COMPARATIVE STUDY OF THE ANTIMICROBIAL ACTIVITY BETWEEN RAW AND POWDERED SPICES

A thesis submitted to the Department of Mathematics and Natural Science (MNS) in partial fulfillment of the requirements for the Bachelor of Science in Biotechnology

By

Tasmia Azam Roza
19236021
Sumaiya Amin
19336032
Masaba Samanzar
18236008
Kazi Tanha Annoor
18236004

Mathematics and Natural Science
BRAC University
September, 2023

For our beloved family members,

who stuck with us through this difficult journey.

Declaration

It is hereby declared that

1. The thesis submitted is our original work while completing the degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. We have acknowledged all main sources of help.

Student's Full Name & Signature:

Tasmia Azam Roza 19236021	**Masaba Samanzar** 18236008
Kazi Tanha Annoor 18236004	**Sumaiya Amin** 19336032

Approval

The thesis titled, “Comparative study of the antimicrobial activity between raw and powdered spices” submitted by

1. Tasmia Azam Roza (19236021)
2. Sumaiya Amin (19336032)
3. Masaba Samanzar (18236008)
4. Kazi Tanha Annoor (18236004)

of Spring, 2023 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of BSc in Biotechnology on 21st September, 2023.

Examining Committee:

Supervisor:

_____

Mahboob Hossain, PhD
Professor, Microbiology Program
Department of Mathematics and Natural Sciences
BRAC University

Program Director
(Member)

_____

Munima Haque, PhD
Associate Professor
and Director Biotechnology Program

Departmental Head:
(Chair)

_____

A F M Yusuf Haider, PhD
Professor and Chairperson
Department of Mathematics and Natural Sciences
BRAC University

Acknowledgement

Our first and foremost thanks towards the Almighty for guiding us through this difficult journey and providing us with the patience required to finish this successfully.

Our sincerest gratitude to **A F M Yusuf Haider**, Professor and Chairperson, Department of Mathematics and Natural Sciences, BRAC University, for his cooperation.

We are much obliged to our thesis supervisor **Mahboob Hossain**, Professor, Department of Mathematics and Natural Sciences, BRAC University, for his continuous supervision and guidance throughout this thesis. His suggestions truly made it possible for us to complete this project with such clarity.

We are also grateful to **Munima Haque**, Associate Professor and Program Director, Department of Mathematics and Natural Sciences, BRAC University, for approving this thesis and acknowledging our work.

Lastly, we would like to appreciate the constant support and encouragement from our family and friends throughout this long journey. Having them by our sides is what helped us be more passionate about this project and finish it with confidence.

Abstract

Currently, pathogen-led infectious diseases and food poisoning caused by microbial spoilage are some of the biggest concerns for human health all over the world. However, the efficiency level of some antimicrobial agents that inhibit disease-causing microorganisms has weakened over time. This has given rise to the need for discovering new antimicrobial agents that can lower the rate of harmful microorganisms in food and medicine.

This study focuses on the commonly consumed spices and investigates their antimicrobial effect on various multidrug-resistant organisms. Furthermore, it focuses on comparing the effectiveness of raw and powdered spices. Four every day consumed spices (Turmeric, Ginger, Cinnamon, and Black Pepper) were tested against a total of eight organisms (ETEC, EIEC, *Klebsiella pneumonia, Acinetobacter baumannii, Bacillus cereus, Staphylococcus aureus, Staphylococcus saprophyticus* and *Pseudomonas aeruginosa*), following three different protocols (non-standardized disc diffusion, non-standardized well diffusion and standardized disc diffusion).

On an overall observation, the only spice that showed antimicrobial activity in all 3 methods and against almost every bacteria, is Ginger. It is more effective in the non-standardized methods compared to the standardized methods. This led us to believe that abundance encourages effect for Ginger. Observations also concluded that powdered Ginger is effective in all methods whereas raw Ginger is not. The second most effective spice was Turmeric. However, it only showed antimicrobial activity in non-standardized disc diffusion. The highest zone of inhibition was found in *Staphylococcus aureus* (Raw = 21 mm, Powder = 26 mm). Cinnamon showed the weakest results across all 3 methods. Only in a few cases zones were observed in *Bacillus cereus* (21 mm) and *Staphylococcus aureus (*15 mm) for raw Cinnamon. On the other hand, powder Cinnamon showed inhibition for *Bacillus cereus* (20 mm), *Staphylococcus aureus* (15 mm), and ETEC (18 mm).

To summarize the study according to our goal, powdered spices were more effective under various conditions in comparison to raw unprocessed spices. Ginger can be labeled as the most effective antimicrobial spice.

Table of Contents

List of Tables

List of Figures

List of Acronyms

HLGR	High level gentamicin-resistant
MRSA	Methicillin-resistant *Staphylococcus aureus*
HEPA	High Efficiency Particulate Air
MHA	Mueller Hinton Agar
NA	Nutrient Agar
HSV	Herpes simplex virus
HIV	Human immunodeficiency virus
E. coli	*Escherichia coli*
ETEC	*Enterotoxigenic E. coli*
EIEC	*Enteroinvasive E. coli*

Chapter 1

Introduction

1.1 Background

Historically, spices have been used for flavor, aroma, colour, and preservation of foodstuffs. Spice extracts are well known to exhibit antibacterial properties, but there is a lack of a comprehensive evaluation of the antibacterial effect of spices against antimicrobial resistance (Zhang et al., 2019). Recent literature has increasingly reported on the antibacterial activity of spices against common Gram-positive and Gram-negative bacteria responsible for human infectious diseases and food safety problems. Few studies have focused on the inhibitory effects of raw spices on antimicrobial-resistant bacteria. (Liu et al., 2017) such as Ginger, cumin, Cinnamon, clove etc. The methanolic and ethanolic extracts of Cinnamon, which was the most studied spice, were reported to have inhibitory effects on high-level gentamicin-resistant (HLGR) enterococci, multi-drug resistant *Escherichia coli* AG100, methicillin-resistant *S. aureus* (MRSA), βlactamase producing multi-drug *Klebsiella pneumonia* and *Pseudomonas aeruginosa* . Bacteria can be killed or inhibited by many spices, or their active chemical ingredients. The purpose of this study is to find out the antimicrobial activity of natural raw spices and powder spices on multi-drug /antimicrobial resistance in vitro.The sensitivity of some human pathogenic bacteria to various spice extracts and demonstrating a positive interaction between spices and conventional antimicrobials will be compared in this study.

Unprocessed or minimally processed plant-derived ingredients used to enhance the flavor, aroma, and colour of food are referred to as raw spices. They are typically obtained from various parts of plants, such as seeds, fruits, bark, roots, or flowers. Raw spices have been utilized for centuries in cooking, traditional medicine, and food preservation owing to their rich flavors and medicinal

properties (Kumar, 2020). For instance, black pepper, Ginger, garlic, Cinnamon, etc., are just a few examples of the wide variety of raw spices employed in cooking. Each spice is characterized by its own unique flavor profile and potential health benefits.

On the other hand, powdered commercial spices are referred to as spices that have been processed and ground into a fine powder form for commercial distribution and use. These spices are widely available in grocery stores and are convenient for use in cooking and seasoning dishes.

Here are some key points about powdered commercial spices:

Processing: The raw spices are typically harvested and then undergo processing steps such as drying, cleaning, grinding, and packaging. This process is employed to preserve the flavors, aromas, and nutritional properties of the spices.

Convenience: Convenience in cooking is offered by powdered commercial spices as they are ready to use. The need for grinding or crushing whole spices before use is eliminated by them, thus saving time and effort in the kitchen.

Variety: A wide range of options is offered by powdered commercial spices, including popular spices like turmeric, cumin, coriander, chili powder, paprika, garlic powder, onion powder, and many others. These spices are often available individually or as pre-mixed spice blends for specific cuisines or dishes.

Standardization: Quality control measures are often applied to commercial spice powders to ensure consistency in flavor, aroma, and colour. This allows a consistent taste profile to be relied upon by consumers when the same brand or type of spice powder is used.

Shelf Life: A longer shelf life is generally possessed by powdered commercial spices compared to whole spices. When properly stored in airtight containers away from heat and moisture, their flavors and qualities can be retained for an extended period.

Additives: Additives like anti-caking agents, preservatives, or colourants may be contained in some commercial spice powders to enhance their appearance, prevent clumping, or increase shelf stability. Spices without unwanted additives should be chosen if desired, and the ingredient list should be read.

While convenience is offered by powdered commercial spices, they may not have the same freshness and potency as freshly ground or whole spices. Over time, the volatile compounds responsible for the aroma and flavor of spices can gradually diminish. Therefore, grinding whole spices at home or using fresh herbs is preferred by some people for a more pronounced flavor. Ultimately, the choice between powdered commercial spices and other forms of spices depends on personal preference and the desired culinary outcome (Spices Manufacturing Process, 2022).

1.2 Turmeric

Turmeric (*Curcuma longa*) is a spice that has been used for centuries in traditional medicine, particularly in India and other parts of Asia. Its vibrant yellow colour is the result of a compound called curcumin, which is believed to have various health benefits, including antibacterial activity.

The antibacterial properties of turmeric and its active compound curcumin have been investigated by several studies. Here are some key points about the antimicrobial effect of turmeric:

Broad-Spectrum Activity: Antibacterial activity against a wide range of bacteria, including both Gram-positive and Gram-negative bacteria, has been demonstrated by curcumin. Common pathogens like *Staphylococcus aureus, Escherichia coli (E. coli),* and *Salmonella* species are included in this spectrum.

Mechanisms of Action: Curcumin's antibacterial effects are believed to arise from its ability to interfere with bacterial cell membranes, inhibit bacterial enzymes, and disrupt bacterial cell communication.

Synergistic Effects: In some studies, the enhancement of the effects of certain antimicrobials by curcumin has been observed, suggesting a potential role in combating antimicrobial-resistant bacteria.

Biofilm Inhibition: The potential for curcumin to inhibit biofilm formation, communities of bacteria encased in a protective matrix that are highly resistant to antimicrobials, has been explored. This suggests its utility in combating biofilm-associated infections.

Wound Healing: Research suggests that wound healing may be aided by turmeric or curcumin due to its antibacterial properties. They may assist in preventing infection and promoting faster healing.

Dental Health: Turmeric's antibacterial effects have also been studied in the context of oral health, potentially combating bacteria associated with dental plaque and gum disease.

Food Preservation: Turmeric and its active components have been researched for their potential use as natural food preservatives due to their antibacterial properties, which could extend the shelf life of certain foods (Adamczak et al., 2020).

1.3 Black pepper

The antimicrobial properties of black pepper, scientifically known as *Piper nigrum*, have been recognized for centuries. (Srinivasan, 2022) The bioactive compounds, such as piperine and other alkaloids, are primarily attributed to black pepper for its antimicrobial effects.

Key points about the antimicrobial effect of black pepper are as follows:

Enhancement of Nutrient Absorption: The absorption of certain nutrients, particularly curcumin from turmeric, can be enhanced by piperine in black pepper. When combined with turmeric, curcumin's bioavailability can be increased, potentially enhancing its potential health benefits.

Digestive Aid: The secretion of digestive enzymes can be stimulated by black pepper, aiding in the breakdown of food and promoting efficient digestion.

Anti-Inflammatory Properties: Anti-inflammatory effects have been demonstrated by piperine in black pepper. It might help reduce inflammation in the body, potentially benefiting conditions like arthritis and other inflammatory disorders.

Antioxidant Activity: Antioxidants contained in black pepper help neutralize harmful free radicals in the body, contributing to overall health and reducing the risk of chronic diseases.

Blood Sugar Regulation: A positive impact on blood sugar levels and insulin sensitivity, which could be beneficial for individuals with type 2 diabetes, might be from piperine.

Respiratory Health: Black pepper's traditional use for respiratory issues, owing to its warming and stimulating properties, might help relieve symptoms of coughs and colds.

Cancer Prevention: While ongoing research is being conducted, some studies have suggested that piperine might have anticancer properties by interfering with the growth of cancer cells.

Antimicrobial Activity: The inhibition of the growth of certain bacteria has been demonstrated by piperine, showing its antimicrobial properties.

1.4 Ginger

The rhizome of the *Zingiber officinale* plant, Ginger, has been recognized for its antimicrobial properties. The antimicrobial effects of Ginger are attributed to its bioactive compounds, including Gingerol, shogaol, and zingerone.

Key points about the antimicrobial effect of Ginger are as follows:

Digestive Health: Ginger, long used to alleviate digestive issues, can help stimulate saliva production and digestive enzymes, aiding in the digestion process. It's often used to relieve nausea, including motion sickness, morning sickness during pregnancy, and chemotherapy-induced nausea.

Anti-Inflammatory Properties: Ginger contains compounds like Gingerol and zingerone, which have anti-inflammatory effects. Regular consumption of Ginger might help reduce inflammation in the body, potentially benefiting conditions like osteoarthritis and other inflammatory diseases. Some studies suggest that Ginger's anti-inflammatory properties can also contribute to pain relief, potentially helping alleviate menstrual pain, muscle soreness, and other types of pain.

Cardiovascular Health: A positive impact on heart health might be from Ginger, potentially helping lower blood pressure and improve cholesterol levels, thus reducing the risk of heart disease.

Immune System Support: Ginger contains antioxidants that can help strengthen the immune system and protect the body from oxidative stress and damage.

Anti-Nausea and Vomiting: Known for its ability to reduce nausea and vomiting, Ginger is commonly used as a natural remedy for morning sickness in pregnant women and for motion sickness during travel.

Cancer Prevention: While more research is needed, some studies have indicated that Ginger might have potential anticancer properties. Its compounds could potentially inhibit the growth of certain cancer cells.

Respiratory Health: Ginger's anti-inflammatory and antimicrobial properties might be beneficial for respiratory health, potentially helping soothe sore throats and reduce congestion.

Type 2 Diabetes Management: Preliminary research suggests that Ginger might have a positive impact on blood sugar levels and insulin sensitivity in people with type 2 diabetes.

Cognitive Function: Some studies suggest that Ginger might have neuroprotective properties that could contribute to brain health and cognitive function. (Mao et al., 2019)

1.5 Cinnamon

Derived from the bark of trees belonging to the *Cinnamomum* genus, Cinnamon has been recognized for its antimicrobial properties for centuries. The bioactive compounds, including cinnamaldehyde and other phenolic compounds, are primarily attributed to Cinnamon for its antimicrobial effects.

Key points about the antimicrobial effect of Cinnamon are as follows:

Antibacterial Activity: Cinnamon has demonstrated antibacterial activity against a wide range of bacteria, including both Gram-positive and Gram-negative species. Effectiveness against bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* species, and others has been shown. The antibacterial properties of Cinnamon are believed to be due to the presence of active compounds that disrupt bacterial cell membranes, inhibit bacterial enzymes, and impede bacterial growth.

Antifungal Activity: Effectiveness against various fungal strains has been demonstrated by Cinnamon, including *Candida* species, *Aspergillus* species, and other common fungal pathogens. Mechanisms such as disruption of fungal cell membranes, inhibition of fungal enzymes, and prevention of fungal growth are involved in Cinnamon's antifungal properties.

Antiviral Activity: Studies have been conducted on Cinnamon for its antiviral activity against the influenza virus, HSV, and HIV. Interference with viral replication and inhibition of viral attachment to host cells may be involved in the antiviral properties.

Mechanisms of Action: Cinnamon's active compounds, particularly cinnamaldehyde, are attributed to the antimicrobial effects. Antimicrobial properties have been found in cinnamaldehyde by disrupting the integrity of microbial cell membranes, interfering with microbial enzymes, and inhibiting the production of microbial toxins.

Synergistic Effects: Cinnamon has been reported to exhibit synergistic effects when combined with certain antimicrobials or antimicrobial agents. Enhanced antimicrobial activity against bacteria and fungi has been demonstrated by these combinations. Such synergistic effects may help overcome antimicrobial resistance and increase the efficacy of existing antimicrobial treatments.

Oral Health: In the context of oral health, Cinnamon's antibacterial effects have been investigated. Some studies suggest that Cinnamon extracts or oils might help combat oral bacteria associated with dental plaque and bad breath.

Biofilm Inhibition: Similar to turmeric, Cinnamon has been studied for its potential to inhibit the formation of bacterial biofilms. This is important because biofilms can make bacteria more resistant to antimicrobials and the immune system. (Nabavi et al., 2015)

1.6 Bacterial strains selected for study

The antimicrobial effect of raw spices compared to commercial powdered spices in different microorganisms such as *E. coli*, *Pseudomonas sp.*, *Streptococcus sp.*, *Staphylococcus sp.*, and *Acitenobactersp.* was examined. Bacterial strains are variations or subtypes of bacteria within a particular species. Different traits, such as resistance to antimicrobials, the ability to cause specific diseases or tolerance to certain environmental conditions, may be exhibited by each bacterial strain as a result of variations in their genetic composition, physical characteristics, and behavior.

Here are a few examples of well-known bacterial strains that were utilized:

Escherichia coli (E. coli): The intestines of humans and animals commonly host the *E. coli* species. Numerous strains with distinct characteristics exist within the *E. coli* species. Some strains are harmless and are part of the normal gut flora, while others can cause severe gastrointestinal infections. Examples of pathogenic *E. coli* strains include *E. coli* O157:H7, which is associated with foodborne illness, and enterotoxigenic *E. coli* (ETEC), known for causing traveler's diarrhea.

Staphylococcus aureus: *Staphylococcus aureus* is a bacterium commonly found on the skin and in the respiratory tract of humans. It encompasses various strains, including methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA strains are resistant to many antimicrobials and can cause difficult-to-treat infections, particularly in healthcare settings.

Streptococcus pneumoniae: *Streptococcus pneumoniae* is a bacterium responsible for respiratory infections such as pneumonia, sinusitis, and otitis media. Numerous serotypes or strains of *S. pneumoniae* exist based on differences in the polysaccharide capsule surrounding the bacteria. Some serotypes are more virulent and associated with invasive diseases, while others are less pathogenic.

Bacterial strains can be further classified and identified through molecular techniques such as DNA sequencing and genetic analysis. Understanding the characteristics and behaviors of different bacterial strains is crucial for diagnosing infections, determining appropriate treatment strategies, and studying their epidemiology and evolution.

1.7 Antimicrobial resistance

Antimicrobial resistance is a phenomenon in which the ability to survive and grow in the presence of antimicrobials that would normally kill or inhibit their growth is developed by bacteria. This resistance can occur naturally through genetic mutations or the acquisition of resistance genes from other bacteria through horizontal gene transfer.

Here are some key points about antimicrobial resistance:

Mechanisms of Resistance: Resistance to antimicrobials can be developed by bacteria through various mechanisms. These include:

- Enzymatic Inactivation: Enzymes are produced by bacteria that can chemically modify antimicrobials, rendering them ineffective.
- Target Modification: The target site of the antimicrobial is altered by bacteria, preventing the drug from binding and exerting its effect.
- Efflux Pump: Efflux pumps that can actively pump out the antimicrobial, reducing its concentration within the bacterial cell, are developed by bacteria.
- Reduced Permeability: The outer membrane or cell wall of bacteria can be modified, limiting the entry of antimicrobials into the cell.

Consequences of antimicrobial Resistance: Significant challenges in the treatment of bacterial infections are posed by antimicrobial resistance. It can lead to prolonged illness, increased healthcare costs, and higher rates of treatment failure. In some cases, infections caused by resistant bacteria may become untreatable, resulting in increased morbidity and mortality.

Factors Contributing to Resistance: Several factors contribute to the development and spread of antimicrobial resistance. These include the misuse and overuse of antimicrobials in human and veterinary medicine, inadequate infection control measures, poor sanitation and hygiene practices, and the widespread use of antimicrobials in agriculture and livestock production.

Global Health Impact: Antimicrobial resistance is a global public health concern. It affects both developed and developing countries, and the spread of resistant bacteria can transcend geographical boundaries. Infections caused by resistant bacteria are more difficult and costly to treat, leading to increased hospitalizations, longer durations of illness, and higher mortality rates.

Combating Antimicrobial Resistance:

Addressing antimicrobial resistance requires a multifaceted approach. Key strategies include:

- **Rational Antimicrobial Use:** Promoting appropriate antimicrobial prescribing practices and educating healthcare professionals and the public about the responsible use of antimicrobials.
- **Infection Prevention and Control:** Implementing robust infection control measures in healthcare settings and promoting good hygiene practices in the community.
- **Surveillance and Monitoring:** Monitoring the emergence and spread of antimicrobial-resistant bacteria through surveillance systems and sharing data nationally and internationally.
- **Research and Development:** Investing in the development of new antimicrobials, diagnostics, and alternative treatment options to combat resistant infections.

Efforts to combat antimicrobial resistance require collaboration between healthcare providers, policymakers, researchers, and the public to ensure the responsible use of antimicrobials and the preservation of these life-saving medications for future generations. (Arias & Munita, 2016)

1.8 Objective

The main objective of this study was to determine the antimicrobial activity of various spices against different multidrug-resistant microorganisms. The actions were also to be compared to identify a health preference for consumers, between raw unprocessed spice and powdered processed spice.

Chapter 2

Materials and Methods

2.1 Instrument used:

Petri dish: Shallow cylindrical containers with fitted lids were used to hold the experiment's growth medium and for cell culture.

Wire loops: Also known as Nichrome Wire Inoculating Loops, used for transferring inoculum for streaking.

Cotton swab: Sterile swabs were used for biological sample collection to avoid sample contamination and for lawning petri dishes.

Bunsen burner: A Bunsen burner was used to sterilize loops.

Sterile Cork Borer: A metal tool that was used for cutting a hole in the solid media so that the holes could be filled with the spice sample.

Filter paper: As spices were used as samples, it was necessary to filter the sample to avoid tiny pieces of spices present in the mixture. Using filter paper gives a clear solution as the particles are trapped in the pores of filter paper.

Glass Funnel 75 mm: This was used for pouring liquids or powder through a small opening and for holding the filter paper in filtration.

Screw-capped tubes or small Vials: Were used for dry spice samples and antimicrobial discs.

Eppendorf tube: This tube was used for preparing, mixing, centrifuging, transporting, and storing solid and liquid samples and reagents.

Micropipette (30-300 µl): Micropipettes were used for transferring liquids.

Centrifuge machine: It was used to separate particles suspended in a liquid according to particle size and density, viscosity of the medium, and rotor speed.

Forceps (sterile): Was used for moving and sterling small objects.

Glass spreader: A glass cell spreader or plate spreader was used to smoothly spread cells and bacteria on a culture plate, such as a petri dish.

Glass Beaker: Scientific glass beakers were used for mixing liquids or powders.

Vortex machine: This was used in the rapid mixing of samples.

Fume hood: The purpose of a chemical fume hood is to prevent the release of hazardous substances into the general laboratory space by controlling and then exhausting hazardous and/or odorous chemicals as the samples of spice mixtures were mixed with chemicals.

Antibiotic disc: The discs are used to find out which antimicrobial an infective organism is sensitive to. At first, the discs were autoclaved by putting them inside vials and then used by placing them on the solid media on a petri dish.

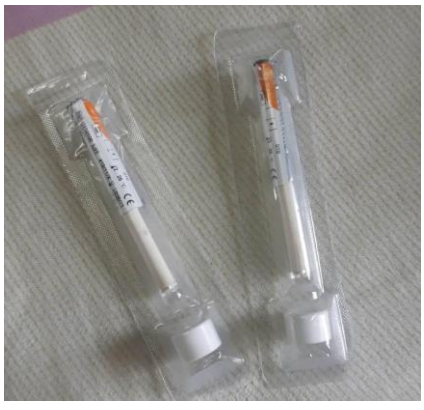


Figure 1: Antibiotic discs

Electronic balance: An electronic balance was used to measure spice amounts.

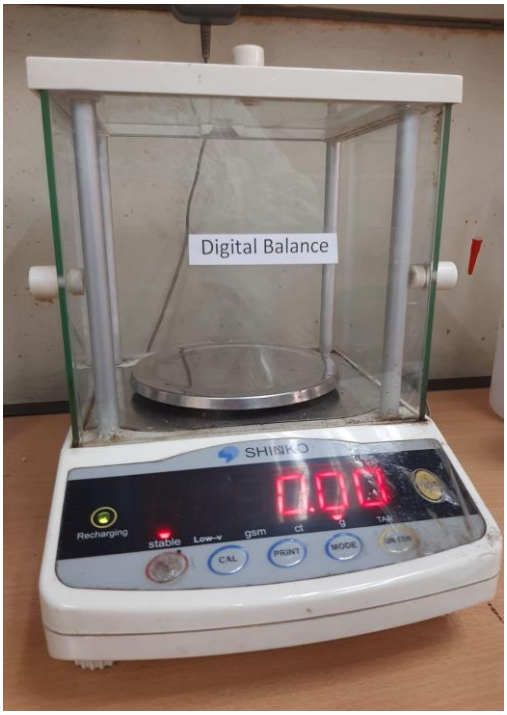


Figure 2: Electronic balance

Measuring Cylinder: A measuring cylinder is a vessel used for determining liquid volume.

Fine thin Cloths or Cheesecloth: The cloths were used for doing the spice filtration work.

Autoclave Machine: Items are heated to an appropriate sterilization temperature for a given amount of time.

Borer: A stainless steel tool used to create a round hole in the media for well-diffusion.

Laminar hood: Was used for media plate preparation, sample preparation, and culturing. Laminar flow hoods protect the working environment from dust and other airborne contaminants by maintaining a constant, unidirectional flow of HEPA-filtered air over the work area.

2.2 Chemical used:

Agar (MHA, NA): In a microbiology lab, scientists use agar to create a solid growing area mixed with nutrients for the bacteria they want to culture and identify. Agar has several properties that make it the ideal solidifying agent which are:

1. It becomes a transparent molten solution at about the boiling point of water (100^0C) and remains liquid down to about 38-40^0C.
2. Because most microorganisms are not killed at 45^0C, they can be added to a liquid medium containing molten agar before the medium is poured into tubes or Petri dishes.
3. Once the agar medium cools and solidifies, it will remain solid at the usual incubation temperatures (30-50^0C) and can be inoculated with microorganisms.
4. It is not a nutrient for most microorganisms and is not metabolized during microbial growth (except for a few marine bacteria).

Microorganisms vary widely in their requirements for growth. However, all organisms require:

a) A suitable energy source

b) Suitable sources of carbon and nitrogen

c) Water

d) Adequate amounts of certain mineral salts and trace elements.

e) Many organisms require particular organic growth factors such as amino acids and vitamins.

MH (Mueller–Hinton) agar was mainly used because it is a microbiological growth medium that is commonly used for antimicrobial susceptibility testing, specifically disc diffusion tests. Mueller Hinton agar is made up of a couple of components, including beef extract, acid hydrolysate of casein, and starch, as well as agar to solidify the mixture.

MH agar is considered the best medium to use for routine susceptibility testing of non-fastidious bacteria for the following reasons:

- It shows acceptable batch-to-batch reproducibility for susceptibility testing
- It is low in sulphonamide, trimethoprim, and tetracycline inhibitors
- It supports satisfactory growth of most non-fastidious pathogens

A large body of data and experience had been collected concerning susceptibility tests performed with this medium.

NA(Nutrient agar) agar media was also used which is a liquid medium supporting the growth of a wide range of non-fastidious organisms. The key difference between nutrient agar and Mueller Hinton agar is that nutrient agar is a media mainly used for the general isolation of bacteria, while Mueller Hinton agar is a media used for antimicrobial susceptibility testing of bacteria.

Ethanol: It is known that ethanol is used in the purification and precipitation of biomolecules and disinfection. In this experiment, it was used to disinfect the workspace and the experimental specimens. The laminar hood was cleaned with ethanol every time. Hands and all used specimens were thoroughly disinfected before inserting them in the hood.

Distilled water: The distilled water was used to dilute the spice in the first two methods.

Methanol: Methanol is used as a solvent in producing synthetic drugs. In the third method, the spice was precipitated in ethanol for around 45 days to collect the purest and concentrated spice for the experiment.

2.3 Specimen collection and processing:

For this study specimens were collected from the university laboratory.

Microorganisms used:

- *Klebsiella pneumonia*
- *Enteroinvasive Escherichia coli* (EIEC)
- *Bacillus cereus*
- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*
- *Enterotoxigenic Escherichia coli* (ETEC)
- *Acinetobacter baumannii*

NA media plates were used to grow this bacterial organism. A correct and timely laboratory test result is dependent on proper specimen collection and management. Specimens must be obtained in the proper tubes or containers and correctly labeled. So, in this process, the following steps were orchestrated-

At first, bacterial organisms were taken from a single colony from the sample (collected from the lab)by a sterile wire loop .Then it was mixed in a special collection tube with distilled water .

1) Then the streak plate method to obtain single isolated pure colonies.
2) After incubation for 48 hours at 37°C growth was seen.

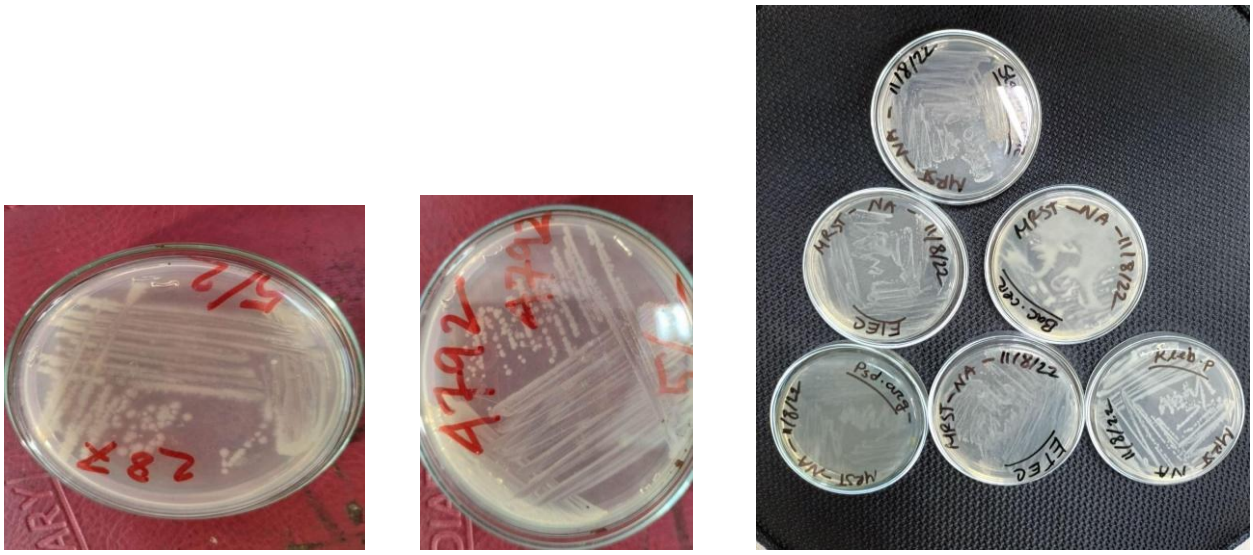


Figure 3: Appearance of bacterial colonies after incubation

2.4 Maintenance and preservation of bacterial sample:

Bacterial culture was grown on an appropriate solid medium. Once it has been isolated and grown in pure culture, it becomes necessary to maintain the viability and purity of the microorganism by keeping the pure culture free from contamination. The pure cultures were transferred periodically onto a fresh medium (subculturing) to allow continuous growth and viability of microorganisms. To avoid contamination it is always subject to aseptic conditions by transfering it.

Pure cultures were successfully stored at 0 to 4°C by using the culture refrigerators. Because the metabolic processes of the microorganisms are considerably slowed but not stopped by this approach for a limited period (2-3 weeks for bacteria).

2.5 Preparation of Nutrient Agar:

Nutrient agar was made with various nutrients which allow the growth of a wide variety of microorganisms that do not usually require specific nutrients or supplements. Nutrient agar was used from the laboratory and prepared from the dehydrated powder supplied by vendors. The preparation process is given below.

1. The appropriate quantity of the ingredients of nutrient agar for 200 mL medium (0.3% beef extract, 0.5% peptone, 0.85% NaCl, and 1.5% agar) was weighed by measuring.
2. Two hundred milliliters of distilled water was added and the ingredients were dissolved by heating at 100℃ on a hot plate with continuous agitation.
3. The medium was dispensed in the conical flask.
4. The opening of the flask was closed with a cotton plug and the plug was covered by using a piece of aluminum foil.
5. The flasks of the medium were sterilized by autoclaving for 15 minutes at 121℃.
6. After autoclaving, the flask of the medium was placed in the 50℃ water bath until ready to pour.
7. Sterile Petri dishes were placed in smooth rapid motion.
8. The cotton plug of the flask medium was removed and discarded.
9. The opening was flamed briefly.

10. The agar medium (about 20-25 mL) was poured rapidly and gently into the five dishes by lifting the lid of the dish no more than necessary.
11. If any bubbles persisted, they were removed with a brief exposure to the Bunsen burner flame moved over the surface to the agar in the dish.
12. The agar was allowed to cool and solidify with the Petri dish by raising the lid slightly to allow the escape of steam. After about 10 minutes the lid was closed.
13. The plates were inverted and incubated overnight at 37℃ by using the incubator to check for sterility.

2.6 Preparation of Saline water:

The normal saline solution is simply the 0.85% Sodium chloride (NaCl) solution which can be prepared in the laboratory by dissolving the calculated amount of Sodium chloride crystals in the required quantity of Distilled water. The preparation process was-

1. 0.85g of NaCl was dissolved in a beaker with distilled water to bring the final volume to 100 mL.
2. Autoclave 15 min at 121°C.
3. Cool to room temperature.
4. Stored it in a screw cap flask

2.7 Preparation of Mueller Hinton Agar:

Mueller–Hinton agar is a microbiological growth medium that is commonly used for doing antimicrobial susceptibility testing, specifically disc diffusion tests.

The preparation process is given below-

1. 38g of Mueller Hinton agar powder was added in 1 L of distilled water. (Every time before making MHA plates, 200 ml of distilled water was used with 7.6 gm of MHA powder to prepare the MHA media.)
2. Mixed and dissolved.
3. Then the suspension was boiled to dissolve the medium completely by using heat.
4. Sterilized by autoclaving at 121°C for 15 minutes.
5. Poured the liquid into the petri dish and waited for the medium to solidify.

The Agar was prepared in a clean environment to prevent any contamination. The laminar hood was properly sterilized by using ethanol before any work.

2.8 Methods

2.8.1 Method-1

1. In the case of powdered spices, 100 gm of commercial spice was mixed with 100 mL of distilled water and, in the case of raw spices, 100 gm of blended raw spices was mixed with 100 mL of distilled water. Cinnamon, Ginger, Turmeric, and Black Pepper were taken as spice samples, and about 20 filter papers were cut into 80 circles which were then penetrated the spice solution.

2. Prepared the inoculum by suspending four or five isolated colonies of the organism with a sterile inoculating loop or needle in 2 ml of sterile saline. Vortexed the saline tube and created a smooth suspension.

3. Inoculated the MH plate with a sterile swab which was dipped into the inoculum tube and rotated against the side of the tube (above the fluid level) using a firm pressure, to remove excess fluid. Inoculated the dried surface of an MH agar plate by streaking the swab three times over the entire agar surface; rotated the plate approximately 60 degrees each time to ensure an even distribution of the inoculums and discarded the swab afterward into an appropriate container.

4. Used the forceps carefully to remove each disc from the conical flask and placed the disc on the plate. The disc was pressed gently with the forceps to ensure complete contact with the agar surface. The process was continued to place one disc at a time onto the agar surface until all discs had been placed. Once all discs were in place, the plates were inverted , and placed in a 37°C air incubator for 18 to 24 hours.

5. After incubation, the zone of inhibition was measured sizes to the nearest millimeter with a ruler or caliper; including the diameter of the disc in the measurement. Recorded the zone size on the recording sheet.

2.8.2 Method-2

1. The spice solutions were made by mixing distilled water and spices in conical flasks.

2. In the case of powdered spices, 100 gm of commercial spice was taken and 100 ml of distilled water was added to it, in the case of raw spices, 100 gm of raw spices was taken which was then blended into powdered form and 100 ml of distilled water was added. 3 spices were taken, they were: chili, Ginger and turmeric.

3. Then inoculum was prepared by suspending four or five isolated colonies of the organism with a sterile inoculating loop or needle in 2 ml of sterile saline. Vortexed the saline tube and created a smooth suspension.

4. Inoculated the MH plate with a sterile swab which was dipped into the inoculum tube and rotated against the side of the tube (above the fluid level) using a firm pressure, to remove excess fluid.

5. Inoculated the dried surface of an MH agar plate by streaking the swab three times over the entire agar surface; rotated the plate approximately 60 degrees each time to ensure an even distribution of the inoculums. Discarded the swab afterward into an appropriate container.

6. A hole with a diameter of 6 to 8 mm was punched aseptically with a sterile cork borer. 20 µL of spice solution was introduced into the well in different amounts (20 µL, 40 µL, 60 µL, 80 µL and 100 µL). Without inverting the plate, place them in a 37°C air incubator for 18 to 24 hours.

7. After incubation, the zone sizes were measured to the nearest millimeter with a ruler or caliper; including the diameter of the disc in the measurement.

2.8.3 Method-3

1. 100 gm of each spice was dissolved in 250 mL of methanol and was placed in a beaker, covering the mouth with cotton and foil paper. It helped prevent contamination.

2. The sealed beaker was then placed inside a laminar hood with exposure to sunlight. It was soaked for around 45 days. The goal was to completely soak the spices and eventually have the alcohol dry up due to the sunlight. However, this attempt was not successful as even after 45 days the methanol had not completely evaporated. Therefore, a different approach was required.

3. The spice and methanol mixtures were placed in Eppendorf tubes containing 2 ml each. Then they were centrifuged. For the first cycle, the Eppendorf tubes were placed in the machine with their lids tightly closed at 5000 rpm for 10 mins. This caused the methanol and soaked spice to form two separate layers. The top layer containing only methanol was then carefully pipetted out.

4. The soaked spice remaining in the tube was then again centrifuged. This time the lid of the tubes was kept open to allow the alcohol to air out at 5000 rpm and ran for 20 mins. Finally, the dry powdered format of the spices was obtained. The dried spices were again dissolved in methanol at a 15 µL : 4 mg ratio and stored in Eppendorf tubes for preservation.

5. After the spice preparation was done, the inoculum was prepared, and inoculated the MH plate and placed six blank discs around the circumference of the plate in a circular format. Used a 1-20 µL range pipette to pour different concentrations of the diluted spice and methanol on the discs. The ratios are as follows-

    - Disc 0 = 10 µL methanol : 0 µL diluted spice
    - Disc 5 = 5 µL methanol : 5 µL diluted spice
    - Disc 8 = 2 µL methanol : 8 µL diluted spice
    - Disc 10 = 0 µL methanol : 10 µL diluted spice

6. After incubation, the zone size was measured with a ruler; recorded the result. Included the diameter of the disc in the measurement.

Chapter 3

Result and Observation

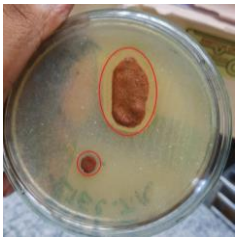
3.1 Method-1: **Disc diffusion** (Distilled water + spice)

Table 1: Antimicrobial activities of processed and raw **Turmeric** against different bacteria

	Zone of inhibition (mm)	
Organism	Raw spice	Powder spice
Acinetobacter baumannii	15	20
EIEC	**10**	**17**
ETEC	11	22
Bacillus cereus	20	20
Staphylococcus aureus	**21**	**26**
Klebsiella pneumonia	18	21

Acinetobacter baumannii

EIEC



ETEC

Bacillus cereus

Staphylococcus aureus

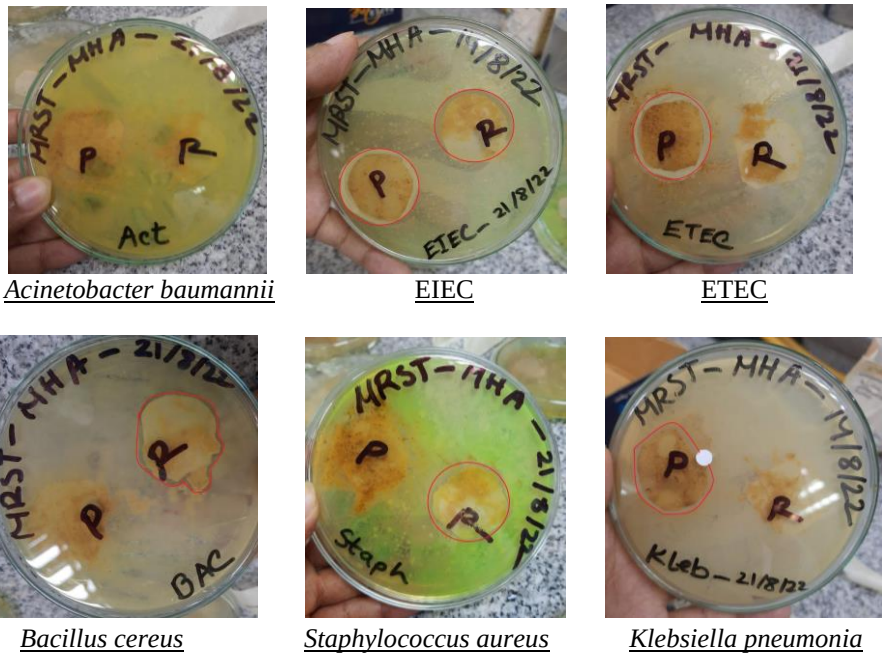
Klebsiella pneumonia

Figure 4: Antimicrobial activities of Turmeric against multiple organisms

Summary: Results were observed in all bacteria for both formats of spice. In comparison, powdered turmeric showed better antimicrobial effects than raw turmeric.

Table 2: Antimicrobial activities of processed and raw **Ginger** against different bacteria

	Zone of Inhibition (mm)	
Organism	Raw spice	Powder spice
Acinetobacter baumannii	-	-
EIEC	22	**21**
ETEC	-	**32**
Bacillus cereus	**20**	-
Staphylococcus aureus	**29**	-
Klebsiella pneumonia	-	30



Acinetobacter baumannii EIEC ETEC

Bacillus cereus *Staphylococcus aureus* *Klebsiella pneumonia*

Figure 5: Antimicrobial activity of Ginger against multiple organisms

Summary: Antimicrobial activity was observed for all organisms except *Acinetobacter baumannii*. The powder format was observed to be more effective against ETEC and *Klebsiella pneumonia*. Whereas, raw format showed a better antimicrobial effect in EIEC, *Bacillus cereus,* and *Staphylococcus pneumonia*.

Table 3: Antimicrobial activities of processed and raw **Cinnamon** against different bacteria

	Zone Of Inhibition (mm)	
Organism	Raw spice	Powder spice
Acinetobacter baumanii	-	-
EIEC	-	-
ETEC	-	18
Bacillus cereus	**21**	**20**
Staphylococcus aureus	**15**	**13**
Klebsiella pneumonia	-	-

Acinetobacter baumanii

EIEC

ETEC

Bacillus cereus

Staphylococcus aureus

Klebsiella pneumonia

Figure 6: Antimicrobial activity of Cinnamon against multiple organisms

Summary: No antimicrobial activity were observed for *Acinetobacter baumanii*, EIEC, and *Klebsiella pneumonia.* ETEC is the only organism against which the powder format showed a better effect. For *Bacillus cereus* and *Staphylococcus aureus*, raw format displayed slightly better antimicrobial ability.

3.2 Method-2: **Well diffusion** (Distilled water + spice)

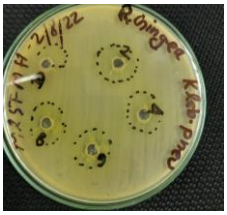
Table 4: Antimicrobial activities of **Raw Ginger** against different bacteria

	Zone Of Inhibition (mm)				
Organism\ Dilution (µl)	20	40	60	80	100
ETEC	17	20	20	20	20
Pseudomonas aeruginosa	13	20	**10**	**10**	20
Bacillus Cereus	14	-	20	**10**	-
Klebsiella pneumonia	**23**	20	20	20	**10**

ETEC

Pseudomonas aeruginosa

Bacillus Cereus



Klebsiella pneumonia

Figure 7: Antimicrobial activity of raw Ginger against multiple organisms

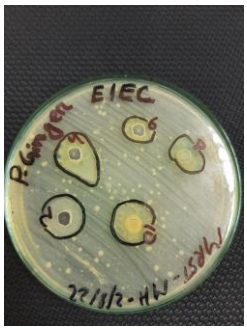
Summary: For ETEC, the best results were observed in dilution above two ml [>2ml]. For *Pseudomonas aeruginosa* a specific dilution of 4 ml and 10 ml seems to have shown the most effect. Similarly, a specific dilution of 6 ml and 2 ml displayed the most results against *Bacillus Cereus* and *Klebsiella pneumonia* respectively.

Table 5: Antimicrobial activities of **Powdered Ginger** against different bacteria

	Zone Of Inhibition (mm)				
Organism \Dilution (µl)	20	40	60	80	100
Klebsiella pneumonia	-	22	-	-	-
Bacillus Cereus	-	13	-	-	**12**
EIEC	25	**29**	**12**	22	25
Pseudomonas aeruginosa	23	26	25	24	27

Klebsiella pneumonia

Bacillus Cereus



EIEC

Pseudomonas aeruginosa

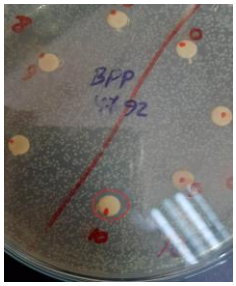
Figure 8: Result of powdered Ginger against multiple organisms.

Summary: For all of *Klebsiella pneumonia*, *Bacillus Cereus*, and EIEC, the highest antimicrobial effect was seen at a specific dilution of 4 ml. Only for *Pseudomonas aeruginosa* a slightly better result was observed at 10 mL dilution.

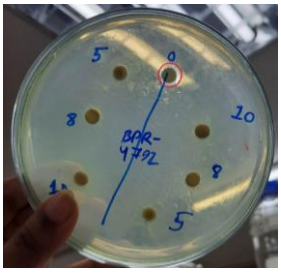
3.3 Method-3: **Blank disc diffusion** (methanol + spice)

Table 6: Antimicrobial activities of different spices against ***Pseudomonas aeruginosa***

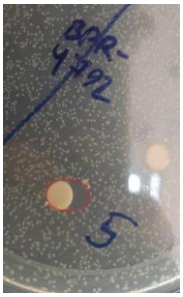
	Zone Of Inhibition (mm)			
Spice\Dilution (µl)	0	5	8	10
Powder Black Pepper	-	-	-	10
Raw Black pepper	8	**9**	-	-
Raw Cinnamon	6	-	-	-
Powder Ginger	8	-	-	**13**
Raw Ginger	7	-	-	-



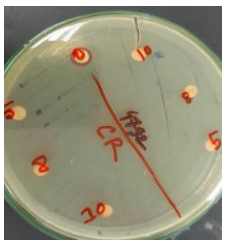
Powder Black Pepper (10)



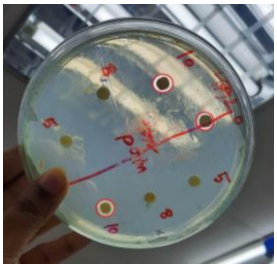
Raw Black Pepper (0)



Raw Black Pepper (5)



Raw Cinnamon (0)



Powder Ginger (0, 10)

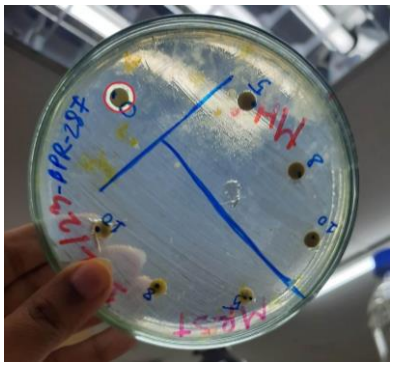
Raw Ginger (0)

Figure 9: Result of *Pseudomonas aeruginosa* against multiple dilutions of spices.

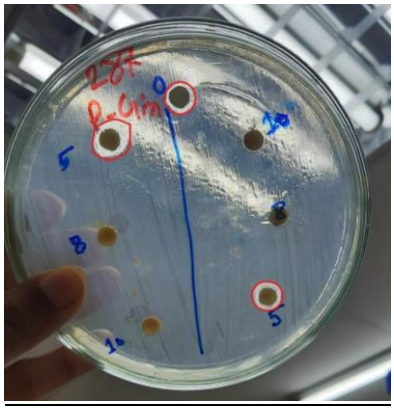
Summary: Powder Ginger at a specific dilution of 10 ml shows the best antimicrobial effect against *Pseudomonas aeruginosa*.

Table 7: Antimicrobial activities of different spices against ***Staphylococcus saprophyticus***

	Zone Of Inhibition (mm)			
Spice\Dilution	0	5	8	10
Raw Black Pepper	12	-	-	-
Powder Ginger	6	**10**	-	



Raw Black Pepper (0)



Powder Ginger (0, 5)

Figure 10: Antimicrobial activity of *Staphylococcus Saprophyticus* against multiple dilutions of spices.

Summary: The best antimicrobial effect against *Staphylococcus Saprophyticus* is seen by powder Ginger at a specific dilution of 5 ml.

Chapter 4

Discussion

Method 1 was performed using hand-made filter paper discs. The spices were mixed only with distilled water. Antimicrobial activities were observed for the organisms *Acinetobacter baumannii*, EIEC, ETEC, *Bacillus cereus*, *Staphylococcus aureus,* and *Klebsiella pneumonia* in Turmeric, Ginger, Cinnamon, but not in case Black Pepper.

Method 2 was performed by forming wells in the agar, using a borer. In this case, as well, the spices were mixed with distilled water only. Ginger is the only spice that showed antimicrobial activity although not significant in this method, for the organisms EIEC, ETEC, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia.*

Method 3 was performed with professional blank discs. The spices were sterilized with methanol, then dried and re-extracted. Only two organisms were used - *Pseudomonas aeruginosa* and *Staphylococcus saprophyticus.* Antimicrobial activity was observed only in the case of Ginger.

4.1 TURMERIC

Turmeric showed antimicrobial activity against the organisms *Acinetobacter baumannii*, EIEC, ETEC, *Bacillus cereus*, *Staphylococcus aureus,* and *Klebsiella pneumonia,* for both raw and powder. Turmeric contains Curcumin which works as an antimicrobial agent against these organisms. In 2014, a study stated that the promising results for the antimicrobial activity of curcumin made it a good candidate to enhance the inhibitory effect of existing antimicrobial agents through synergism. Indeed, different investigations have been done to increase the

antimicrobial activity of curcumin, including synthesis of different chemical derivatives to increase its water solubility. (Moghadamtousi et al., 2014)

According to a 2015 study, water-extracted samples of turmeric stored at room temperature inhibits the growth of *Escherichia coli*, and Methanol extracted samples stored at room temperature or autoclaved at 121 °C were effective in controlling the growth of all microbes. (Gul & Bakht, 2013)

A 2020 study states that curcumin is a type of antimicrobial agent that has selective activity. According to the authors, this study exhibited a significantly larger variation in the curcumin activity than previous works and suggested that numerous clinical strains of widespread pathogens have poor sensitivity to curcumin. However, curcumin was effective against some species and strains:

- *Streptococcus pyogenes* (median MIC = 31.25 µg/mL)
- Methicillin-sensitive *S. aureus* (250 µg/mL)
- *Acinetobacter lwoffii* (250 µg/mL)
- Individual strains of *Enterococcus faecalis*
- Individual strains of *Pseudomonas aeruginosa* (62.5 µg/mL).

The sensitivity of test organisms was not associated with their affiliation to the genus. Hence, this study concluded curcumin is a promising antibacterial agent, but with a very selective activity. (Adamczak et al., 2020)

In the study for method 1, the most antimicrobial activity was found to be in *Staphylococcus aureus* (Raw = 21 mm, Powder = 26 mm). Moderate activity was observed in *Bacillus cereus* and

Klebsiella pneumonia. Weak activity was observed in EIEC, ETEC, and *Acinetobacter baumannii*.

Similar to the aforementioned study, the antimicrobial activity did not vary due to genus or species. However, selective antimicrobial activities were observed as not all spices showed a similar amount of zone.

Hypothesis - We hypothesize that the effect of turmeric is selective but not restricted to its genus or species. **To quantify the goal of this study, the observations suggest that powder turmeric shows better antimicrobial results compared to raw turmeric.**

Troubleshooting - Method 1 was not performed under standardized conditions. The discs varied in size and for most cases the discs used for powder turmeric were bigger in diameter than the ones used for raw turmeric. Due to this, it may be suggested that the results varied and the observations made can not be documented as accurate information.

4.2 GINGER

The antimicrobial activities observed in the case of Ginger varied for powder and raw. Zones were seen in ETEC and *Klebsiella pneumoni*a in the case of powder Ginger. On the other hand, for raw Ginger, zones were visible in *Bacillus cereus* and *Staphylococcus aureus.* EIEC is the only organism that showed a zone for both raw and powdered Ginger. Whereas, against *Acinetobacter baumannii* no zones were observered.

As mentioned before, in Method 1 the spices were dissolved only with water. As per other studies, this could be a reason for weak results. A 2019 study stated that phenolic compounds (eugenol, shogaols, zingerone, Gingerdiols, Gingerols, etc.) and their synergistic relationship with other compounds such as β-sesquiphellandrene, cis-caryophillene, zingiberene, α-farnesene,

α- and β-bisabolene, are mainly responsible for the antimicrobial activity found in Ginger. Most of these compounds are insoluble in water; thus, aqueous extracts exhibit lower antimicrobial activity than essential oil, oleoresins, and organic extracts. (Beristain-Bauza et al., 2019)

Hypothesis 1 - So, it can be hypothesized that this experiment showed relatively weak antimicrobial activity due to the presence of water, which restricted the phenolic compounds from exhibiting their antimicrobial effects properly.

On the other hand, in method 2 Ginger was similarly dissolved in water and placed directly in the wells. However, this method showed distinctly better results than method 1 despite the mixtures having similar contents. Antimicrobial activity was observed for 5 different organisms and zones in almost all concentrations except a few. The best-found concentrations for each organism combined with raw or powdered Ginger, are as follows -

Dilution ratio = spice: methanol

- ❖ Raw Ginger
  - ➢ ETEC = > (2:8)
  - ➢ *Pseudomonas aeruginosa* = 4:6 ; 10:0
  - ➢ *Bacillus cereus* = 6:4
  - ➢ *Klebisella pneumoniae* = 2:8
- ❖ Powder Ginger
  - ➢ EIEC = 4:6
  - ➢ *Pseudomonas aeruginosa* = 10:0
  - ➢ *Bacillus cereus* = 4:6
  - ➢ *Klebisella pneumoniae* = 4:6

Hypothesis 2 - It can be hypothesized that the restrictions faced by phenolic compounds due to the presence of water, can be avoided up to a certain extent by specifying the dilution concentration.

In method 3, the spice was sterilized with methanol first. Powder Ginger at 1 ml concentration shows a 13mm zone for *Pseudomonas aeruginosa*. For *Staphylococcus Saprophyticus* powder Ginger shows a zone of 10 mm at 0.5 ml concentration.

Hypothesis 3 - Diluting with methanol caused any external impurities that may affect the antimicrobial function to be removed. So it can be hypothesized that purified extracts provide more specific results.

To quantify the goal of this study, it can be understood that for non-standardized method 1, raw and powdered Ginger showed similar antimicrobial activity, providing slight changes due to the disc size difference. The more standardized method 2 presented the same result for 3 organisms (*Pseudomonas aeruginosa, Bacillus cereus* and *Klebsiella pneumoniae*) in the case of both raw and powdered Ginger. However, only raw Ginger showed inhibition for ETEC and only powder Ginger showed inhibition for EIEC. Whereas for method 3, only powder Ginger showed results that too on specific concentrations.

Troubleshooting - The main difference between methods 1 and 2 is the presence of standardization. Unlike method 1, method 2 was performed with standard concentrations, and specific amounts were introduced into the plate. On the other hand, method 3 was diluted with methanol and standard amounts were given.

4.3 CINNAMON

Across all the 3 methods performed, Cinnamon was the spice with the least antimicrobial activity. Only in case of method 1, a few zones were observed.

Raw Cinnamon -

- *Bacillus cereus* = 21 mm
- *Staphylococcus aureus* = 15 mm

Powder Cinnamon -

- ➢ *Bacillus cereus* = 20 mm
- ➢ *Staphylococcus aureus* = 13 mm
- ➢ ETEC = 18 mm

Method 2 and 3 produced no zone of inhibition at all. The main antimicrobial ingredient present in Cinnamon is Cinnamaldehyde. It is obtained from the extraction of *C. zeylanicum* barks.

In a 2015 study, the antibacterial activities of several *C. zeylanicum* bark extracts, obtained with different organic solvents, as ethyl acetate, acetone and methanol, were tested in vitro against *Klebsiella pneumonia* 13883, *Bacillus megaterium* NRS, *Pseudomonas aeruginosa* ATCC 27859, *Staphylococcus aureus* 6538 P, *Escherichia coli* ATCC 8739, *Enterobacter cloacae* ATCC 13047, *Corynebacterium xerosis* UC 9165, *Streptococcus faecalis* DC 74, by the disc-diffusion method. The results showed that the antibacterial activity, expressed as the inhibition zone, ranges from 7 to 18 mm for the application of 30 µL, suggesting a high antibacterial activity. (Nabavi et al., 2015)

Hypothesis 1 - Comparing the findings of this study with the above-mentioned study, it can be hypothesized that Cinnamon shows better results when the extraction is done purely from the tree bark rather than using raw spice or commercialized powder.

Hypothesis 2 - Better antimicrobial activities were observed when diluted with water rather than methanol. Following this observation, a hypothesis can be made that the antimicrobial quality of Cinnamon as a spice is more effective when used in high amounts and non-standardized forms. Therefore, abundance is equivalent to the effect.

To quantify the goal of this study, it can be deduced that raw and powdered Cinnamon produce almost similar results in the same organisms despite differences in extraction method or dilution concentration.

References

Adamczak, A., Ożarowski, M., & Karpiński, T. M. (2020, July 16). *Curcumin, a Natural Antimicrobial Agent with Strain-Specific Activity*. NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7408453/

Arias, C. A., & Munita, J. M. (2016, October 1). *Mechanisms of Antibiotic Resistance - PMC*. NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4888801/

Beristain-Bauza, S. D. C., Hernández-Carranza, P., Cid-Pérez, T. S., Ávila-Sosa, R., Ruiz-López, I. I., & Ochoa-Velasco, C. E. (2019, February 04). *Antimicrobial Activity of Ginger (Zingiber Officinale) and Its Application in Food Products*. https://www.tandfonline.com/doi/full/10.1080/87559129.2019.1573829#:~:text=Results%20indicate%20that%20ginger%20contains,interesting%20alternative%20to%20synthetic%20antimicrobials

Gul, P., & Bakht, J. (2013, November 8). *Antimicrobial activity of turmeric extract and its potential use in food industry*. NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4375173/

Kumar, S. (2020, July 1). *(PDF) Medicinal uses of spices used in our traditional culture: World wide*. ResearchGate. https://www.researchgate.net/publication/342591176_Medicinal_uses_of_spices_used_in_our_traditional_culture_World_wide

Liu, Q., Meng, X., Li, Y., Zhao, C.-N., Tang, G.-Y., & Li, H.-B. (2017, June 16). *Antibacterial and Antifungal Activities of Spices - PMC*. NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5486105/

Mao, Q.-Q., Xu, X.-Y., Cao, S.-Y., Gan, R.-Y., Corke, H., Beta, T., & Li, H.-B. (2019, May 30). *Bioactive Compounds and Bioactivities of Ginger (Zingiber officinale Roscoe)*. NCBI. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6616534/

Moghadamtousi, S. Z., Kadir, H. A., Hassandarvish, P., Tajik, H., Abubakar, S., & Zandi, K. (2014, April 29). *A Review on Antibacterial, Antiviral, and Antifungal Activity of Curcumin*. Hindawi. https://www.hindawi.com/journals/bmri/2014/186864/

Nabavi, S. F., Lorenzo, A. D., Izadi, M., Sobarzo-Sánchez, E., Daglia, M., & Nabavi, S. M. (2015, September 11). *Antibacterial Effects of Cinnamon: From Farm to Food, Cosmetic and Pharmaceutical Industries*. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4586554/

***Spices** Manufacturing Process*. (2022, February 21). Indpro. https://indpro.com/blog/spices-manufacturing-process/

Srinivasan, K. (2022, October 2). . . . https://pubmed.ncbi.nlm.nih.gov/17987447/

Zhang, D., Gan, R.-Y., Farha, A. K., Kim, G., Yang, Q.-Q., Shi, X.-M., Shi, C.-L., Luo, Q.-X., Xu, X.-B., Li, H.-B., & Corke, H. (2019, May 29). *Discovery of Antibacterial Dietary Spices That Target Antibiotic-Resistant Bacteria*. NCBI. Retrieved August 2, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6617121/