol extract both moderate polar and nonpolar, chloroform extract nonpolar and distilled water extract polar bioactive compounds. Organic solvents had a strong effect on these pathogens and as compared to aqueous solvents. Previous studies highlighted that *Datura* plants had strong alkaloids and these alkaloids can be extracted by these organic solvents that were used. As a reason, that's why they showed a strong antimicrobial activity against these pathogens than distilled water.

5.5 Comparison of plant susceptibility between tested microbes

The antibacterial activity of plant extracts against *Candida albicans* and *Staphylococcus aureus* demonstrated that the bacteria were frequently more susceptible to the extracts. This was evidenced by larger zones of inhibition observed in *Staphylococcus aureus* compared to *Candida albicans* at similar concentrations and solvents.

Staphylococcus aureus's greater vulnerability than *Candida albicans* may be due to structural variations between bacteria and fungi. Peptidoglycan makes up the majority of the very simple cell wall structure of the Gram-positive bacteria *Staphylococcus aureus*. This structure enables antimicrobial chemicals to quickly enter and disrupt cellular processes. Antibacterial drug penetration may be slowed by *Candida albicans'* complex cell wall structure, which is composed of chitin, glucans, and mannoproteins (Kigigha & Kalunta, 2017).

Fungi have an additional resistance to antimicrobial compounds since the cell wall is composed of ergosterol. Ergosterol improves the cell wall's resilience and reduces its susceptibility to antibiotics. It helps account for why *Staphylococcus aureus* has greater zones of inhibition compared to *Candida albicans*.

5.6 Comparison of Solvents Used

Based on the zone of inhibition against these microbes, the study's findings demonstrated that pure alcohol extract had the strongest antimicrobial action against both *Candida albicans* and *Staphylococcus aureus*, followed by chloroform and distilled water. This implies that the kind of solvent utilized is important when extracting bioactive compounds. However, sometimes the differences might not be very effective due to the type of plant and the targeted compounds. This explains that the phytochemistry of plants differs, meaning some have alkaloids that are very effective against a particular strain, whereas in some plants those alkaloids might be less effective, vice versa.

Chloroform is a nonpolar solvent with the ability to extract nonpolar bioactive substances like alkaloids and terpenoids which claimed to have strong antimicrobial activities. Absolute alcohol, which is moderately polar, removes both polar and non-polar molecules, yielding moderate antibacterial action. Distilled water, being extremely polar, extracts primarily polar molecules, which may lack substantial antibacterial activity. These findings are consistent with prior research that found organic solvents to be more successful at extracting antimicrobial chemicals than aqueous solvents.

In terms of statistics, the Analysis of variance proved that there was a tremendous effect on the selection of solvent used for the phytochemicals because all the p-values were less than 0.05, hence H0 is rejected, and H1 accepted; meaning there is a considerable difference between the use of different solvents for plant extracts in assessing the antimicrobial activity of Datura stramonium leaf extract.

5.7 Limitations

This particular study is limited to *in vitro* without any clinical trial or the use of animal models for trials. In addition, *Datura* plants are known to have toxic alkaloids that may cause serious problems to living organisms. So, this toxic part was not exploited in this study, making it more difficult to create agents before the toxicology of this particular plant. Moreover, this study was mainly focused on two species, not including other pathogenic organisms that also cause skin diseases. In addition, only 3 solvents were used, whilst many solvents and methods can be used for phytochemical extraction. This study was mainly focused on antimicrobial effects as a whole without finding out the specific active compounds that are more effective in this plant.

5.8 Summary

This experiment sought to establish the efficacy of *D. stramonium* on two organisms, namely *S. aureus* and *Candida albicans*, which are known causes of skin diseases and many other ailments. Despite this plant's use in herbal medicine as a remedy for numerous bacterial and fungal infections, there is no evidence that this plant is effective against the aforementioned pathogens. So, this project focused on this area, on the other hand, using different solvents for phytochemical extraction. It also focused on the MIC, MBC and MFC of the extracts extracted using different solvents against these microbes.

A lot of things were discovered after the experiment was carried out, such as the choice of solvent being used for extraction. The extract solvent determines the type of phytochemical you want to extract, such as polar or nonpolar compounds. In this scenario, three solvents were used, which are distilled water, chloroform, and absolute alcohol. Absolute alcohol was the solvent that produced a large inhibition zone on both *S. aureus* and *C. albicans* after agar well diffusion at different concentrations of 100µg/ml, 300µg/ml, and 600µg/ml, and incubated respectively, followed by chloroform and distilled water, with a little inhibition zone.

A good explanation for this effect is the type of compounds being extracted by each solvent. Some of the solvents they extract moderate both polar and nonpolar compounds, such as chloroform and absolute alcohol. It was discovered before that chloroform and absolute alcohol extracts alkaloids more than other compounds, and the *Datura stramonium plant* also contains more toxic alkaloids. The results showed chloroform and absolute alcohol with a large inhibition zone, which explains why it is due to the extraction of more toxic alkaloids than distilled water.

In simple words, *Datura stramonium* exhibits antimicrobial effects on *S. aureus* and *C. albicans*. The zone of inhibition increased exponentially as the concentration increased. In addition, a 24-well plate microdilution was used to determine the MIC for every extract that was used against both organisms. It was discovered that the chloroform solvent extract requires a concentration of 12.5µg/ml for *S. aureus*, and 25µg/ml for *C. albicans*, the absolute alcohol extract, which requires 6.25µg/ml for *S. aureus* and 12.5µg/ml for *C. albicans*, and finally distilled water with 25µg/ml for *S. aureus* and 50µg/ml for *C. albicans,* respectively.

CHAPTER 6
CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study looked into the antibacterial efficacy of *Datura* plant leaf extracts against *Candida albicans* and *Staphylococcus aureus*, two prevalent pathogens linked with skin diseases. The result of this study indicated the presence of antibacterial properties in the extracts from *Datura* leaves against both microbes tested. Agar well diffusion analysis proved that antibacterial properties were found in varying degrees in the extracts of various solvents used. Absolute alcohol extracts demonstrated more antibacterial activity than chloroform and distilled water extracts, implying that the bioactive molecules responsible for antimicrobial action are more soluble in organic solvents.

Furthermore, it was found that the antibacterial activity of the extracts increased with the increase in concentration, implying a dose-dependent effect. High concentrations caused large zones of inhibition towards these microbes. This shows that the active ingredients in *Datura* leaf extracts can prevent the growth of these bacteria. The MIC and MBC/MFC were determined to demonstrate how efficiently the extracts inhibited microbial growth; most significantly, the extracts were bactericidal and fungicidal. This suggests that the plant's extract has a higher ability to eradicate certain infections.

The difference in antibacterial activity between the various solvents shows how important the extraction procedure is for obtaining useful bioactive compounds. The antibacterial activity seen in this study may be explained by the phytochemicals present in *Datura* leaves. The observed inhibitory effects on the test organisms may be explained by these chemicals' known antibacterial properties.

However, further investigation will be needed to prove and enhance the safety and effectiveness of *Datura* leaf extracts in vivo regardless of the fact that the current study was done only in vitro despite the promising outcomes. Nevertheless, the toxic nature of *Datura* genus stresses the necessity of careful examination before employing this plant for medicinal purposes. Nonetheless, the findings of the present study can prove useful for developing antibacterial remedies from the plant kingdom and reveal important data concerning the antibacterial potential of the mentioned plant.

In conclusion, the investigation performed using *Datura* plants revealed that its leaves may serve as an alternative source for antibacterial compounds that could aid in treating skin ailments. Further studies are required to isolate the active ingredients, carry out toxicity testing, and develop formulations.

6.2 Recommendations

There are various recommendations based on the findings of this study concerning the use of *Datura* leaf extract as antibacterial substance against *Candida albicans* and *Staphylococcus aureus*. The findings of this study have shown that there are antibacterial effects associated with the extracts of *Datura* leaves, hence, suggesting the plant to be used as substitute antimicrobials.

Further analysis through phytochemical analysis needs to be conducted so as to identify the exact chemical compounds that are responsible for the observed antibacterial action in the plant extract. This is despite the fact that there were observed antibacterial effects in the plant extracts as crude form; however, isolating the active ingredients would further help to better understand the mechanisms involved.

Second, prior to their prescription for use in medication, the toxicity and safety of these substances need to be tested. It is well known that different *Datura* plants include poisonous alkaloids like atropine, scopolamine, and hyoscyamine that can cause harm in case they are not handled with caution. Therefore, studies such as acute, subacute, and chronic toxicity

testing must be carried out in order to figure out the dosage and potential adverse reactions of consumers.

The variety of test microorganisms should be expanded in future research meaning some microbes that causes skin infection must also be included to accompany a variety pathogens. The study concentrated on two common microorganisms that cause skin conditions: *Staphylococcus aureus* and *Candida albicans*. Future research may include more microbes and this would increase the possibilities for using *Datura* extracts to treat a range of microbial infections by assessing whether they have broad-spectrum antibiotic action.

To increase the yield and efficacy of bioactive chemicals, additional study should be done to identify the optimal extraction techniques and solvents. Better yields of bioactive chemicals require consideration of Soxhlet extraction, maceration, and novel extraction techniques such ultrasonic or microwave-assisted extraction. To maximize the extraction of strong bioactive chemicals, a range of solvents with different polarities can be used to enhance antibacterial activity and extraction efficiency. By optimizing extraction variables including temperature, duration, and solvent concentration, the extracts' efficiency might be further increased.

Furthermore, formulation research should be carried out to create acceptable pharmaceutical products using *Datura* extracts. Because the investigated organisms are linked to skin illnesses, topical formulations such as creams, ointments, gels, and lotions could be created. These formulations would make it easier to apply the extracts directly to affected areas, hence improving their practical use in healthcare settings. Additionally, stability studies should be carried out to assess these formulations' shelf life and storage circumstances.

Furthermore, in vivo studies using animal models should be conducted to show that *Datura* extracts are safe and effective against bacteria in physiological conditions. In vivo examinations are necessary to ascertain how the extracts work in living animals, even though in vitro studies offer useful preliminary data. Determining aspects like absorption, metabolism, and potentially detrimental consequences might also be facilitated by these investigations.

Future studies should also look at the synergistic effects of *Datura* extracts and conventional antibacterial drugs. Antimicrobial efficacy can be increased, treatment dosages can be decreased, and the danger of antimicrobial resistance can be decreased by combining plant extracts with conventional antibiotics or antifungal drugs.

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Appendices

Appendix A *Datura stramonium* plant



Appendix B Phytochemical analysis for alkaloids, glycosides, flavonoids and saponins.





Appendix C Total percentage yield of the extract from different solvents used was calculated by Yield (%) = (extract dry weight / powdered leaf weight) × 100

Solvent used	**Petri dish weight(g)**	**Petri dish weight + extract(g)**	**Percentage Yield (%)**
Absolute alcohol	48.9	50.1	6
Chloroform	48.5	50.2	8.5
Distilled water	48.3	49.0	3.5

Appendix D Analysis of Variance for *S. aureus* antibacterial susceptibility test.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	3	28	9.333333	6.333333
Column 2	3	52	17.33333	12.33333
Column 3	3	40	13.33333	6.333333

Key

Column 1 – Distilled water

Column 2 – Absolute Alcohol

Column 3 – Chloroform

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	96	2	48	5.76	0.040165	5.143253
Within Groups	50	6	8.333333			
Total	146	8				

Appendix E Analysis of Variance for *C. albicans* antifungal susceptibility test.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*

Column 1	3	22	7.333333	2.333333
Column 2	3	43	14.33333	6.333333
Column 3	3	34	11.33333	6.333333

Key

Column 1 – Distilled water

Column 2 – Absolute Alcohol

Column 3 – Chloroform

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	74	2	37	7.4	0.024003	5.143253
Within Groups	30	6	5			
Total	104	8				

Appendix F Post Hoc Test using Turkey's HSD

Formular

HSD = $q\sqrt{\frac{MSE}{n}}$, where

HSD = Honestly Significant Difference

q = Studentized range statistic (obtained from Turkey's table)

MSE = Mean Square Error (from the ANOVA table)

n = Number of observations per treatment (equal sample sizes).

Pairwise for extracting solvents against *Staphylococcus aureus*

Mean Difference	**HSD=7.23**

Distilled	Absolute	8	Differ significantly
Absolute	Chloroform	4	Not differ significantly
Distilled	Chloroform	4	Not differ significantly

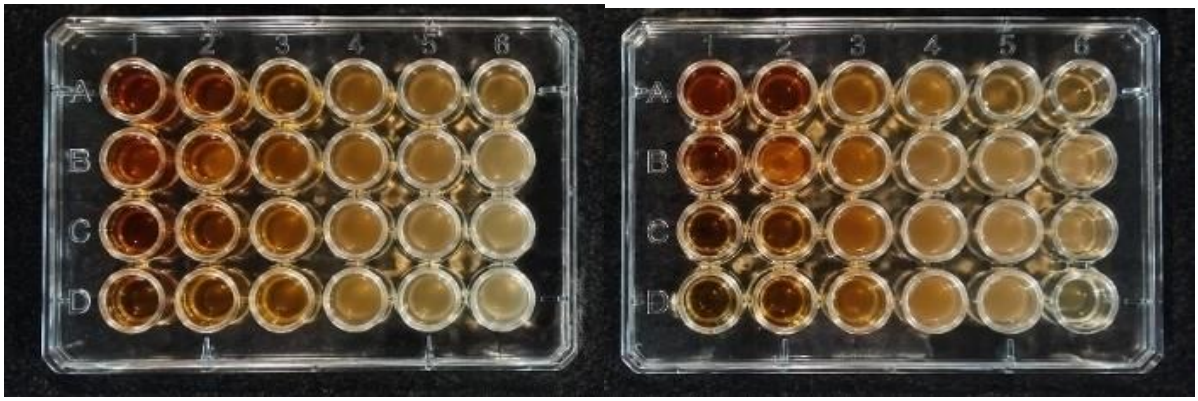
Pairwise for extracting solvents against *Candida albicans*

		Mean Difference	**HSD=5.60**
Distilled	Absolute	7	Differ significantly
Absolute	Chloroform	3	Not differ
Distilled	Chloroform	4	Not differ

Appendix G MBC/MIC and MFC/MIC of the extracts against *S. aureus* and *C. albicans* and how data was interpreted.

Solvent used	**MBC/MIC**	**If ≤ 4 = Bactericidal** **If >4 = Bacteriostatic**	**MFC/MIC**	**If ≤ 4 = Fungicidal** **If > 4 = Fungistatic**
Chloroform	2	Bactericidal	2	Fungicidal
Absolute alcohol	2	Bactericidal	2	Fungicidal
Distilled water	2	Bactericidal	2	Fungicidal

Appendix H 24-well plate microdilution method; *S. aureus*(left), *C. albicans*(right)



Appendix I Antimicrobial susceptibility test

