Testing for Antibiotic Properties: *Momordica charantia*

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Abstract

Natural products derived from plants are a source of many biologically active components, many of which are the basis of many medicines today. Extracts that are isolated from medicinal plants have been seen to have many biological activities, such as antimicrobial, anti-inflammatory, and antioxidant activities. *Momordica charantia*, better known as bitter melon, is a popular vegetable used in Cambodian and Vietnamese cuisine due to its many health benefits. These benefits include reducing blood sugar, having cancer-fighting properties, and decreasing cholesterol levels. The fruits and leaves of the *Momordica* species are rich in phytochemicals, which are chemicals found in plants to protect them against bacteria, viruses, and fungi. Although traditional medicinal practices have involved mainly ingesting the *Momordica charantia* fruit, the question arises as to whether any parts of the plant contain any potential antibiotic properties that could aid in the fight against antibiotic resistance. To determine this, methanol and ethanol extracts of the different parts of *Momordica charantia* were prepared by maceration. Extracts were tested for antibacterial activity using Kirby Bauer diffusion and Minimum Inhibitory Concentration (MIC) assays. The research is conducted on extracts from the leaf, stem, seed, and fruit of the *Momordica charantia* plant to four types of bacteria: *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and *Bacillus subtilis.* The experiments show that the ethanol extract of the parts of *Momordica charantia* were seen to be ineffective against *E. coli, B. subtilis, S. aureus,* and *P. aeruginosa.* It is also seen that the methanol extracts of *Momordica charantia* are not effective against *E. coli, B. subtilis,* and *P. aeruginosa.* However, the seed, leaf, and fruit methanol extracts were seen to be effective against the bacterium *S. aureus.*

I. Introduction

Antibiotics are antimicrobial substances that fight against bacteria that infect humans or animals (Medline, 2022). Antibiotics have become one of the most significant factors in encouraging a longer life span in humans and animals. However, the emergence of antibiotic-resistant bacteria has resulted in the prevention of treating common infections and performing surgical procedures (WHO, 2023). Due to the significance of antibiotics, it is essential to find more kinds to help fight against antibiotic resistance. The antimicrobial components found in medicinal plants may prevent the growth of bacteria, viruses, fungi, and protozoa in different ways than current antimicrobials (Vaou et al., 2021). Within this thesis, experimentation will be conducted with parts of the plant *Momordica charantia* in hopes of exhibiting antibiotic activity.

1. Bacteria

1.1 General

Bacteria are diverse microscopic single-celled organisms that are ubiquitous. They are found almost everywhere on Earth, including humans. Bacteria are considered prokaryotes. This means they do not have a membrane-bound nucleus or other internal structures (Kadner & Rodgers, 2024). They are much simpler in structure than eukaryotes, which would have a membrane-bound nucleus and organelles. Bacteria can be classified in several ways: through their shape, need for oxygen, genetic makeup, staining, and whether they are gram-positive or gram-negative (Cleveland, 2022). Bacteria can be classified by shape: sphere-shaped, rod-shaped, and spiral-shaped. They are named according to their shape: cocci, bacilli, and spirochete.

Bacteria can also be classified by whether they need or do not need oxygen to survive and grow. Bacteria that require oxygen are called aerobes. Aerobes can use gaseous oxygen in metabolism and have the enzymes needed to process toxic oxygen products (Cowen et al., 2021). Anaerobes do not require oxygen since they do not have the metabolic enzyme systems for using oxygen in respiration (Cowen et

al., 2021). Some bacteria can metabolize in oxygen and no-oxygen environments, called facultative anaerobes. Staining bacteria through a process called gram-staining can cause bacteria to be classified as either gram-positive or gram-negative. Gram-negative bacteria are characterized by their cell envelope, which comprises an inner cytoplasmic cell membrane, peptidoglycan cell wall, and outer membrane (See Figure 1.1.1). The outer membrane comprises a lipid bilayer, associated proteins, and lipopolysaccharide (LPS), resulting in a toxic reaction if lysed. LPS protects the bacterium against certain types of molecules. Gram-positive bacteria do not have an outer membrane but thicker peptidoglycan layers. Gram-positive bacteria include *Mycobacterium tuberculosis*, *Staphylococcus aureus*, and *Bacillus subtilis*. Peptidoglycan layers assist in resistance to osmotic pressure, and lipopolysaccharides help preserve cell integrity (Silhavy et al., 2010). If a bacterium is gram-positive, it will stain purple; if it is gram-negative, it will stain pink or red.

Bacteria are typically harmless to humans and, at times, even beneficial. The bacteria in and on the body are resident flora or the microbiome (Cleveland Clinic, 2022). There are bacteria located in many parts of the human body, such as the gut. Bacteria in the gut help aid in the digestion of food and assist in the way energy is stored from food. Bacteria in the gut can also help the body fight off disease by maintaining resistance against pathogenic bacteria by competing for nutrients and attachment sites on the mucosal surfaces of the colon (Zhang et al., 2015).

There are also times when bacteria can harm humans, resulting in disease. Bacteria that cause harm to humans are termed pathogenic. Virulence determines the relative severity of disease caused by a particular microbe (Cowen et al., 2021). The virulence of bacteria is determined by the ability to establish itself in the host and its ability to cause damage. For the bacteria to establish itself in the host, it must be able to enter the host, attach to the host tissues firmly, negotiate the host biome, and survive the host defenses (Cowen et al., 2021). The microbe must then be able to produce toxins or initiate a harmful response to the host. The pathogen can have virulence factors, which are structures or characteristics that

contribute to the activities of a pathogenic microbe (Cowen et al., 2021). This research will focus on four bacteria: *Escherichia coli, Bacillus subtilis, Staphylococcus aureus,* and *Pseudomonas aeruginosa.*

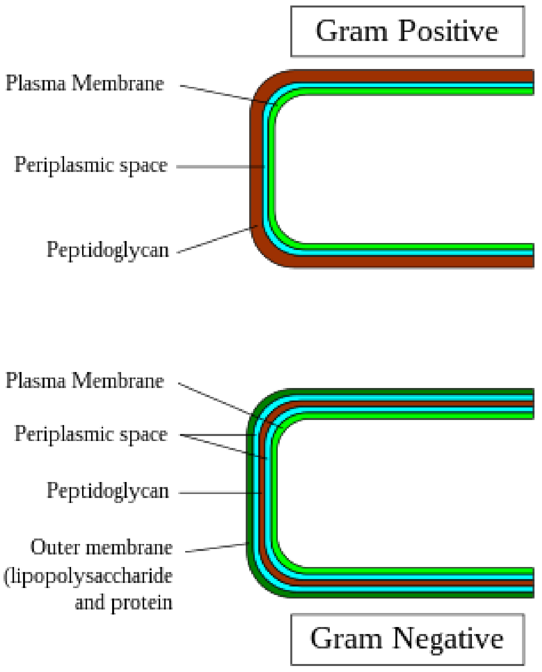

Figure 1.1.1: The Outer barriers of grampositive vs gram negative (Wikipedia, 2008) Comparison of gram-positive and gram-negative bacteria.

1.2 Escherichia coli

Escherichia coli is a gram-negative, rod-shaped, facultative anaerobic bacterium classified in the family *Enterobacteriaceae*. *E. coli* has a flagellum, a tail-like projection that allows the bacterium to mobilize (see Figure 1.2.1). Within hours to days after birth, these bacteria colonize the gastrointestinal tract of most warm-blooded animals. They typically reside as a part of the normal gastrointestinal tract flora of humans and animals. Under normal conditions, a symbiotic relationship occurs because the enteric flora provides a source of vitamin K (menaquinones) and B-complex vitamins (Jnani & Ray, 2022). However, some strains of *E. coli* have evolved by acquiring virulence factors through plasmids, transposons, bacteriophages, and pathogenicity islands, which makes *E. coli* pathogenic (Lim et al., 2010). Due to this, some strains of *E. coli* can cause diarrhea while others can cause urinary tract

infections, respiratory illness, pneumonia, and other disease. Factors like infection or chemotherapy may affect the balance of the bacterium's habitat and favor the growth of the more virulent species or make the symbiotic relationship dysfunctional. *E. coli* infections cause approximately 265,000 illnesses and about 100 deaths annually in the United States (Jnani & Ray, 2022). *E. coli* is intrinsically susceptible to almost all clinically relevant antimicrobial agents, but this bacterial species has a significant potential to accumulate resistance genes, mainly through horizontal gene transfer. (Poirel et al., 2018).

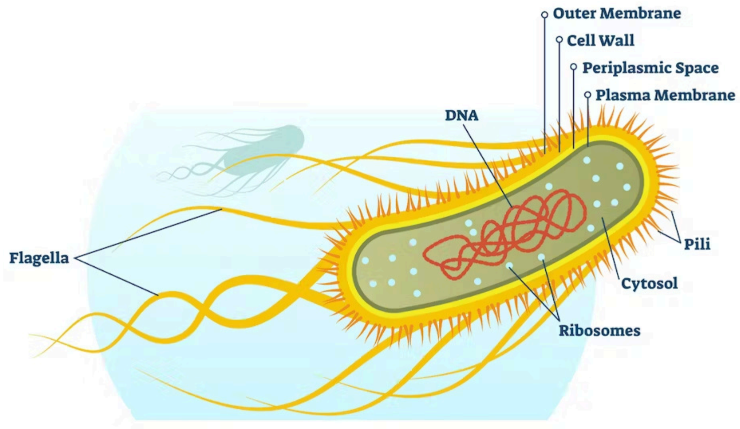

Figure 1.2.1: Structure of *E.coli* (VectorMine, 2018) Depiction of *E.coli* bacterium. Displays parts of a gram-negative bacteria cell.

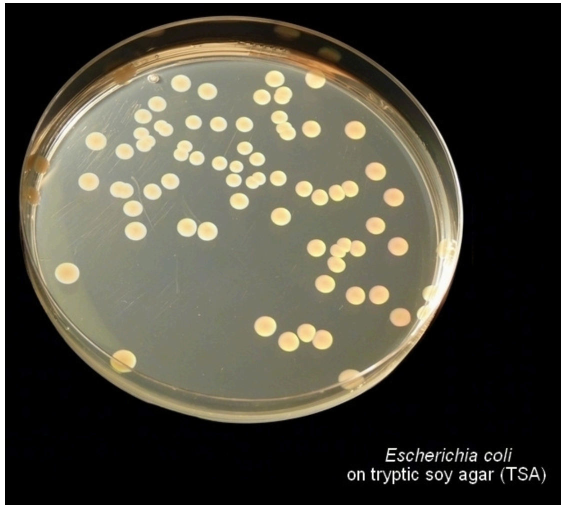

Figure 1.2.2: *E. coli* growth on agar (MicrobiologyinPictures) Growth of *E.coli* on tryptic soy agar (TSA). Cultivation 24 hours, 37°C in an aerobic atmosphere.

1.3 Bacillus subtilis

Bacillus subtilis is a gram-positive, aerobic bacterium classified in the family *Bacillaceae*. *B. subtilis* are rod-shaped cells usually 2–6 µm long and less than 1 µm in diameter. (Errington & Aart, 2020). The active and growing cells can move around due to their flagellum (see Figure 1.3.2). It is often found in soil and is considered nonpathogenic (Piggot, 2009). When cultured on ordinary nutrient agar, the morphology of the circular colony of this bacteria is rough, opaque, fuzzy white, or slightly yellow with jagged edges (see Figure 1.3.1) (Lu et al., 2018). *B. subtilis*, under starvation conditions, forms endospores, dormant bodies formed when the environment is unfavorable. Bacterial endospores are the hardiest of all life forms, capable of withstanding extreme heat, drying, freezing, radiation, and chemicals that readily kill vegetative cells (active and growing cells) (Cowan et al., 2022). The endospore initially exists inside the vegetative cell, but eventually, the cell disintegrates, and the endospore is on its own. The heat resistance of endospores is due to the high calcium and dipicolinic acid content. Due to *B. subtilis'* ability to form endospores, it has become a prevalent gram-positive bacteria worked with since it has become a tractable system for studying fundamental aspects of cellular development and differentiation (Errington & Aart, 2020).

Figure 1.3.1: Growth of *B. subtilis* on agar (Pandav, 2021) *B. subtilis* growth on nutrient agar. Colonies are circular, rough, opaque, fuzzy white or slightly yellow with jagged edges.

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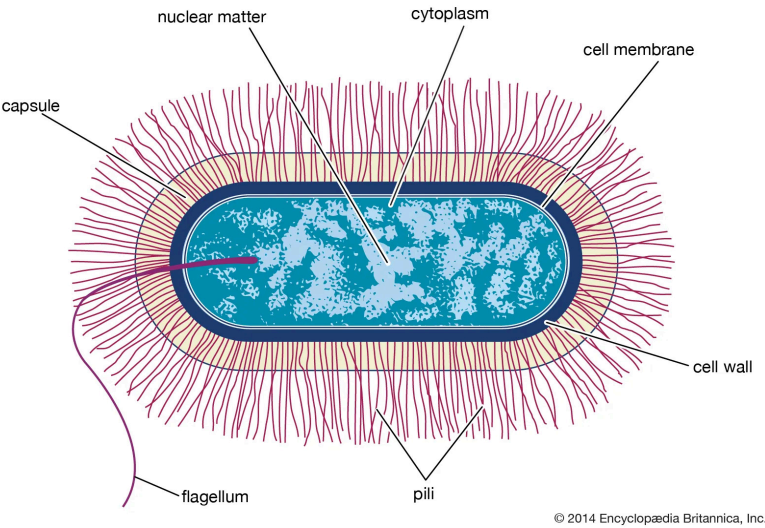

Figure 1.3.2: *B. subtilis* structure (Britannica, 2014) Depiction of *B. subtilis* bacterium structure

1.4 Staphylococcus aureus

Staphylococcus aureus is a gram-positive bacteria strain often found on the human skin, nose, armpit, and other areas. It often resides on the mucous membranes and skin, and humans are considered the primary reservoir of *S. aureus* (Taylor & Unakal, 2023). Usually, this bacterium does not cause infection in a healthy individual; however, if allowed into the bloodstream or internal tissues, it can cause dangerous infections (Taylor & Unakal, 2023). Like grapes, *S. aureus* is cocci-shaped (round) and often arranged in groups. These organisms can grow in up to 10% salt on media; their colonies are usually golden or yellow (Taylor & Unakal, 2023) (Figure 1.4.1). They can grow aerobically, meaning with oxygen, and anaerobically, meaning they can grow without oxygen. *S. aureus* grows between temperatures of 18 and 40 degrees Celsius. It is estimated that about one-half of all human adults are colonized by *S. aureus*, and about 15% of the population carry the bacteria in the outer portion of their nose (Taylor & Unakal, 2023). The populations of people that have higher rates of *S. aureus* colonization (about 80%) are healthcare workers, hospitalized patients, people who often use needles daily (such as people with diabetes or intravenous drug users), and immunocompromised people (Taylor & Unakal, 2023). *S. aureus* is the most common bacterial infection in humans. It can cause many infections, such as

meningitis, urinary tract infections, bacteremia, and infective endocarditis. The organism can be spread within the body through the bloodstream to the internal organs, which can set up a focus of infection. Two types of virulence determinants generally cause disease caused by S. aureus: cell surface-associated proteins and extracellular-associated proteins (Foster, 2002). It can express many surface proteins that bind to parts of the extracellular matrix and the parts that contribute to blood clots and damaged tissue. The surface proteins may help the bacteria attach itself more quickly and may help it multiply. There are many ways in which *S. aureus* fights the host defenses. It can release cytolytic toxins, cause cells to burst, injure the cell membrane of the host cells, and create superantigens that contribute to the symptoms of toxic shock (Foster, 2002).

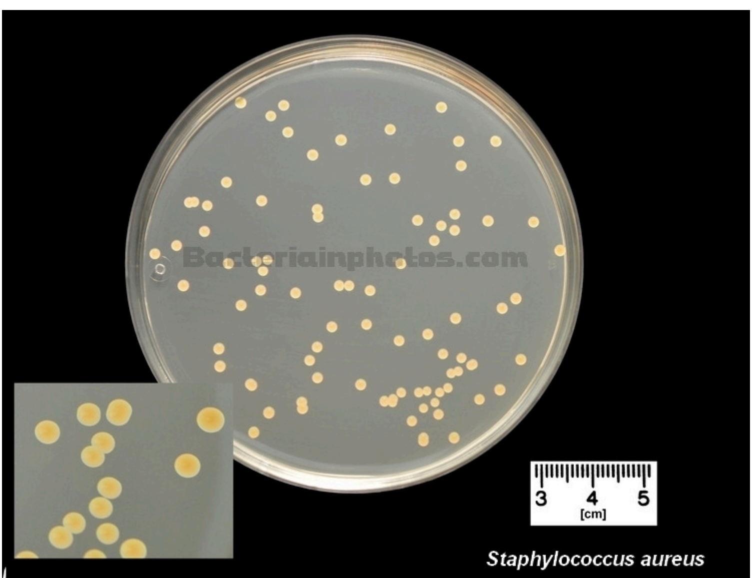

Figure 1.4.1: *S. aureus* growth on agar *S. aureus* colony growth on tryptic soy agar (TSA). Cultivation 24 hours, aerobic atmosphere, 37°C.

1.5 Pseudomonas aeruginosa

Pseudomonas aeruginosa is a gram-negative, aerobic rod bacteria often found in freshwater environments. *P. aeruginosa* is commonly found in freshwater in community reservoirs such as swimming pools, jacuzzis, and hot tubs (Wilson & Pandey, 2023). It can cause many community-acquired infections, such as pneumonia, folliculitis, and otitis externa. *P. aeruginosa* is considered an opportunistic pathogen; it is not usually pathogenic unless there is an impaired immune system where the body's host

defenses are disrupted. It is often the cause of healthcare-associated infections like ventilator-associated pneumonia and catheter-associated urinary tract infections.

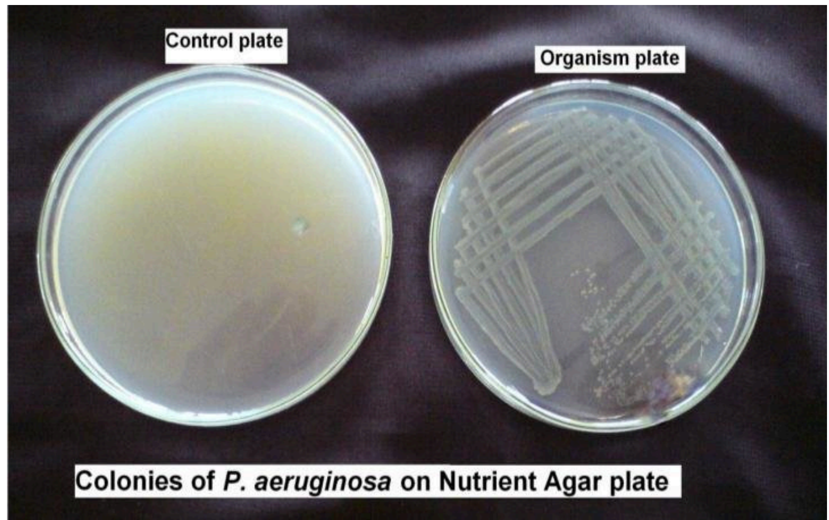

Figure 1.7: *P. aeruginosa* growth on agar plate (Verma, 2018). Growth of *P. aeuruginosa* bacterium on Nutrient Agar (NA) plates

2. *Antibiotics*

The development of antibiotics has allowed humans to live longer, treat diseases, and practice modern medical procedures. Antibiotics are antimicrobial substances used to fight against pathogens. They can be taken orally, topically, or through injections. Antibiotics work by disrupting the vital processes of bacteria, preventing the spread of bacteria by destroying them (MedicineWise, 2017). This assists the body's immune system fight against the infection caused by the bacteria. Antibiotics are initially the metabolic products of bacteria and fungi (Cowan et al., 2022). Microorganisms produce antibiotics to reduce competition for space and nutrients within their habitat. Antibiotics have successfully prevented or treated infections that can occur in patients who are receiving chemotherapy treatments, who have chronic diseases such as diabetes, end-stage renal disease, or rheumatoid arthritis, or who have had complex surgeries such as organ transplants, joint replacements, or cardiac surgery (Ventola, 2015).

2.1 Origin and Discovery

Antibiotic-producing microbes have been used to prevent and treat disease for thousands of years in several cultures. The oldest preserved medical document was Eber's papyrus from 1550 BC (Hutchings et al., 2019). It listed many remedies, such as medicinal soil and moldy bread. Practices such as using moldy bread were used by Serbia, China, Greece, and Egypt to help treat open wounds over 2000 years ago (Hutchings et al., 2019). Paul Ehrlich is considered the person who developed chemotherapy and anti-infective drugs (Hutchings et al., 2019). From 1878 to 1887, Ehrlich developed the detection of bacteria through dye-staining, which provided the basis for acid-staining reactions (Valent et al., 2016). Through this, Ehrlich developed a synthetic- arsenic-based prodrug called salvarsan and neo-salvarsan in 1910. This antibiotic drug was used to treat the bacterium *Treponema pallidum,* which is the bacterium that causes syphilis (Hutchings et al., 2019). After salvarsan was discovered, it was replaced by the sulfonamide prodrug Protonisil, which Gerhard Domagk discovered (Hutchings et al., 2019). Domagk and others continued Ehrlich's work utilizing the selective dying of bacteria developed by Ehrlich. Although sulfonamides were the first effective antibiotic that killed a broad spectrum of bacteria, penicillin later replaced them. Through observing a contaminated petri dish, Alexander Flemming discovered penicillin in 1928. The discovery of penicillin resulted in many more discoveries of other antimicrobial compounds produced by microbes (Hutchings et al., 2019). This resulted in chemists developing more drugs by changing the structure of naturally occurring antibiotic compounds. The discovery of antimicrobial compounds led Selman Waksman to systematically study microbes as producers of antimicrobial compounds in the 1930s (Hutchings et al., 2019). Waksman defined an antibiotic as "a compound made by a microbe to destroy other microbes." (Hutchings et al., 2019). This led him to identify *Actinomycetales* as plentiful producers of antibiotic compounds (Figure 2.1.1). Waksman also identified the genus *Streptomyces* as a producer of many secondary metabolites (NPs). Secondary metabolites are compounds not needed for an organism's average growth, development, or reproduction in a lab (Hutchings et al., 2019). This discovery began the Golden Age of antibiotic

discovery in the 1940s and the 1960s. Because of antibiotic resistance, many antibiotics discovered are not as effective as before. Multiple classes of NPs found during that short period resulted in the overuse of those drugs. Since then, no new classes of antibiotics have been discovered, and new drugs have developed by changing existing antibiotics (Adedeji, 2016).

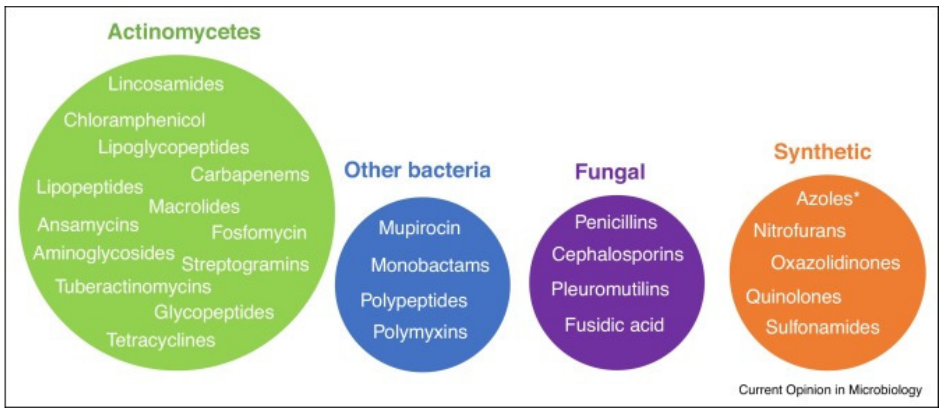

Figure 2.1.1: Antibiotic classes made from natural products (Hutchings et al., 2019).
Shows the most clinically relevant classes of antibiotics that are derived by natural products.

2.2 Antibiotic Mechanisms

Many kinds of antibiotics work in several ways. There are narrow-spectrum antibiotics, effective against a few types of bacteria, and broad-spectrum antibiotics, effective against diverse bacteria. However, the best antibiotics are the ones that are selectively toxic, where they can kill or prevent microbial cells without damaging the host cells at the same time. However, the more closely the infectious agent resembles the host cell, the more difficult it is to achieve selective toxicity, which results in more severe side effects in the host (Cowan et al., 2022). Antibiotics are classified by their mechanism to destroy bacteria: targeting the cell wall, inhibiting protein biosynthesis, inhibiting DNA replication, and inhibiting folic acid metabolism (figure 2.2.2) (Kapoor et al., 2017). Antibiotics that target the bacteria's cell wall either prevent or interfere with cell wall formation. The cell walls of bacteria are mainly made of peptidoglycan (see section 1.1). When antibiotics target the formation of peptidoglycan, it affects the integrity of the cell. These antibiotics are effective in animals since animals do not have a cell wall. For example, penicillin works by blocking the production of the bacteria's cell wall while not directly

affecting human cells (figure 2.2.1). Antibiotics that interfere with protein biosynthesis target the 30s or 50s subunits of the bacterial ribosome to block protein synthesis (Kapoor et al., 2017).

Examples of antibiotics that affect protein biosynthesis include tetracyclines (Figure 2.2.2). Tetracyclines inhibit the 30s ribosomal subunits by blocking the acceptor site on the mRNA-ribosome complex, preventing them from binding to aminoacyl-tRNA (Shutter & Akhondi, 2023). Without this process, the bacterial cell cannot function properly and will not grow or replicate. Antibiotics that inhibit DNA replication target the enzymes used during bacterial DNA replication. An example would be Quinolones (Figure 2.2.2). Quinolones target two essential bacterial enzymes of bacteria: DNA gyrase and topoisomerase IV. The enzymes are needed by bacterial cells to regulate chromosomal supercoiling, which is needed for DNA synthesis, transcription, and cell division (Correia et al., 2017). Antibiotics can also inhibit folic acid metabolism in bacterial cells. Bacteria need folic acids to make the nucleic acids that make up their DNA and RNA. An example of an antibiotic that targets folic acid metabolism would be trimethoprim (Figure 2.2.2). Trimethoprim interferes with dihydrofolate reductase, an enzyme needed to accelerate the last step of making bacterial folic acid (Kester et al., 2012). When trimethoprim is used by itself, it is bacterial static, meaning it slows down the growth of bacteria. However, when used with another antibiotic, sulfamethoxazole, it is bactericidal, killing the bacteria. Therefore, trimethoprim and sulfamethoxazole are usually used together.

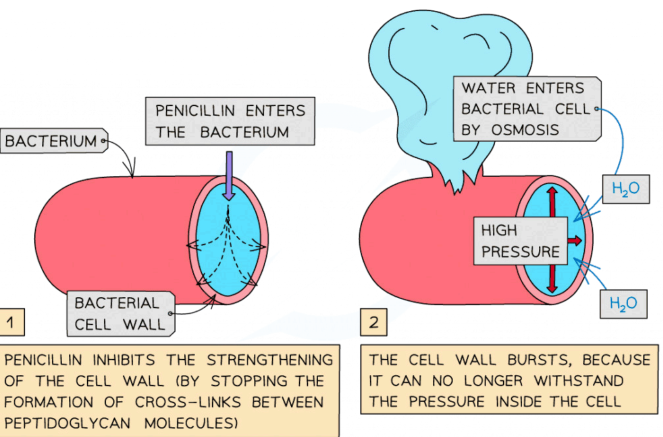

Figure 2.2.1: Mechanism of penicillin Mechanism of penicillin drug against bacterium. Penicillin inhibits the strengthening of the cell wall, which then bursts because it cannot handle the pressure inside the cell.

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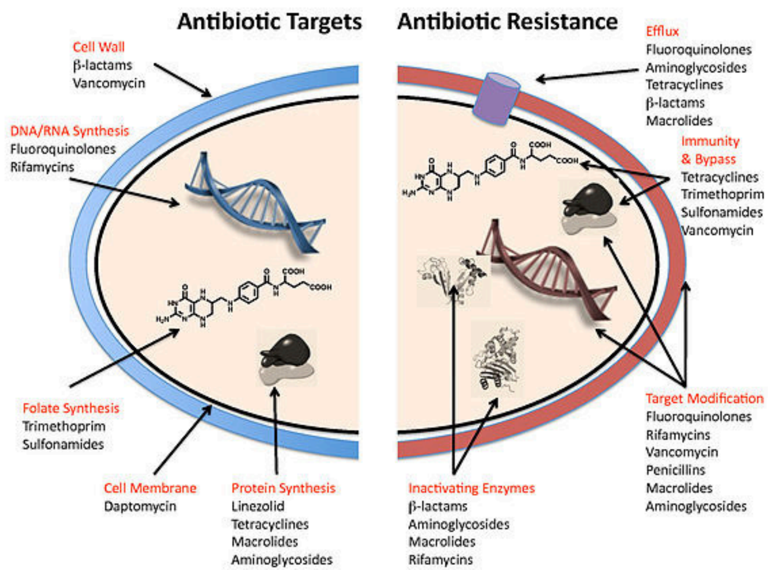

Figure 2.2.2: Mechanisms of antibiotics & antibiotic resistance (Wikipedia, 2010). This shows what the antibiotic targets in bacteria as well as the methods of antibiotic resistance that bacteria exhibits.

2.3 Gentamicin

Gentamicin is a broad-spectrum antibiotic often used for common bacterial infections that may occur in different body parts. It is bactericidal, meaning that this antibiotic kills aerobic, gram-negative bacteria. The microorganisms that gentamicin is often used for are bacteria that are classified in the *Enterobacteriaceae* family (such as *E. coli*), *Pseudomonas aeruginosa*, and some strains of *Neisseria*, *Moraxella*, and *Haemophilus* genera (Chaves & Tadi, 2023). Gentamicin enters the membrane of gram-negative bacteria through oxygen-dependent active transport (Chaves & Tadi, 2023). Gentamicin is ineffective against anaerobic bacteria since oxygen is required for the mechanism of this antibiotic. When the antibiotic enters the cytoplasm of the bacteria, gentamicin binds to the 16s rRNA at the 30s ribosomal subunit, interrupting mRNA translation and producing nonfunctional protein in the bacterial cell (Chaves & Tadi, 2023). The details of what gentamicin does are still unclear; however, further research is still being conducted. Gentamicin is an antibiotic that exhibits concentration-dependent killing, meaning that the more concentrated the dose is, the more antimicrobial killing (Chaves & Tadi, 2023). Due to this fact, it is essential for physicians who are prescribing gentamicin to monitor the dose closely.

3. ***Antibiotic Resistance***

Antibiotics were first introduced to treat severe infections in the 1940s; penicillin became effective against bacterial infections among World War II soldiers (Ventola, 2015). However, shortly after, the medication began to stop working against the *Penicillin-R-Staphylococcus* bacteria since the bacterium had mutated to become resistant to the Penicillin drug, which became a significant issue. As a result, new beta-lactam antibiotics were developed and distributed, thus helping reduce the number of infections. Antibiotic resistance occurs when a bacterium mutates or changes to protect itself against an antibiotic. Due to these mutations, the antibiotic previously used to treat the bacteria will no longer be effective. When a bacterium is considered resistant to an antimicrobial agent, its daughter cells would also be resistant (Reygaert, 2018). Antibiotic resistance has resulted in an urgent public health threat where medicines once treated for certain bacterial infections are no longer effective. Epidemiological studies have provided strong evidence for a direct relationship between the overuse of antibiotics and the prevalence and spread of antibiotic-resistant bacteria strains (Ventola, 2015). Due to the lack of treatment options, antibiotic resistance has resulted in killing at least 1.27 million people worldwide and is associated with nearly 5 million deaths in 2019 (CDC, 2022).

3.1 How Antibiotic Resistance Occurs

The emergence of antibiotics is mainly due to the overuse and misuse of these medications and the lack of time to develop new drugs (Ventola, 2015). Healthcare providers may prescribe medication to a patient with a viral infection or something not yet diagnosed to alleviate symptoms (NIH, 2011). When antibiotics are not used correctly or not at the correct dose, bacteria may not be killed and pass those traits of survival to more bacteria. The combination of bacteria exposure to antibiotics, the spread of those bacteria, and their resistance mechanism has increased antibiotic resistance in bacteria (CDC, 2022). This can lead to increased illness, worse infections, or death.

Antibiotic resistance occurs when random mutations during bacterial cell division allow the bacterium to express the trait of defense against certain antibiotics. The antibiotic resistance gene can be spread through inheritance or spread to other bacteria of the same species through horizontal gene transfer (HGT). HGT is the transfer of DNA that results in organisms having new genes that did not come directly from the parent organism (Cowan et al., 2022) (Figure 3.1.1). Changes in antibiotic-induced gene expression can increase virulence, while increased mutagenesis and HGT promote antibiotic resistance and spread (Ventola, 2015).

Different horizontal gene transfer types exist in bacteria: conjugation, transformation, and transduction (Figure 3.1.1). Conjugation is a mode of genetic exchange where plasmids or other genetic material are transferred from a donor to a recipient cell by direct connection (Cowan et al., 2022)(Figure 3.1.1). Plasmids are small circular DNA molecules separate from the chromosomal DNA of bacterial cells (NIH, 2024). Plasmids are not essential to bacterial growth and metabolism but provide protective traits such as resisting drugs and making toxins or enzymes (Cowan et al., 2022). The genes commonly transferred using conjugation in nature include drug resistance, resistance to metals, toxin production, and enzymes. Transformation is when a bacterium takes a DNA fragment from a dead, degraded bacterium in exchange for a part of its DNA; it is indirect (Kaiser, 2023) (see Figure 3.1.1). The genes that are commonly transferred using transformation in nature are polysaccharide capsules. Polysaccharide capsules are necessary to protect bacteria from environmental and host factors, such as the host's immune system (Hsieh & Allen, 2020). Transduction is when a bacterial virus transfers DNA from one bacterium to another by a bacteriophage; it is indirect (Kaiser, 2023) (see Figure 3.1.1). A bacteriophage is a virus that infects bacteria (Cowan et al., 2022). When the bacteriophage infects another bacterium, it injects the fragment of donor bacterial DNA it is carrying onto the recipient, which is then exchanged for a piece of the recipient's DNA (Kaiser, 2023). The genes typically transferred in nature include toxins, enzymes for sugar fermentation, and drug resistance.

Bacteria are not all the same; some may be more resistant to certain antibiotics than others. The susceptibility and resistance of bacteria are usually measured by the minimum inhibitory concentration (MIC). The minimum inhibitory concentration (MIC) is the lowest concentration (highest dilution) of the drug that visibly inhibits the growth of bacteria (Cowan et al., 2022). This is useful in determining the smallest effective dosage of a drug and providing a comparative index to other antimicrobials (Cowan et al., 2022). If the average MIC of a species is in the resistance range, the species is considered to have an intrinsic resistance against a drug (Reygaert, 2018). Resistance may be natural, where it is always expressed in a species (intrinsic), or induced, where the genes naturally occur in the bacteria but are only expressed to resistance levels after exposure to antibiotics (Reygaert, 2018). Gene mutations in bacteria occur randomly and then are selected for by antibiotic exposure. Antibiotics kill competitor bacteria since they are more sensitive, which leaves the drug-resistant bacteria to reproduce due to natural selection (Ventola, 2015). The bacteria with the gene mutation multiply, leading to resistance to the once-effective antibiotic.

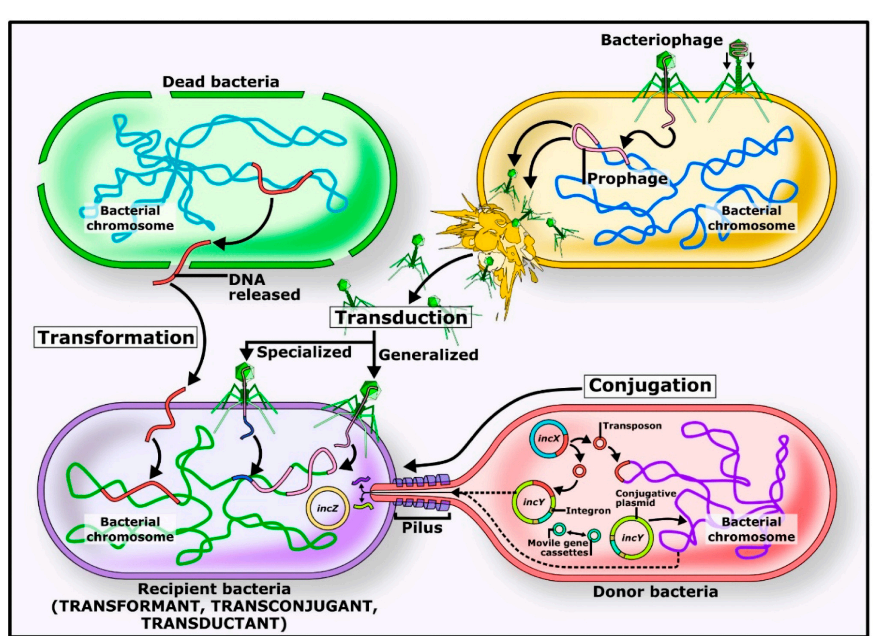

Figure 3.1.1: Types of horizontal gene transfer (Bello-Lopez et al., 2019) Shows the types of horizontal gene transfer in bacteria:transduction, conjugation, and transformation.

3.2 Mechanisms of Resistance

Bacteria have evolved different resistance mechanisms against drugs. Since there are many different kinds of bacteria, many different qualities can contribute to their resistance against antibiotics. Bacteria exhibit either intrinsic or induced resistance to antibiotics. They can also acquire resistance through HGT (see section 3.1). The mechanisms of bacterial resistance within bacteria fall into four categories: limiting uptake of a drug, modifying a drug target, inactivating a drug, and drug efflux (Reygaert, 2018) (Figure 3.2.1).

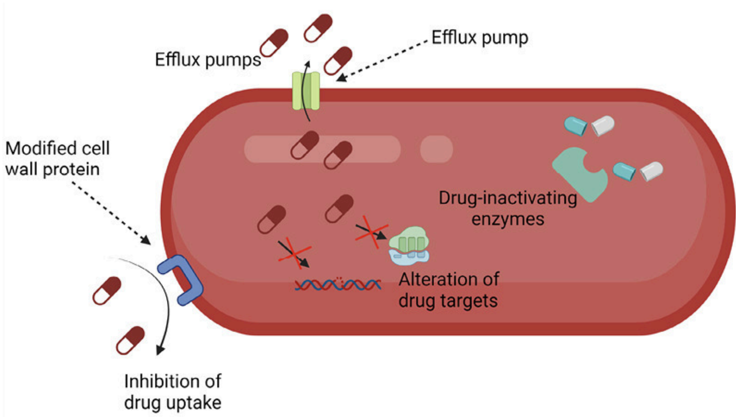

Figure 3.2: Mechanisms of antibiotic resistance (Zagaliotis et al., 2022). Shows mechanisms of antibiotic resistance in bacteria: limiting drug uptake, modifying drug targets, drug efflux, and inactivating the drug.

3.2.1 Natural Resistance

Bacteria can exhibit natural resistance, which can be either intrinsic or induced. Intrinsic resistance is considered a trait that is always expressed and naturally shared within its species (Reygaert, 2018). Common intrinsic resistance mechanisms include less outer membrane permeability, specifically the lipopolysaccharide (LPS) in gram-negative bacteria, and efflux pumps' natural activity (Reygaert, 2018). Other examples of intrinsic resistance exhibited in bacteria are shown in Table 1. Bacteria's susceptibility and resistance to a drug are typically measured using MIC (see section 3.1). A bacteria that falls within the resistance range can be considered to have intrinsic resistance. Induced resistance occurs when the resistance trait of bacteria occurs naturally; however, it is only expressed in resistant levels after

exposure to an antibiotic (Raygaert, 2018). The common induced resistance mechanisms include multidrug efflux pumps.

Table 1: Intrinsic resistance seen in bacteria (Reygaert, 2018). Examples of intrinsic resistance exhibited in different bacteria.

Organism	**Intrinsic Resistance Exhibited by Bacteria**
All gram negatives	glycopeptides, lipopeptides
All gram positives	aztreonam
Escherichia coli	macrolides
Pseudomonas aeruginosa	sulfonamides, ampicillin, 1st and 2nd generation cephalosporins, chloramphenicol, tetracycline
Bacteroides (anaerobes)	aminoglycosides, many β-lactams, quinolones
Listeria monocytogenes	cephalosporins

3.2.2 Limiting Drug Uptake

One of the ways that bacteria can resist drugs is by limiting drug uptake. Antibiotics are seen to target the cell membranes of bacterial cells to destroy them (see section 2.2). This is especially important for gram-negative bacteria since the outer membrane is the first line of defense for bacterial cells against toxic substances such as antibiotics (Munita & Arias, 2016). Gram-negative bacteria are intrinsically less permeable to specific molecules than gram-positive bacteria (Uddin et al., 2021). Since gram-negative bacteria have an LPS (see section 1.1) in their outer membrane layer, their outer membrane can act as a shield against certain antibiotics. The outer membrane of gram-negative bacteria also has a higher lipid content, meaning that hydrophilic (water-loving) molecules such as tetracyclines or β-lactams are ineffective in permeating the bacterial cell (Reygaert, 2018). However, bacteria that do not have an outer membrane (gram-positive) are less likely to limit the uptake of a drug. Bacteria with more extensive outer membranes have porin channels where substances typically enter. The porin channels of gram-negative bacteria let in hydrophilic molecules inside the cell. The porin channels can limit drug uptake by either decreasing the number of porins present or having mutations that change the selectivity of the porin

channel (Raygaert, 2018). This allows the bacteria to limit drug uptake. Examples of bacteria that exhibit this mechanism of antibiotic resistance include bacteria of the family *Enterobacteriaceae.*

Enterobacteriaceae become resistant by reducing the number of porin channels or stopping the production of specific porin channels they have (Raygaert, 2018). Biofilms formed by bacteria also exhibit the mechanism of limiting drug uptake. Bacteria form a biofilm in the bodies of humans or animals, which are communities of bacteria or microbes that attach to a surface and each other, making a multilayer conglomerate of cells (Cowen et al., 2021). Biofilm helps protect the pathogenic organism from the host's immune system. The surface of a biofilm is sticky, slimy, and complex, making it difficult for the antibiotic to work through (Prinzi & Rohde, 2023). Due to this challenge, a higher dose of the drug may be necessary to be effective.

3.2.3 Modifying Drug Target

Another mechanism evolved by bacteria involves modifying the drug target. Antibiotics target specific areas or processes of the bacterial cell (see section 2.2). Bacteria can change those different targets in several ways to ensure resistance against those drugs (Raygaert, 2018). An example of this mechanism is through resistance against β-lactam drugs that are exclusively seen by gram-positive bacteria. Gram-positive bacteria can change the structure or the number of penicillin-binding proteins (PBP). PBPs are enzymes that assist in the formation of peptidoglycan in the cell wall (Raygaert, 2018). Changing the number of PBPs can affect the amount of drugs that can be bound to that target. They change the structure of PBPs, which results in either fully inactivating the drug or lowering the ability of the antibiotic to bind (Raygaert, 2018). Another example is when bacteria can exhibit resistance against antibiotics that target specific ribosomal subunits. This is due to bacteria being able to mutate or change their ribosomes, which results in the antibiotic not effectively binding to the ribosome.

3.2.4 Inactivating Drug

Bacteria can inactivate drugs in two main ways: by transferring a chemical group to the drug or by actually breaking down the drug (Reygaert, 2018). Hydrolyzing is the process of chemically breaking down a product with water. β-lactamases are large hydrolyzing groups that break down an antibiotic. As illustrated in Figure 3.2.4.1, they inactivate β-lactam drugs by hydrolyzing a specific area of the β-lactam chemical ring structure, which causes the ring to open (Reygaert, 2018). Due to this, the open-ring β-lactam drugs cannot bind to their target proteins, making them ineffective. The production of β-lactamases is the most common mechanism gram-negative bacteria use and is the most significant resistance against penicillin and cephalosporin drugs (Reygaert, 2018). Drug inactivation can also occur by transferring a chemical group, such as acetyl, phosphoryl, and adenyl groups. Acetylation is the most diversely used mechanism and is known to be used against aminoglycosides, chloramphenicol, streptogramins, and fluoroquinolones (Reygaert, 2018).

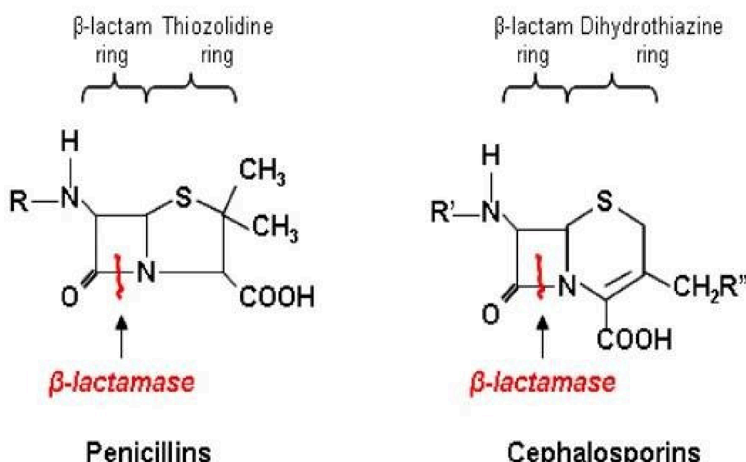

Figure 3.2.4.1: Structure of β-lactam antibiotics (Rocha et al., 2019). Shows the chemical structure of β-lactam antibiotics, penicillin and cephalosporin. The image shows how the enzyme β-lactamase can hydrolyze the area of the ring structure, causing the ring to open and become ineffective.

3.2.5 Drug Efflux

Another mechanism used by antibiotic-resistant bacteria is active drug efflux. Bacteria have chromosomally encoded genes for efflux pumps (Reygaert, 2018). Efflux pumps allow the microorganisms to regulate their internal environment by removing toxic substances, including antimicrobial agents, metabolites, and quorum-sensing signal molecules (Soto, 2012). Quorum-sensing molecules allow microbes to monitor the size of their population; it also allows interaction with other members of the same species nearby (Cowen et al., 2021). Some bacteria express efflux pumps because they are essential, induced, or even overexpressed. Efflux pumps use active transport to move antibiotics out of the cell (Uddin, 2021). The resistance capability of many of these pumps is influenced by the available carbon source (Reygaert, 2018). Bacteria can have multiple families of efflux pumps (figure 3.2.5.1) based on the structure and energy source, including the ATP binding cassette (ABC) family, the multidrug and toxic compound extrusion (MATE) family, the small multidrug resistance (SMR) family, the major facilitator superfamily (MFS), and the resistance-nodulation-cell division (RND) family (Reygaert, 2018). Most efflux families, except for the RND family, are singular pumps that transport substrates across the cytoplasmic membrane (Uddin, 2021). The RND family consists of pumps with multiple parts that move substrates along the entire cell envelope (Raegert, 2018). Efflux pumps found in gram-positive bacteria indicate intrinsic resistance since efflux pumps are coded on the chromosome (Raegert, 2018). The efflux pumps in gram-negative bacteria are seen to come from all five families and are distributed throughout the bacterial cell (Raegert, 2018). Most of the pumps found in gram-negative bacteria are seen to belong to the RND family. An example of a bacteria exhibiting the efflux pump mechanism would be *P. aeruginosa,* which contains the RND family efflux pumps. Many RND pumps can transport drugs such as β-lactams, tetracycline, and chloramphenicol (Raegert, 2018).

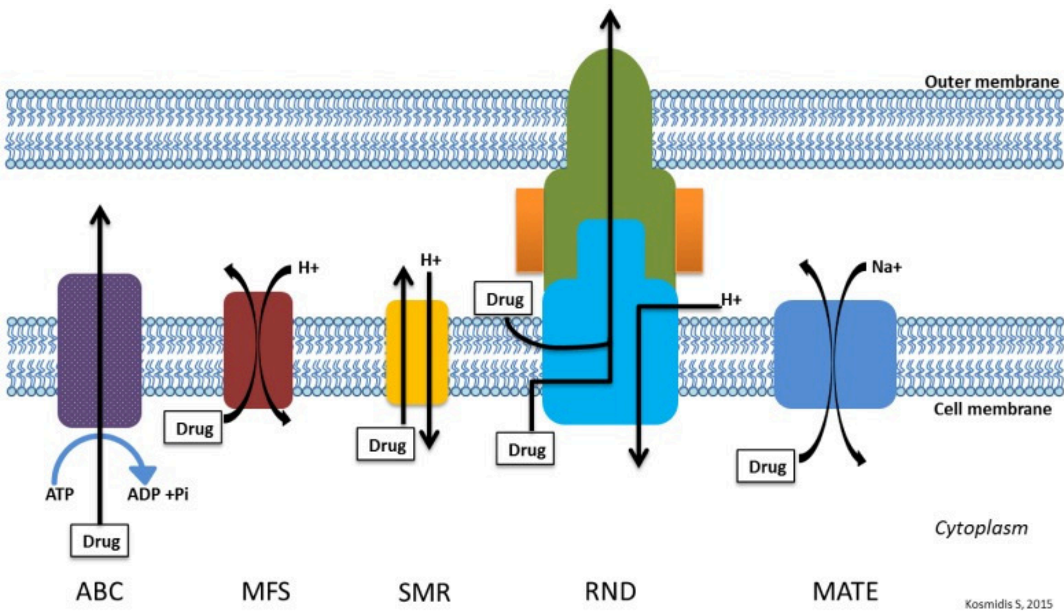

Figure 3.2.5.1: Families of efflux pumps (Reygaert, 2018). Depicts the structure of the different families of efflux pumps: the ATP binding cassette (ABC) family, the multidrug and toxic compound extrusion (MATE) family , the small multidrug resistance (SMR) family, the major facilitator superfamily (MFS), and the resistance-nodulation-cell division (RND) family (Raegert, 2018).

4. Using Plants for Medicinal Purposes Throughout History

Plants have been used for medicinal purposes for centuries, even before recorded history. The oldest written evidence of plant medicinal usage was found on a Sumerian clay slab from Nagpur, about 5000 years old (Petrovska, 2012). It includes drug recipes using over 250 different plants, such as poppy, henbane, and mandrake. Plants have been seen to have many benefits and even be able to treat illness before the creation of drugs.

4.1 Herbal Medicine

Herbal medicine uses parts of a plant for medicinal properties. These include the plant's seeds, leaves, stems, bark, fruits, and flowers. Herbal medicine is often utilized in traditional medicine. Traditional medicine is the prevention, diagnosis, improvement, or treatment of physical and mental illness based on beliefs, theories, or experiences (Wachtel-Galor & Benzie, 2011). People often turn to traditional medicine for many reasons. People use traditional medicine because it is more affordable and accessible. Pharmaceuticals require a prescription and can cost more if the person does not have adequate

insurance coverage, while alternative medicines can be purchased over the counter without a prescription (Mamleeva, 2022). Traditional medicine also aids worries about the adverse effects of chemical (synthetic) medicines, satisfies a desire for more personalized health care, and allows greater public access to health information (Wachtel-Galor & Benzie, 2011). However, herbal medicine is mainly used for its health benefits and as therapy for chronic conditions. Sometimes, standard medicine and treatment are seen as ineffective in patients with illnesses such as advanced cancer. This can also cause people to turn to traditional remedies for other ways to treat illness. In China, in 2003, traditional herbal medicines played a significant role in the strategy to prevent the spread of and treat severe acute respiratory syndrome (SARS) (Wachtel-Galor & Benzie, 2011). In Africa, a traditional herbal medicine, the African flower, has been used for decades to treat wasting symptoms associated with HIV (Wachtel-Galor & Benzie, 2011).

4.2 Properties of Plants

Presently, plants can treat chronic and acute conditions such as prostate problems and cardiovascular disease. Natural products derived from medicinal plants have been seen to be a great source of biologically active compounds, and many of them have helped develop new chemicals for the medicine used currently (Hotwani et al., 2014). Herbs and plants can be processed and taken in several ways and forms. People can use whole herbs, teas, syrup, essential oils, ointments, salves, rubs, capsules, and tablets that contain a ground or powdered form of a raw herb or its dried extract.

Plants contain a wide variety of active phytochemical compounds. Phytochemicals are bioactive compounds that plants produce to protect them against viruses, bacteria, and fungi. In a way, they are part of the plant's immune system. Phytochemicals are classified as primary and secondary metabolites based on how the phytochemical works during plant metabolism (Rabizadeh et al., 2022). Primary metabolites such as carbohydrates, lipids, and proteins are essential for plant life. Secondary metabolites are the other plant chemicals the cells make through metabolic pathways from the primary metabolic pathways (Rabizadeh et al., 2022) (also see section 2.1). Plants are rich in secondary metabolites such as flavonoids,

terpenoids, alkaloids, and saponins (Cowan, 1999). The antimicrobial components due to the secondary metabolites in medicinal plants may prevent the growth of bacteria, viruses, fungi, and protozoa in different ways than current antimicrobials (Vaou et al., 2021), as will be described below. Because the second metabolites in plants potentially prevent the growth of microbes in different ways, bacteria are less likely to have evolved to have resistance against them. This is important for searching for more treatments against resistant microorganisms.

4.2.1 Flavonoids

Flavonoids are found naturally in many plants, such as fruits and vegetables. In plants, flavonoids are produced in specific areas and are responsible for the aroma and color of the flower. In fruits, flavonoids attract pollinators, in fruit dispersion, and germinating seeds and spores. Flavonoids can protect plants from biotic and abiotic stresses and act as a unique UV filter (Panche et al., 2016). Flavonoids are also known to be synthesized by plants in response to microbial infection (Cowan, 1999). They are classified into different types based on their chemical structure, degree of unsaturation, and oxidation of their carbon ring (Ullah, 2020). Flavonoids have different types: anthoxanthins (flavanone and flavanol), flavanones, flavanonols, flavans, chalcones, anthocyanidins, and isoflavonoids (Figure 4.2.1.1). The antimicrobial activity of flavonoids may be due to their ability to complex with bacterial cell walls and extracellular and soluble proteins (Cowan, 1999). More lipophilic flavonoids may also interfere with microbial membranes (Cowan, 1999). They are widely found in nature and have many health benefits. For example, flavonoids are found to be rich in antioxidants. This provides human bodies with natural immune protection against toxins when exposed. It is encouraged to eat a flavonoid-rich diet to reduce the risk of disease. The different classes of flavonoids have different biological activities, such as being anticancer, antifungal, anti-inflammatory, and cardio-protective (Ullah, 2020). The commonly used herbs that provide significant amounts of flavonoids include chamomile, dandelion, ginkgo, green tea, hawthorn, licorice, passionflower, milk thistle, onions, rosemary, sage, thyme, and yarrow (Craig, 1999).

Due to their many benefits, flavonoids are now considered significant in various nutraceutical, pharmaceutical, medicinal, and cosmetic applications (Panche et al., 2016).

Figure 4.2.1.1: Chemical structure of flavonoid. This image depicts the chemical structure of a flavonoid, as well as its different subgroups.

4.2.2 Terpenes & Terpenoids

Terpenoids make up a significant chemical component of the essential oils of plants. They have antioxidant activities, antimalarial activity, anticancer properties, and anti-inflammatory effects (Adefegha et al., 2022) (Figure 4.2.2.1). Terpenoids are derived from terpenes, which are compounds responsible for plants' aroma, flavor, and color. Terpenes are found highly distributed in the leaves, stems, flowers, and roots of conifers, citrus, and eucalyptus plants (Ninkuu et al., 2021). The general structure of terpene is typically $C_{10}H_{16}$, with different types depending on the number of isoprene units (Masyita et al., 2022). Different terpenes include diterpenes, triterpenes, tetraterpenes, hemiterpenes, and sesquiterpenes (Cowan, 1999). When these compounds have other elements like oxygen, they are called terpenoids. Common terpenoids include carvacrol, citronellal, geraniol, linalool, linalyl acetate, piperitone, menthol, and thymol (Masyita et al., 2022). Terpenoids are effective against bacteria, fungi, protozoa, and viruses (Cowan, 1999). The antimicrobial mechanism of terpenes has yet to be determined. However, it may involve membrane disruption by the lipophilic compounds (Cowan, 1999).

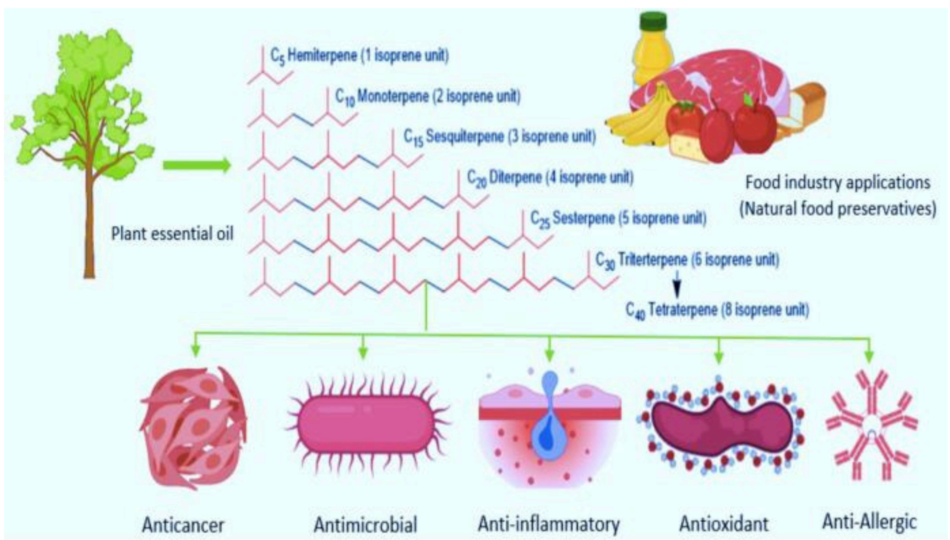

Figure 4.2.2.1: Benefits of terpenes and terpenoids in essential oils (Masyita et al., 2022). This image depicts the potential benefits of terpenes and terpenoids found in essential oils.

4.2.3 Alkaloids

Alkaloids are compounds seen in humans and plants as a part of their natural defense. Alkaloids protect plants from predators and help regulate growth (Heinrich et al., 2021). Alkaloids have many benefits for humans, such as being cardioprotective and anti-inflammatory, and they can be used as anesthetics (Heinrich et al., 2021). Alkaloids that are often used in a clinical setting include morphine, strychnine, quinine, ephedrine, and nicotine (Heinrich et al., 2021). Alkaloids are heterocyclic nitrogen compounds (Cowan, 1999). Heterocyclic compounds have two or more different kinds of atoms in a ring (Katritzky et al., 2008). There are three types of alkaloids: true alkaloids, protoalkaloids, and pseudoalkaloids (Dey et al., 2020). True alkaloids are made from amino acids and are highly reactive in nature. They share a heterocyclic ring that contains nitrogen. Other than nicotine, most true alkaloids are bitter and solid (Dey et al., 2020). Cocaine, morphine, and quinine are the common types of true alkaloids found in nature (Dey et al., 2020) (figure 4.3). Protoalkoids have a nitrogen atom from amino acids but are not part of the heterocyclic ring. The main alkaloids of this kind are yohimbine, mescaline, and hordenine. They are used to help treat health disorders such as pain, neuralgia, and mental illness (Dey et al., 2020). At the same time, true alkaloids and protoalkaloids are made from amino acids, while

pseudoalkaloids are not. The carbon skeletons of pseudoalkaloids are connected to amino acid pathways and are derived from the reactions of forerunners of amino acids (Dey et al., 2020). Common pseudoalkaloids include caffeine, capsaicin, and ephedrine (Dey et al., 2020) (figure 4.2.3.1). Alkaloids have been seen to have several mechanisms to inhibit bacterial growth, including inhibition of the bacterial nucleic acid and protein synthesis, modification of the bacterial cell membrane permeability, damage of the cell membrane and cell wall, inhibition of bacterial metabolism, and inhibition of efflux pumps (Yan et al., 2021).

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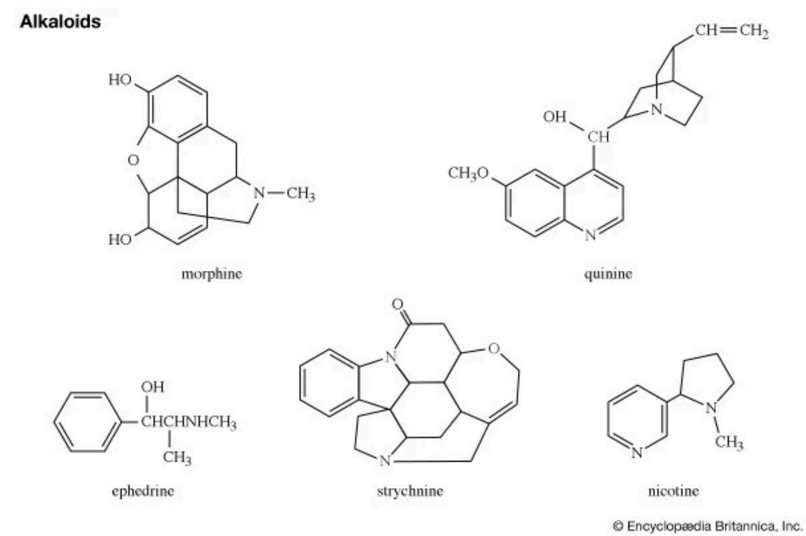

Figure 4.2.3.1: Alkaloid chemical structure (Britannica, 2024). Shows chemical structure of different alkaloids.

4.2.4 Saponin

Saponins are compounds produced by plants to assist with many ecological functions. Many of the saponin compounds help plants defend against microbes and predators. Saponins also have many health benefits for humans because they have anti-inflammatory, antifungal, antibacterial, antiparasitic, antiviral, and anticancer activities (Mugford & Osbourn, 2012). The mechanism of action of saponin is usually puncturing the plasma membrane. Saponin has highly polar sugar parts and non-polar triterpene (class of terpenes) or sterol backbones, which result in a highly amphipathic compound (Mugford & Osbourn, 2012). Because saponins are amphipathic, they are allowed to puncture cell membranes.

Saponins complex with sterols and cause pores to form (Mugford & Osbourn, 2012). They can also have other effects on cells, such as interrupting cell processes such as enzyme activity, organelle integrity, and transport. Saponins are widely found in nature in different plants and some marine animals. Examples of saponins-rich foods and plants include oats, asparagus, quinoa, chickpeas, and sea cucumbers (Timilsena et al., 2023) (figure 4.2.4.1).

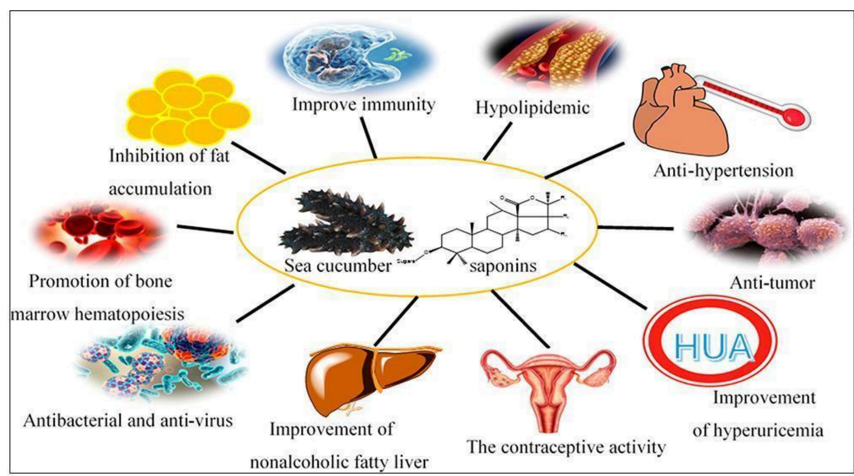

Figure 4.2.4.1: Benefits of saponin in sea cucumbers (Zhao et al., 2018). Saponins are rich in sea cucumbers. With saponins present in sea cucumbers, a number of its benefits are shown.

5. *Traditional Medicine in Cambodian Culture*

Ayurveda and traditional Chinese medicinal traditions have influenced traditional Khmer (or Cambodian) medicine throughout the centuries. Because Cambodia is a developing country, the population does not always have proper access to medicine. Due to this lack of access, the population turned to alternative medicine. An estimated 40-50% of rural Cambodians depend on traditional medicine for their healthcare (Lim et al., 2022).

5.1 Traditional Cambodian Medicine

Cambodian culture has incorporated traditional medicine for hundreds of years; it is more often called Traditional Khmer Medicine (TKM) (Lim et al., 2022). Cambodia is rich in animal and plant life, which has resulted in the people of Cambodia practicing traditional and herbal medicine. According to

Khmer medicine theories, an appropriate hot–cold balance is required to maintain health. Illness and disease may disturb this necessary balance. In response to sickness or to avoid or prevent illness, hot and cold foods may be selected to prevent imbalances in the body or to restore the sick body to health (Burke et al., 2011). Food is classified into hot or cold categories based on its effect on the body. Those that raise the heat of the inner body and improve circulation or nourish the body are considered “hot” foods (Xie et al., 2020). “Hot” foods include ginger, cinnamon, pepper, and nuts. Food that can calm the blood, clear toxins, and reduce heat are considered “cold” foods (Xie et al., 2020). “Cold” foods include milk, bitter melon, cucumber, and melons.

The healers and herbalists, known as Kru Khmer, have passed down the practice of TKM through generations. They were the first people to produce medical wisdom in Cambodia during the Khmer Rouge Revolution since modern medicine was banned. During the Khmer Rouge regime and Cambodian genocide (1975-1979), pre-existing French-style Western medicine was almost destroyed (Lim et al., 2022). The doctors and nurses were forced to move to the countryside to work in agriculture, or they were killed. The Khmer Rouge forced their new system of healthcare called “Khmer Rouge medicine” or “Revolutionary medicine .”The population of Cambodia was forced to pay out of pocket for medical services, which reduced the accessibility of medical care for many of the population. Herbal medicine became the most accessible within communities, allowing the Khmer population to turn to Traditional Khmer Medicine. The traditional Khmer medicine (TKM), deeply intertwined with the history of Cambodia, is a form of natural medicine that relies on natural remedies to encourage the body to heal and maintain itself. (Thnam, 2023) For centuries, the Khmer people have relied on TKM to help cure disease, recover from illness, and heal wounds. In Cambodia, traditional Khmer medicine (TKM) is primary healthcare, especially in rural areas with limited or non-existent access to modern medicine. Nowadays, in Cambodia, doctors often combine traditional Khmer medicine (TKM) with modern medicine, an alternative or complementary treatment for patients.

5.2 Momordica charantia

Momordica charantia, often referred to as bitter melon or bitter gourd, is a plant belonging to the *Cucurbitaceae* family. This plant is native to the tropics, including East Africa, South America, Asia, the Caribbean, India, and Southeast Asia. *M. charantia* is oblong and resembles a small cucumber; the young fruit is emerald green and turns orange-yellow when ripe (Grover & Yadav, 2004) (Figure 5.2.1). Although very bitter, *M. charantia* is popular amongst populations due to its medicinal effects. The whole plant, especially the seeds and fruit, have been used as treatment against diabetes since ancient times (Jia et al., 2017) (Figure 5.1). The parts of *M. charantia* have been used for years as folk medicine to aid in many diseases, such as toothaches, diarrhea, and furuncle (Jia et al., 2017).

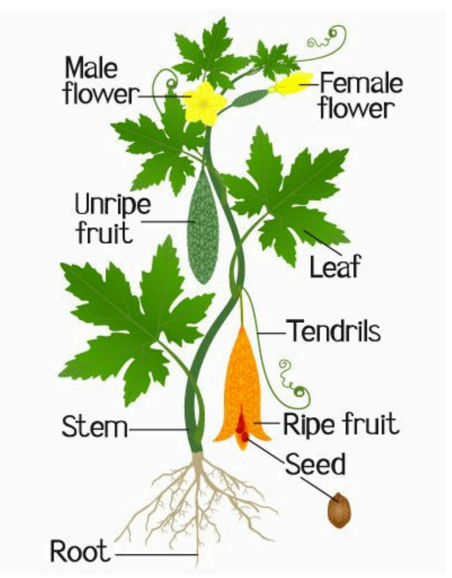

Figure 5.2.1: Parts of *Momordica charantia* (Plantworld, 2021). Anatomical parts of *Momordica charantia.*

5.2.1 Momordica charantia & Cambodian Culture

Momordica charantia has also been used in the Khmer culture as a source of healing. It is not popular with the younger generation since *M. charantia* is known for its sharp, bitter taste. However, Cambodia's older populations enjoy it due to its many health benefits. *M. charantia* has been traditionally used to treat many conditions, such as diabetes, high blood pressure, and digestive disorders. Traditionally, the unripe immature fruits (4-6 in long) are light green, firm, oval-shaped, and with white

flesh and harvested for consumption (Raina et al., 2016) (Figure 5.2.1.1). When the *M. charantia* plant matures, the surface of the fruit is orange-yellow, and the fruit is spongy (Raina et al., 2016). The fruit's flesh is orange-yellow, and the seeds are red (Figure 5.2.1.1).

Traditionally, the part of the *Momordica charantia* that is most popular is the fruit, while the other parts of the plant are not consumed. The fruit of *Momordica charantia* is soaked or boiled in salt water to reduce the bitterness. The fruits are then cooked, stuffed, or stir-fried in the many dishes consumed depending on the practice of the culture. The leaves or fruit extracts can be used for tea as well. For medicinal purposes, the juice of *Momordica charantia* is consumed early in the morning on an empty stomach to control glucose levels of hyperglycemic states (Raina et al., 2016). In Cambodian culture, stuffed bitter gourd soup and stir-fried bitter melon are often consumed. These dishes are also made in Cambodia's neighboring country, Vietnam.

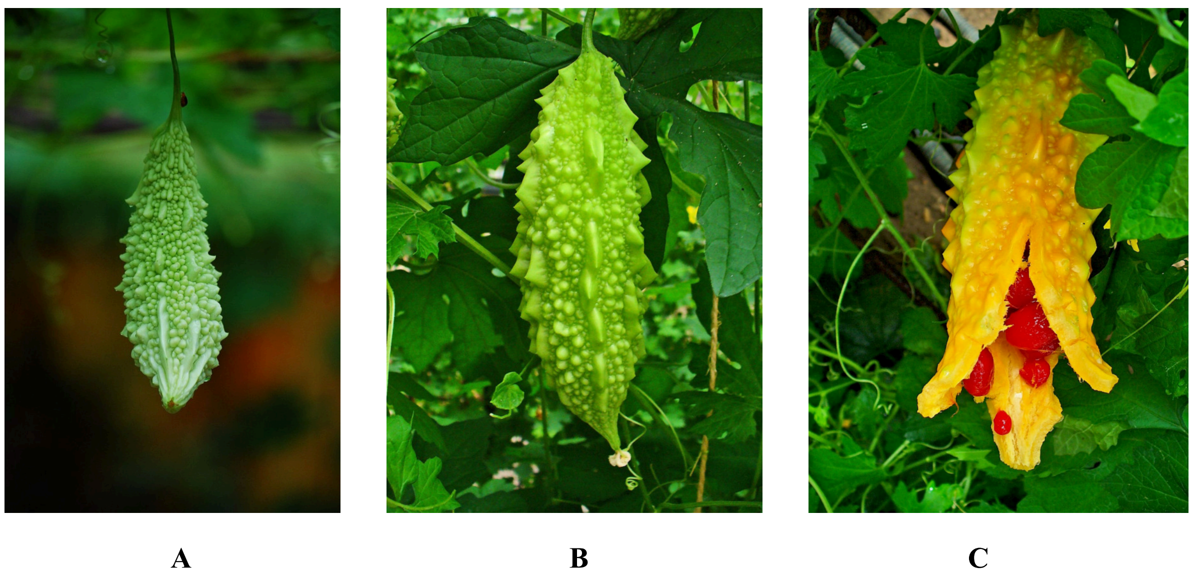

Figure 5.2.1.1: Stages of *Momordica chrantia* growth cycle These images show 3 different stages of the growing cycle of *Momordica charantia.* A shows the *M. charantia* plant being very unripe. B shows the second stage of the *M. charantia* plant where it is still unripe, however, most preferable for consuming. C shows the third stage of the plant where it is ripe, however, this stage is traditionally not consumed.

5.2.2 Bioactive Compounds Within Momordica charantia

Momordica charantia contains several bioactive constituents that allow the plant to provide health benefits beyond the primary nutritional value. The extracts of *Momordica charantia* have been

used as a broad-spectrum antibiotic to fight off infections due to their bioactive components (Jia et al., 2017). The fruits and leaves of the Momordica species are rich in phytochemicals and may have many health-promoting effects by offering nutritional and medicinal components (Bortolotti et al., 2019). Phytochemicals such as flavonoids (see section 4.2.1), saponins (see section 4.2.4), triterpenoids (see section 4.2.2), and alkaloids (see section 4.2.3) have been found in *M. charantia* (Jia et al., 2017).

Past research has shown extracts of the *M. charantia* plant to be effective against several bacteria. The essential oils of *M. charantia* seeds have a significant effect in inhibiting the growth of the bacterium *S. aureus* (see section 1.4); however, they have less impact on *E. coli* (see section 1.2) and *C. albicans* (Jia et al., 2017). It is also seen that the pulp and leaf *M. charantia* extracts have shown antibacterial activity against *E. coli, Staphylococcus, Pseudomonas, Salmonella,* and *Streptobacillus* (Jia et al., 2017).

Objective:

Within this thesis, additional research was done on the antibiotic properties of *Momordica charantia*. However, the parts of the *Momordica charantia* plant (leaf, stem, fruit, seed) are compared. This involved extracting the different parts of the plant *M. charantia* using ethanol and methanol through maceration. *Momordica charantia* was tested against *E. coli, B. subtilis, S. aureus, and P. aeruginosa*. The Kirby Bauer diffusion assay and MIC assay were used to measure the antibacterial activity of the *Momordica charantia* parts. The different parts were compared to see which exhibits more antibiotic activity through statistical analysis.

Research Question:

Is *Momordica charantia* effective in fighting against bacteria? If so, which part of the *Momordica charantia* plant (leaf, stem, seed, or fruit) is most effective against fighting bacteria?

Hypothesis:

Momordica chrantia could have antibiotic activity against *E.coli, B.subtilis, S.aureus,* and *P. aeruginosa.* The fruit of the plant may exhibit the most antimicrobial activity due to it potentially having the highest concentration of phytochemicals.

II. Materials and Methods

Plant retrieval and drying process

The *Momordica charantia* plant was grown in July 2023 by the Cambodian community and purchased in August 2023 from Wat Greensboro. The harvested plant included the stem, fruit, and leaves of the *Momordica charantia* plant. The plant was grown and harvested when it was considered ripe enough to eat, meaning that the fruit was large (4-6 inches) and the skin was light green.

For this study, the parts of the plant were divided into different parts: leaves, seeds, stems, and fruit. Each part of the *Momordica charantia* plant was sliced into ¼ inch pieces to allow faster drying. The parts of the plant were then placed into a dehydrator (NESCO), which utilizes a fan to distribute heat and air to dry out the plant parts. The drying process took approximately 24 hours for the parts of the plants to dry out completely.

Methanol & 70% Ethanol Extract of Momordica charantia

The exaction type of maceration was performed on the dried plant material of *Momordica charantia.* Maceration is suitable for plants that have compounds that can get damaged due to the exposure to heat (Abubakar & Haque, 2020). The method of extraction is based on the procedure described by Abubakar & Haque (2020). After drying, each *Momordica charantia* plant part was separately ground using a mortar and pestle. Grinding the plant material effectively allows the solvent to extract the phytochemicals from the plant part. The mortar and pestle were washed with ethanol between each preparation. Each ground sample was weighed and then placed in a 50 ml sterile conical tube, and then methanol or 70% ethanol was added to the tube. For the ethanol extraction, .5g of plant material was added to 8.5 ml of ethanol. For the methanol extraction, .06g of plant material was added to 1.18 ml of methanol. To prevent evaporation and contamination of the sample, the conical tube was sealed with a screw top cover. The conical tube with the sample was then placed in a shaker for seven days to allow continuous agitation of the sample inside the tube. After seven days, the sample was filtered using

Whatman 90mm filter paper to separate the solid from the liquid sample. The liquid sample was then allowed to evaporate, leaving an oily residue, and then reconstituted with 1 ml of ethanol or methanol. Using a sterile loop, a sample of the methanol/ethanol extract for each plant part extract was spread on nutrient agar plates and then incubated at 37°C for approximately 18-24 hours to ensure no contamination.

Another extraction of the *Momordica charantia* plant was performed. However, it was an extract of a different concentration, with 1 gram of ground plant material added to 20 ml of methanol solvent. This extraction was used for the minimum inhibitory concentration (MIC) assay.

Maintenance of Bacteria

Bacillus subtilis (*B. subtilis*) (Lot # 154921), *Staphylococcus aureus (S. aureus),* and *Pseudomonas aeruginosa (P. aeruginosa)* were obtained from Carolina Biological. These bacteria arrived in a dry powdered form. 1 ml of nutrient broth was added to the dried form of the microorganism and then transferred to a sterile tube containing five ml of nutrient broth (NB). The NB tube was then placed in a shaker for approximately 18-24 hours for the bacteria to grow. The microorganism cultures were then streaked on nutrient agar plates to isolate individual colonies. The nutrient agar plates with the microorganisms were placed in the incubator at 37° for approximately 18-24 hours. The isolated colonies were then inoculated into tubes containing 5 ml of NB and placed in the 37°C shaker for 18 hours for the isolated colonies to grow and be used for further testing. Cultures were used immediately or within 2 hours and maintained at 4°C.

Escherichia coli (E.coli) (Lot # 124500) was obtained as a slant tube from Carolina Biological. A sterile inoculating loop was used to inoculate the *E. coli* into a tube containing 5 ml NB. The NB tube was then placed in a shaker for approximately 18-24 hours for the bacteria to grow. The *E.coli* microorganism was then streaked on a nutrient agar plate to isolate individual colonies. The nutrient agar plate with the microorganism was placed in the incubator at 37°C for approximately 18-24 hours. The isolated colonies

were then inoculated into sterile tubes with nutrient broth and were placed into a shaker for about 18-24 hours. Cultures were used immediately or within 2 hours and maintained at 4°C.

Kirby Bauer Disk Diffusion Assay

A Kirby Bauer assay was performed to determine if there were any antibiotic properties in the different parts of the bitter melon plant extract. Mueller Hinton agar (MHA) (Carolina Biological Supply) was prepared according to the manufacturer's instructions. Briefly, 38 grams of Mueller Hinton (MH) powder was mixed with 1L of distilled water and then sterilized using an autoclave for approximately an hour. Once finished, the MH broth was removed from the autoclave and cooled briefly before being poured into Petri dishes; each dish contained approximately 30 ml of MH broth. The petri dishes were allowed to cool and solidify to room temperature and then stored in plastic sleeves upside down at 4° C.

Ten microliters of each plant extract or methanol were applied to sterile filter disks (7 mm diameter) using a micropipette. The discs were then stored in a sterile petri dish at 4° C until use. The gentamicin discs (BD BBL™ Sensi-Disc™) were used as a positive control.

After preparing the MHA plates and discs, 18-24 hour cultured bacteria was then spread on the plates. A single plate was divided into four quadrants: ethanol or methanol, gentamicin, leaf and fruit, or stem and seed (figure 6). The ethanol or methanol was the negative control, while the gentamicin was the positive control. Each disc containing ten microliters of each extract was placed according to its labeled location. The plates were then incubated at 37°C for approximately 18-24 hours. After removing the plates, each disc's zone of inhibition was measured and recorded in a spreadsheet. A T-test was performed between each plant part in methanol and ethanol trials.

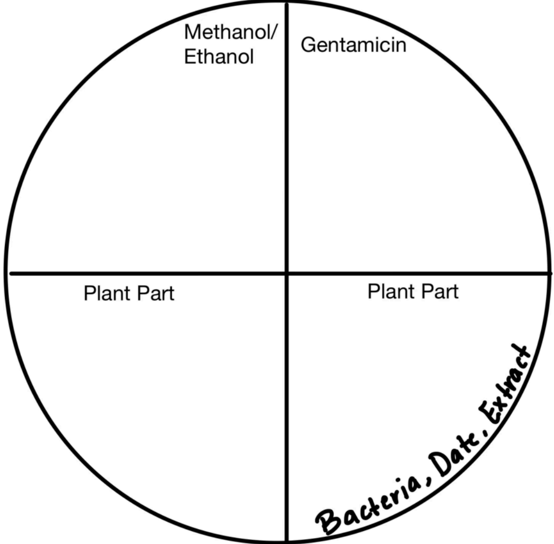

Figure 6: Diagram of Kirby Bauer plate.
Diagram of how the Kirby Bauer plate was divided.

Minimum Inhibitory Concentration (MIC) Assay

A modified minimum inhibitory concentration (MIC) assay was also performed to test the antibiotic activity of *Momordica charantia* and determine the lowest extract concentration needed to inhibit bacterial growth (Cowen et al., 2021). While the Kirby Bauer Assay assesses if the dilution of the extract had antibiotic activity, a MIC assay figures out the lowest dilution of which the compound is effective against the bacterium. Each plant extract was tested against *E. coli, S. aureus,* and *P. aeruginosa.* The *Momordica charantia* extracts were not used against *B. subtilis* due to the results from the Kirby Bauer assay showing that the extracts were ineffective against the bacterium.
In an attempt to normalize the bacteria's concentration, culture dilutions were read in the spectrophotometer. Using 18-hour cultures, dilutions were read to establish a normalized concentration of bacteria. A 1:20 ratio of 2 ml of bacterial culture to 38 ml nutrient broth was made.

Using 48-well plates (Falcon), the following components were added. Two hundred microliters of nutrient broth were added to the wells of the last column, while 100 microliters were added to the rest of the wells. A 100-microliter volume of 1:20 plant extract dissolved in methanol is added to the first column of the well plate. Fifty microliters were withdrawn and serially diluted seven times, resulting in

dilutions ranging from 1:2 to 1:128 (see Figure 7). Subsequently, 100 microliters of bacteria were added to each well (bacteria was a 1:20 dilution of 18-hour culture). Each plate was numbered to differentiate; plates 1-3 were labeled leaf and seed, and plates 4-6 were labeled fruit, gentamicin, and methanol. The rows and columns of the plates were further differentiated (Figure 7). Plates 1 and 4 were tested against *E.coli*, plates 2 and 5 were tested against *S. aureus*, and plates 3 and 6 were tested against *P. aeruginosa.* The plates were wrapped with parafilm and were incubated for 18-24 hours at 37°C. The photos were taken of it.

Using a sterile inoculating loop, individual streaks of each well were applied to nutrient agar plates (Figure 8) to see if bacterial growth occurred at a specific antibiotic dilution. After all streaks were made, the NA culture plates were incubated at 37°C for 18-24 hours. If bacterial growth was seen on the plate, it indicated bacterial growth at that specific dilution. Growth was graded and indicated on a table (see figure 14A, 14B, & 14C in Results) using signs: (-) indicates no growth on the streak plate, (+) indicates little growth, and (++) indicates significant of growth.

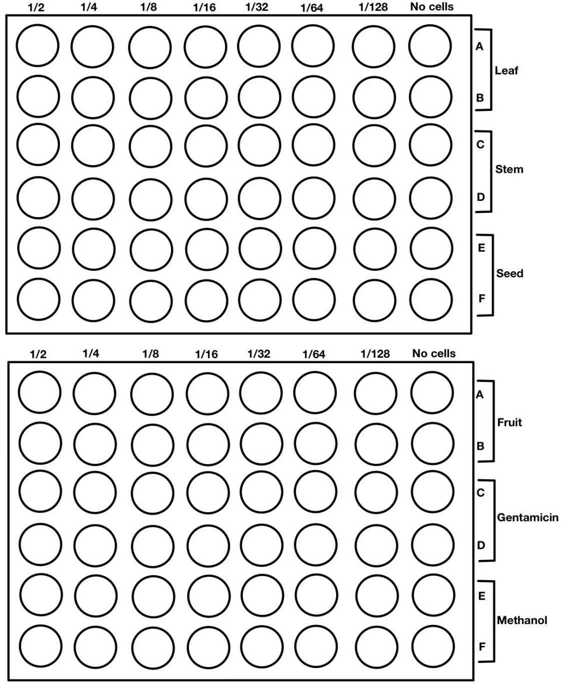

Figure 7: MIC assay microtiter plate division. MIC assay microtiter plate organization

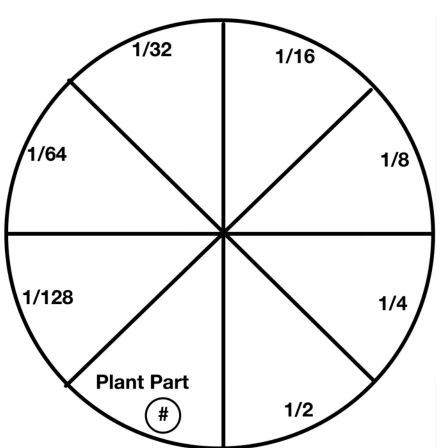

Figure 8: MIC assay Kirby Bauer division. Kirby Bauer nutrient agar plate organization for MIC assay well plate streaks.

Gram-Staining

A gram-staining procedure was performed to identify the bacteria used throughout the experimentation. This was done to confirm that this was the correct bacteria being used by identifying its morphology. This procedure determines the bacterium's gram status and shape. If the bacteria on the slide appeared pink or red, it indicates that it is gram-negative. Bacteria can be shown as either being round (cocci), rod-shaped (bacillus), or spiral-shaped (spirochete). If the bacteria on the slide appeared purple, it was gram-positive. The sterile technique is used throughout this procedure.

Using a sterile inoculation loop, a loopful of sterile water is put on a glass slide and spread. Then, a bacteria colony from a streak plate is retrieved and mixed with the water on the glass slide. The slide is allowed to dry until there is no water on it and is heat-fixed by allowing it to pass through the Bunsen burner flame three times.

Following this step, crystal violet is applied to the slide and stays on for 30 seconds. Then, it is rinsed off with deionized water. Iodine is then applied to the slide and rinsed off after 10 seconds. Ethyl alcohol is then used to rinse the slide for approximately 10 seconds, and deionized water is used to rinse the alcohol off. Safranin is then applied to the slide, left on for 30 seconds, and rinsed off.

These steps are repeated for each of the bacteria. The slides were allowed to dry and then viewed under a microscope under 100x magnification (10x lens) to examine the color. An immersion oil was added to the slide and viewed under 1000x magnification (oil immersion lens) to view the bacterium's shape.

Colony Polymerase Chain Reaction (PCR)

A colony polymerase chain reaction (PCR) was performed for the four bacteria used throughout experimentation to amplify the DNA of the bacteria. This was done to confirm the correct bacteria being used through identifying its DNA sequence. Four microtubes were labeled A, B, C, and D: “A” represents the bacterium *B. subtilis,* “B” represents *E. coli*, “C” represents *S. aureus,* and “D” represents *P.*

aeruginosa. These microtubes represent a short-range PCR. Another four microtubes were labeled the same but circled to represent a long-range PCR. Because of this difference in the range of PCR, different primers were used (Table 4). Finally, one last tube was made and labeled as 'control.' To each tube, 20 µL of nuclease-free (NF) water was added. Then, a sterile pipette tip is lightly dipped into the bacteria colony using a sterile pipette tip and transferred to the tube with NF water. Then, the pipette tip is placed into the tube. This step is repeated for each kind of bacteria tube; however, no bacteria is added to the control tube. Using the appropriate pipette gun, the ingredients were added to the PCR tubes in the order according to Table 1. A microcentrifuge was then used to spin the samples to the bottom of the tube.

Table 2: In the PCR procedure, ingredients are added from top to bottom.

Tube Type	Whole colony	(-) Control
Master Mix	50 µL	50 µL
515 F Primer	2 µL	2 µL
1492 R Primer	2 µL	2 µL
Template DNA	1 µL Whole Cell	0 µL
NF Sterile H2O	45 µL	46 µL
Total Volume:	100 µL	100 µL

The samples were placed in a thermocycler for approximately 3.5 hours and were allowed to run for 30 cycles at these intervals:

Table 3: Steps of thermocycler with PCR samples.

Step	Temperature (C°)	Time (minutes)
Denaturing	92	1:30
Annealing	50	1:30
Elongation/Reannealing	72	:30

This is a total of 3.5 hours and 30 cycles. After this, the thermocycler goes through:

Final elongation/Reannealing: 7 min - 72 C°

Cool down and Storage: 24 hours - 4 C°

The samples are then stored at 4 C°

Table 4: Sequences of short and long primers used in PCR samples.

Primer	Sequences
Short 515 F Primer	5' -GTTACCTTGTTACGACTT-3'
Short 1492 R Primer	5'- GTGCCAGCMGCCGCGGTAA-3'
Long 515 F Primer	5' - GGCTCGAGCGGCCCTTGTTACGACTT-3'
Long 515 F Primer	5'-GCGGATCCTCTAGGCAGCCGCGGTAA-3'

Gel Electrophoresis

A gel electrophoresis of the PCR samples was done to see the array of DNA fragments, allowing the comparison of different individual samples (Cowen et al., 2021). 70 ml of agarose gel was prepared at 1% concentration. The mixture in the flask was microwaved in intervals to create a clear liquid. The mixture was then allowed to cool, and one microliter of Ethidium Bromide (EtBr) was added to the flask. The agarose gel mixture was then added to the gel electrophoresis tray with ten well combs, and 30 minutes was allowed to cool and solidify completely. The gel was covered with about 300 ml of a 1% Tris-acetate-EDTA (TAE) buffer with three microliters of EtBr added. Samples were made using five microliters of the PCR samples of each bacterium and adding two microliters of 6X dye to a 1.5 Eppendorf tube. The samples from the PCR using long primers were used. A DNA ladder (#NO468S) from Quick-Load was also prepared using five microliters of the ladder sample and two microliters of 6X dye. Each sample was loaded into the agarose gel wells: well 2 being the ladder, well 3 being *B.subtilis*, well 4 being *E.coli*, well 5 being *S. aureus*, and well 6 being *P.aeruginosa*. The top was placed on the gel electrophoresis tray, and then the test was run for about 30 minutes to 1 hour at about 85-93 volts. After this step, the gel was placed under a UV light to interpret the DNA fragments of the bacterium.

DNA Cleanup

DNA cleanup was performed to purify the PCR DNA sample so that it could be sent in to be sequenced and compared to the database (BLAST) to identify the type of bacterium used. The QIAquick PCR Purification Kit was used for this procedure, and the directions were followed accordingly. Before use, ethanol (96-100%) was added to the Buffer PE provided. Into separate microtubes, 100 microliters of the PCR sample of the bacteria were added. Then, 500 microliters of phosphate buffer (PB) were added, creating a 1:5 ratio. The dye indicator was not added. The QIAquick column was placed into a 2 ml collection tube (provided) and centrifuged for approximately 30 seconds to bind the DNA.

Once the samples had passed through the filter, the flow-through was discarded, and the column was placed back into the collection tube. To wash the DNA, 750 microliters of Buffer PE was added to the QIAquick column and centrifuged for approximately 30 seconds. The flow-through is discarded, and the column is placed back into the tube. The column is centrifuged in the 2 ml collection tube for one minute to remove the remaining buffer. Each column is then placed into a clean 1.5 ml microcentrifuge tube. Fifty microliters of distilled water are added to the center of the QIAquick membrane, allowed to stand for one minute, and then centrifuged to elute the DNA. This sample is then sent in for sequencing to identify the bacterium.

III. Results

Kirby Bauer Diffusion Assay

70% Ethanol Extract

The ethanol extract of all *Momordica charantia* plant parts against *E. coli, B. subtilis, P. aeruginosa,* and *S. aureus* did not show a zone of inhibition (Figure 10A-D). Three trials were done in triplicate with *E. coli* and *B. subtilis* and two in triplicate with *P. aeruginosa and S. aureus*. Figure 9 shows a representative plate with the only zone of inhibition observed with gentamycin (positive control).

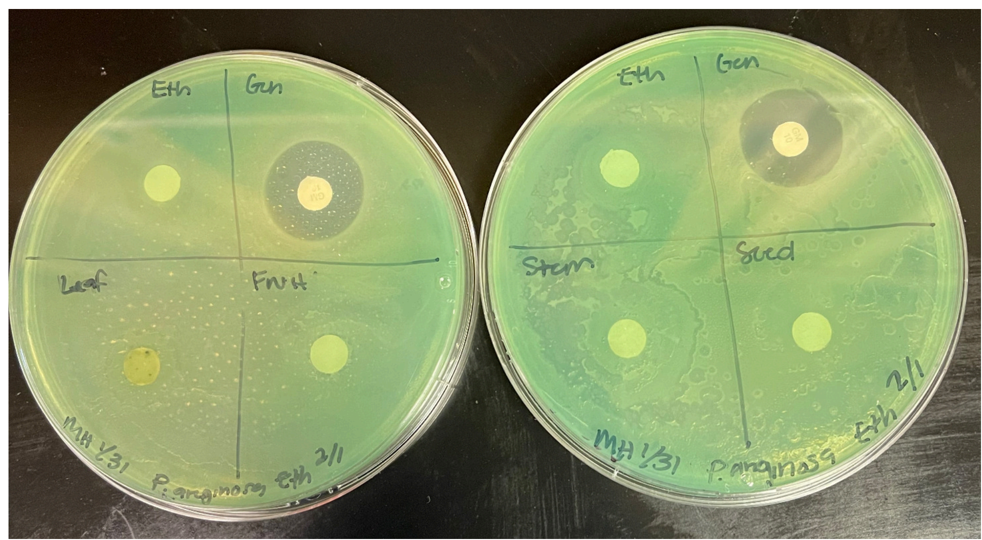

Figure 9: Kirby Baur Assay of *Momordica charantia* ethanol extract against *P. aeruginosa.* *P. aeruginosa* on Mueller-Hinton agar after incubation at 37°C for 24 hours. This is the second trial of *Momordica charantia* 70% ethanol extract against *P. aeruginosa.* The trials were done in triplicate; these are one of them. No zone of inhibition is observed except for the positive control gentamicin. Similar results were seen with *B. subtilis, S. aureus,* and *E. coli.*

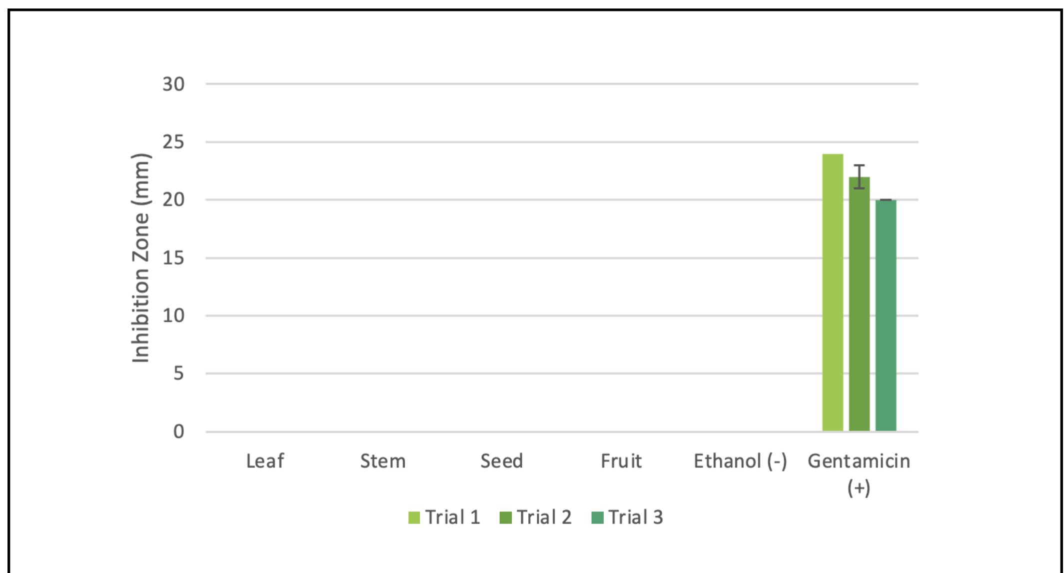

Figure 10A: The statistical data of ethanol extract of *Momorodica charantia* leaf, seed, stem, and fruit extract against *E. coli*. The statistical data of ethanol leaf, seed, stem, and fruit extract against *E. coli*. It also shows that gentamicin is the positive control, while ethanol is the negative control. The error bars indicate the standard deviation within the trials. This graph shows the averages of the zone of inhibition of the triplets in three trials. Zones of inhibition were determined by measuring the diameter of the clearing around the disc containing the compounds. Of the three trials, no zone of inhibition was seen with the ethanol *Momordica charantia* extract.

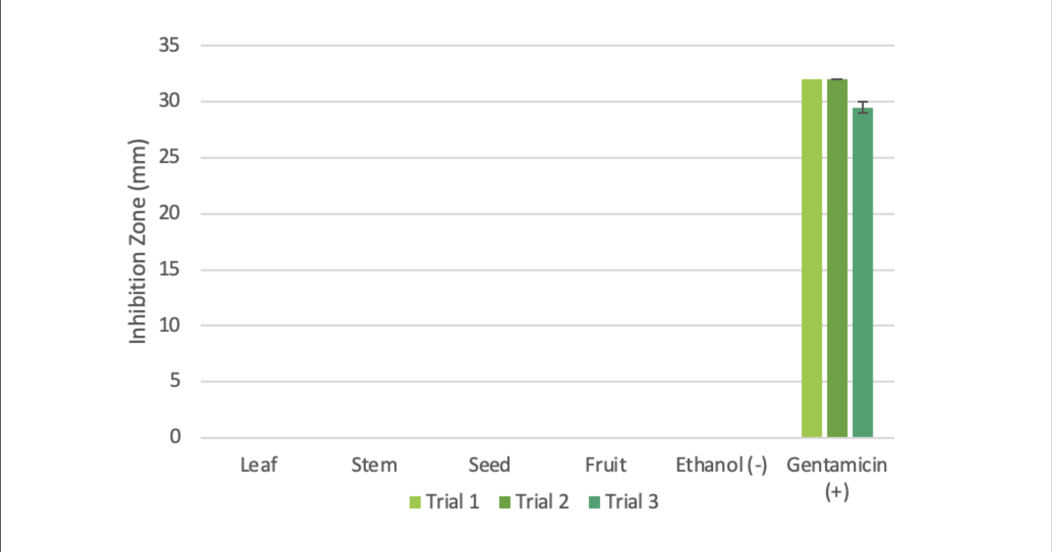

Figure 10B: The statistical data of ethanol extract of *Momorodica charantia* leaf, seed, stem, and fruit extract against *B. subtilis*. The statistical data of ethanol leaf, seed, stem, and fruit extract against *B. subtilis*. It also shows that gentamicin is the positive control, while ethanol is the negative control. The error bars indicate the standard deviation within the trials. This graph shows the averages of the zone of inhibition of the triplets in three trials. Zones of inhibition were determined by measuring the diameter of the clearing around the disc containing the compounds. Of the three trials, no zone of inhibition was seen with the *Momordica charantia* ethanol extract.

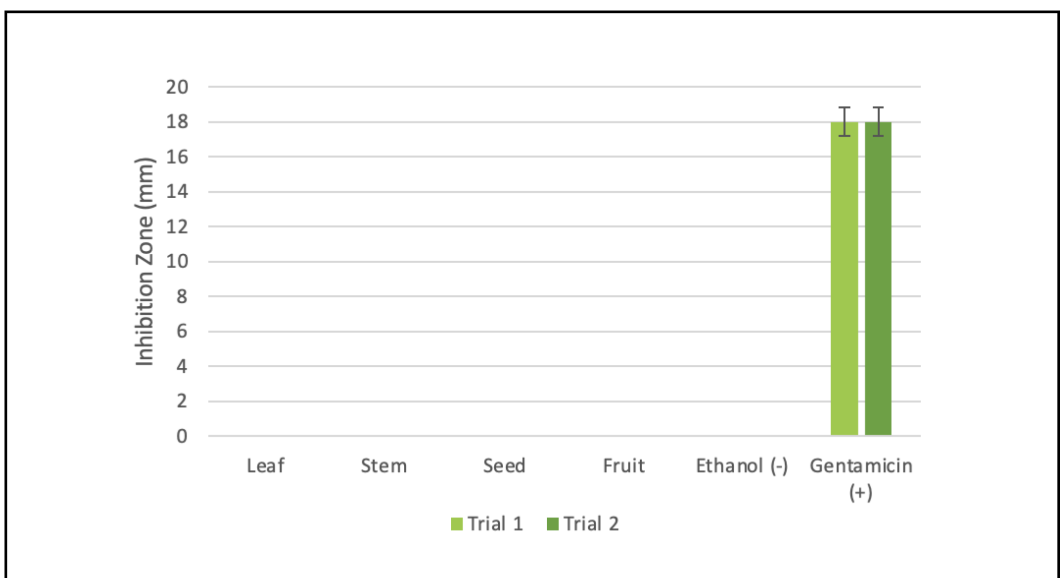

Figure 10C: The statistical data of ethanol extract of *Momorodica charantia* leaf, seed, stem, and fruit extract against *S. aureus*. The statistical data of ethanol leaf, seed, stem, and fruit extract against *S. aureus*. It also shows that gentamicin is the positive control, while ethanol is the negative control. The error bars indicate the standard deviation within the trials. This graph shows the averages of the zone of inhibition of the triplets in two trials. Zones of inhibition were determined by measuring the diameter of the clearing around the disc containing the compounds. Of the two trials, no zone of inhibition was seen with the *Momordica charantia* ethanol extract.

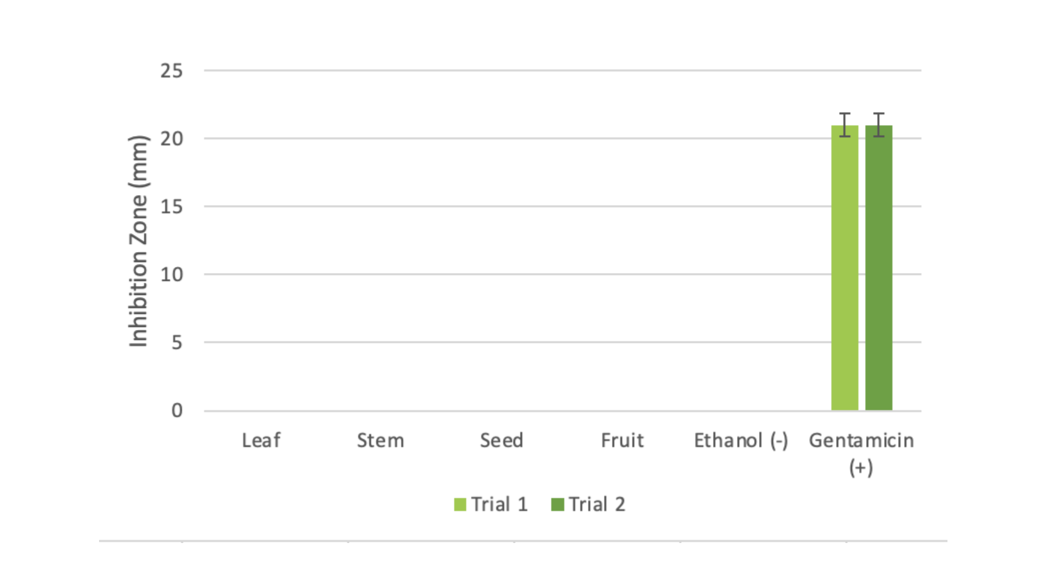

Figure 10D: The statistical data of ethanol extract of *Momorodica charantia* leaf, seed, stem, and fruit extract against *P. aeruginosa*. The statistical data of ethanol leaf, seed, stem, and fruit extract against *P. aeruginosa*. It also shows that gentamicin is a positive control, while ethanol is the negative control. The error bars indicate the standard deviation within the trials. This graph shows the averages of the zone of inhibition of the triplets in two trials. Zones of inhibition were determined by measuring the diameter of the clearing around the disc containing the compounds. Of the two trials, no zone of inhibition was seen with the *Momordica charantia* ethanol extract.

Methanol Extract

Two trials were done in triplicate with *E. coli, B. subtilis*, *P. aeruginosa,* and *S. aureus*. No zone of inhibition was observed with the methanol extract of *Momordica charantia* against *E. coli, B. subtilis*, and *P. aeruginosa* (figure 12A-C). However, with *S. aureus,* there were zones of inhibition present (Figure 11). From the trials against *S. aureus,* the mean of the zone of inhibitions was calculated: fruit is 11.83 mm, seed is 7.5 mm, leaf is 6, and stem is 0. A T-test compared the fruit, seed, and leaf to the stem extraction. The T-test shows that compared to the stem methanol extract, fruit (P = .011 & P = .015) and seed (P = .007) had significant statistical values (Figure 12D).

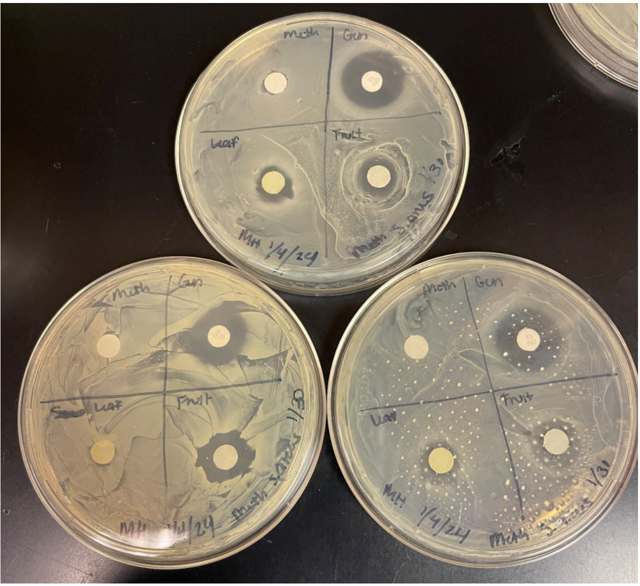

Figure 11: Kirby Baur Assay of *Momordica charantia* ethanol extract against *S. aureus.* *S. aureus* on Mueller-Hinton agar after incubation at 37°C for 24 hours. This is the first trial performed with the methanol extract of *Momordica charantia* parts (leaf, stem, seed, and fruit). This assay was done in triplicate. It is observed that bacteria are cleared from the fruit, leaf, and gentamicin antibiotic.

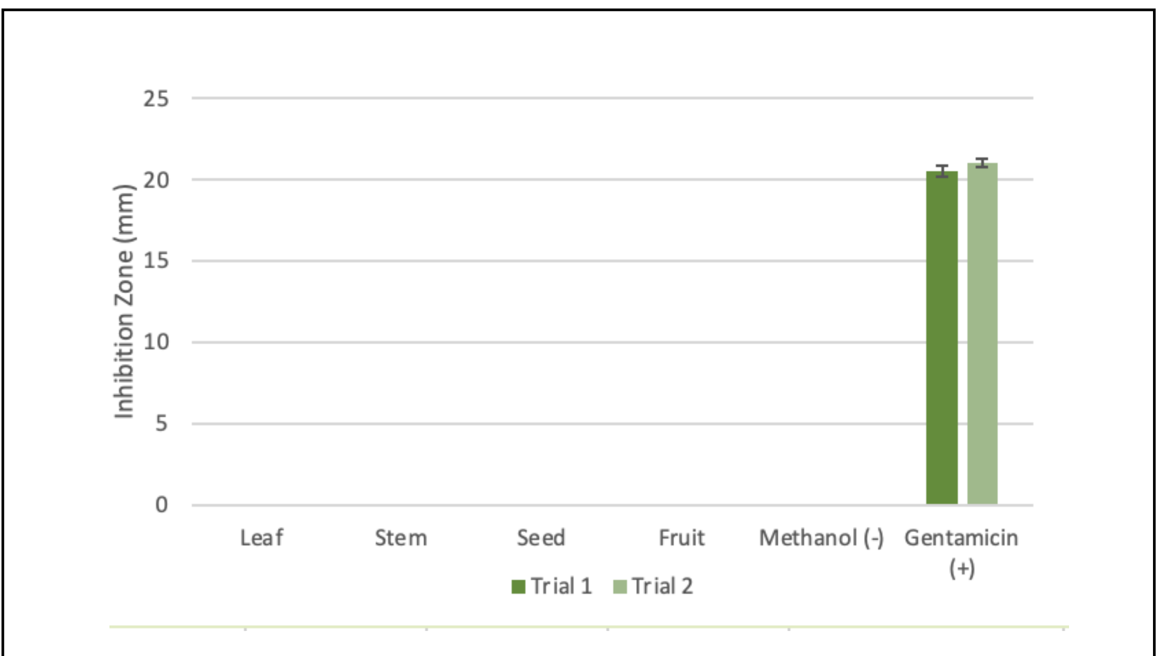

Figure 12A: The statistical data of methanol extract of *Momordica charantia* leaf, seed, stem, and fruit extract against *B. subtilis*.The statistical data of methanol leaf, seed, stem, and fruit extract against *B. subtilis*. It also shows that gentamicin is the positive control, while methanol is the negative control. This graph shows the averages of the zone of inhibition of the triplets in two trials. Zones of inhibition were determined by measuring the diameter of the clearing around the disc containing the compounds. Of the two trials, no zone of inhibition was seen with the *Momordica charantia* methanol extract.

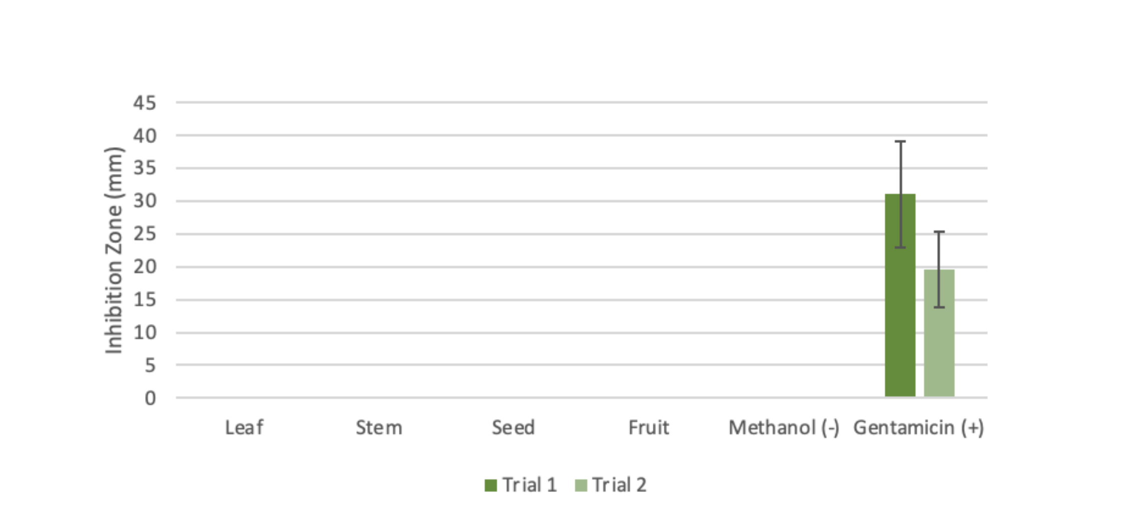

Figure 12B: The statistical data of methanol extract of *Momordica charantia* leaf, seed, stem, and fruit extract against *E.coli*. The statistical data of methanol leaf, seed, stem, and fruit extract against *E. coli*. It also shows that gentamicin is the positive control, while methanol is the negative control. This graph shows the averages of the zone of inhibition of the triplets in two trials. Zones of inhibition were determined by measuring the diameter of the clearing around the disc containing the compounds. Of the two trials, no zone of inhibition was seen with the *Momordica charantia* methanol extract.

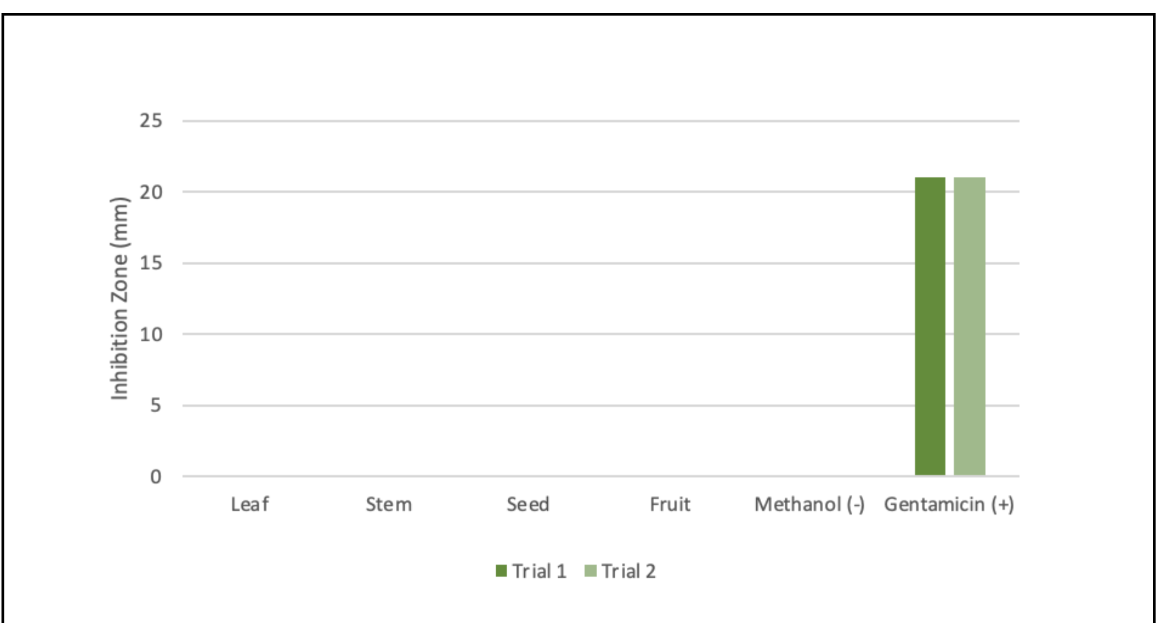

Figure 12C: The statistical data of methanol extract of *Momordica charantia* leaf, seed, stem, and fruit extract against *P. aeruginosa.* The statistical data of methanol leaf, seed, stem, and fruit extract against *P. aeruginosa*. It also shows that gentamicin is the positive control, while methanol is the negative control. This graph shows the averages of the zone of inhibition of the triplets in two trials. Zones of inhibition were determined by measuring the diameter of the clearing around the disc containing the compounds. Of the two trials, no zone of inhibition was seen with the *Momordica charantia* methanol extract.

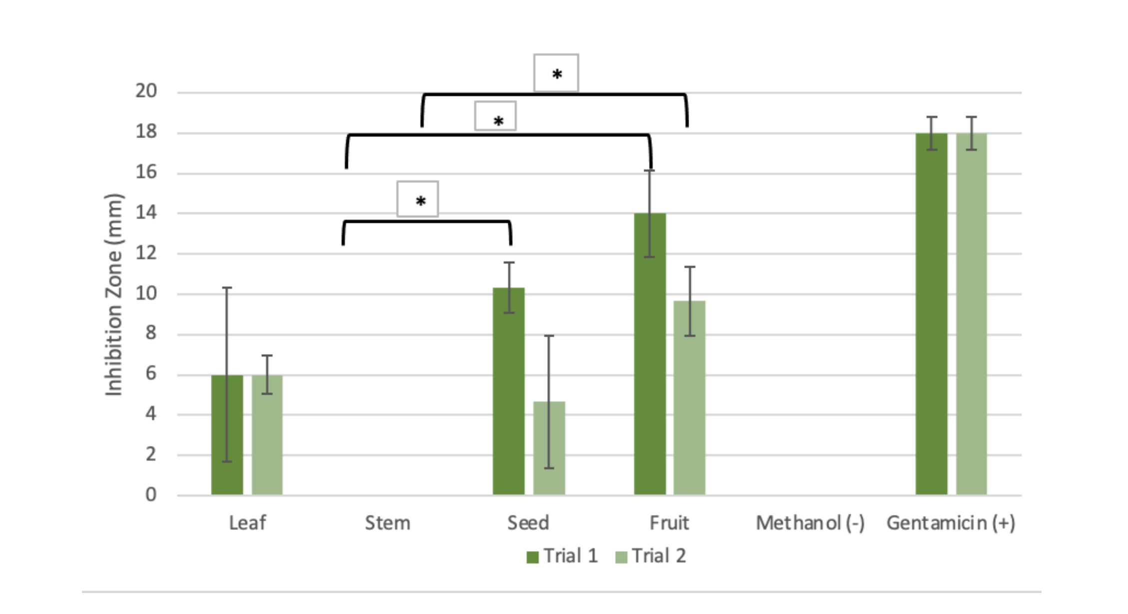

Figure 12D: The statistical data of methanol extract of *Momordica charantia* leaf, seed, stem, and fruit extract against *S. aureus.* The statistical data of methanol leaf, seed, stem, and fruit extract against *S. aureus*. It also shows that gentamicin is the positive control, while methanol is the negative control. This graph shows the averages of the zone of inhibition of the triplets in two trials. Zones of inhibition were determined by measuring the diameter of the clearing around the disc containing the compounds. The leaf, fruit, and seed extracts exhibited a zone of inhibition. The seed and fruit are statistically significant (based on a t-test and alpha level of .05) compared to the stem, indicated using a bracket and "*." The stem, however, was not observed to have any zone of inhibition.

Minimum Inhibitory Concentration (MIC) Assay

A MIC assay was performed testing methanol extractions of *Momordica charantia* against *E. coli, S. aureus,* and *P. aeruginosa*. Streak plates were made from each of the dilution wells to see if bacteria growth was present in the dilutions (see Materials and Methods).

The MIC assay for *E. coli* (Figure 14A) had inhibitory growth at ½ dilution with the methanol control. Compared to the methanol control, reduced bacterial growth was observed at ⅛ dilution for the leaf and ¼ dilution for the stem, seed, and fruit (Figure 14A). The MIC assay for *S. aureus* (Figure 14B) did not have inhibitory growth for the methanol control. Compared to the methanol control, reduced bacterial growth was observed at ⅛ dilution for stem and ¼ dilution for leaves, seed, and fruit (Figure 14B). The MIC assay for *P. aeruginosa* (Figure 14C) had inhibitory growth at ½ dilution with the methanol control. Compared to the methanol control, reduced bacterial growth was observed at ⅛ dilution for stem and ¼ dilution for leaf, seed, and fruit (Figure 14C).

Figure 13: MIC assay well plate streaks of the methanol extract of *Momordica charantia* plant parts against *S. aureus*. MIC assay plate streaks of the methanol extract of *Momordica charantia* plant parts against *S. aureus.* This also shows methanol (negative control) and gentamicin (positive control). It is observed that at ½ dilution, bacterial growth is inhibited. However, the rest of the dilutions do not. Methanol does not inhibit bacterial growth, while gentamicin does inhibit bacterial growth.

Part	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Stem	-	+	++	++	++	++	++
Leaf	-	+	+	++	++	++	++
Fruit	-	+	++	++	++	++	++
Seed	-	+	++	++	++	++	++
Methanol (-)	-	++	++	++	++	++	++
Gentamycin (+)	-	-	-	-	-	-	-

Figure 14A: MIC Assay table with *Momordica charantia* parts against *E.coli.* This table shows the MIC assay done with the methanol extract of *Momordica charantia* against *E. coli*. The top values represent the compound's dilution amounts, and the side of the table shows the extracted parts. (-) indicates no growth on the streak plate, (+) indicates little growth, and (++) indicates a lot of growth.

Part	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Stem	-	+	+	++	++	++	++
Leaf	-	+	++	++	++	++	++
Fruit	-	+	++	++	++	++	++
Seed	-	+	++	++	++	++	++
Methanol (-)	++	++	++	++	++	++	++
Gentamycin (+)	-	-	-	-	-	-	-

Figure 14B: MIC Assay table with *Momordica charantia* parts against *S. aureus.* This table shows the MIC assay done with the methanol extract of *Momordica charantia* against *S. aureus*. The top values represent the compound's dilution amounts, and the side of the table shows the extracted parts. (-) indicates no growth on the streak plate, (+) indicates little growth, and (++) indicates a lot of growth.

Part	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Stem	-	-	+	++	++	++	++
Leaf	-	+	++	++	++	++	++
Fruit	-	+	++	++	++	++	++
Seed	-	+	++	++	++	++	++
Methanol (-)	-	++	++	++	++	++	++
Gentamycin (+)	-	-	-	-	-	-	-

Figure 14C: MIC Assay table with *Momordica charantia* parts against *P. aeruginosa.* This table shows the MIC assay done with the methanol extract of *Momordica charantia* against *P. aeruginosa*. The top values represent the compound's dilution amounts, and the side of the table shows the extracted parts. (-) indicates no growth on the streak plate, (+) indicates little growth, and (++) indicates a lot of growth.

Gram-Staining

The gram-stains of the bacteria were observed at 1000x magnification under an oil immersion lens. The gram stains show that *B. subtilis* is rod-shaped and gram-positive (Table 5). *E. coli* is rod-shaped and gram-negative (Table 5). *S. aureus* is seen to be cocci-shaped and gram-positive (Table 5). *P. aeruginosa* is seen to be rod-shaped and gram-negative (Table 5).

Table 5: Gram-stain results detailing the gram-status, shape, and image of *E. coli, B. subtilis, S. aureus, and P. aeruginosa* at 1000x magnification under an oil immersion lens.

Bacteria	**Gram-status**	**Shape**	**1000x magnification under oil immersion lens**
Escherichia coli	Negative	Rod	
Bacillus subtilis	Positve	Rod	
Staphyloccus aureus	Positive	Cocci	

Pseudomonas aeruginosa	Negative	Rod	

Polymerase Chain Reaction (PCR)/Gel Electrophoresis

A PCR was performed for *E. coli, B. subtilis, S. aureus,* and *P. aeruginosa* to amplify the ribosomal 16s gene. To visualize the DNA fragments, the results of the gel electrophoresis show that *E. coli* or "B," *S. aureus* or "C," and *P. aeruginosa* or "D" have a collection of bands at approximately 4.0-5.0 kilobases (Figure 15A). This also indicates that the DNA masses are approximately 43-33 ng. This uses the ladder or "L" as a reference, where the top of the ladder indicates 10.0 kilobases. For *B. subtilis* or "A," it is approximately 6.0 kilobases (Figure 15B). This indicates that the DNA mass is approximately 42 ng. These values were obtained from the reference ladder provided (Figure 15C).

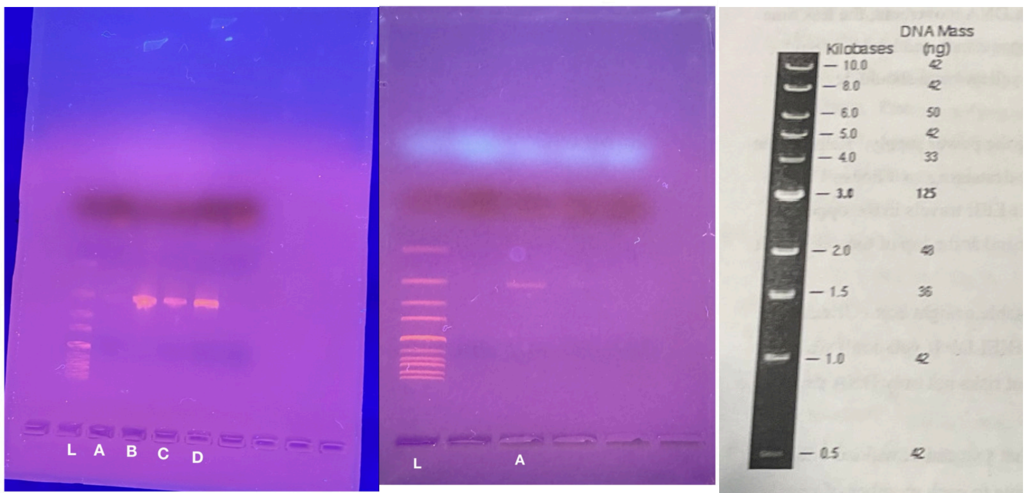

Figure 15A (Left), Figure 15B (Middle), Figure 15C (Right): Gel Electrophoresis of *B. subtilis, E.coli, S. aureus, and P. aeruginosa.* Figure 15A and Figure 15B show the gel electrophoresis bands of PCR samples of bacteria. Figure 15A includes: "L" for the ladder, "A" for B. subtilis, "B" for E. coli, "C" for S. aureus, and "D" for P. aeruginosa. Figure 15B shows "L" for the ladder and "A" for B. subtilis. Figure 15C shows the ladder and is used to analyze the number of kilobases and the DNA mass (ng).

IV. Discussion

The findings from my research have expanded the view of the antibiotic properties of the *Momordica charantia* plant. From the results seen from this study, a 70% ethanol extract of *Momordica charantia* is observed not to exhibit significant antibiotic activity against *B. subtilis, E.coli, S. aureus,* and *P. aeruginosa*. Antibiotic activity is also not observed with the methanol extract against *E.coli, B.subtilis,* and *P. aeruginosa.* However, the leaf, fruit, and seed methanol extract of *Momorodica charantia* is seen to act as a potential antimicrobial agent against *S. aureus.* These results indicate that the fruit (which is traditionally ingested) has the potential to have antibiotic properties, as well as the leaf and seed.
The lack of antibiotic activity may be attributed to the different solvents used to extract the biological compounds from *Momordica charantia*. As seen in the material and methods section, the extraction process was the same for both extract types (either 70% ethanol or 100% methanol).

Similar experiments looking at the antibiotic properties of the *Momordica charantia* plant have been conducted, such as the experiment done by Mada et al. (2013). However, Mada et al. have only investigated the leaves of *Momordica charantia* rather than the other parts that I observed, such as the stem, fruit, and seeds. Their experiment compared the antimicrobial activity and phytochemical screening between the extract of *Momordica charantia* leaves using the solvent ethanol and water. In the study by Mada et al. (2013), the methods were very similar: the plant (leaves) were dehydrated and allowed to soak in a solvent. However, the solvents used were ethanol and distilled water. I did not use water; however, I used methanol and ethanol as solvents. Mada et al. allowed their leaves to soak in water and ethanol in a flask for 48 hours, vigorously shaking it at 3-hour intervals. The samples were filtered and allowed to evaporate using a rotary evaporator. This experiment showed that the antimicrobial activity of the leaves of *Momordica charantia* varied with the ethanol and aqueous extracts against *S. aureus, P. aeruginosa, B. subtilis,* and *E.coli.* The experiment yielded zones of inhibition in the leaves of the *Momordica charantia* plant, which my experiment did not yield using ethanol as a solvent. The variables of the type of solvent,

the location the specimen was obtained, as well as the time of harvesting may have influenced the results from my research.

As one of the things I would do differently with my experiment, I would like to use a different solvent to extract the compounds of the *Momordica charantia* plant, such as water. Water is considered a "universal solvent" due to its ability to dissolve many substances (Sargen, 2019). Biologically, water assists in cell transport and using substances such as oxygen or nutrients (Sargen, 2019). Water is a polar molecule, meaning it has an unequal sharing of electrons. Its polarity allows it to make hydrogen bonds with each other and other polar molecules, which contributes to its ability to dissolve substances. Since most biological molecules have some electrical asymmetry, they are considered too polar. Water can surround polar molecules of another substance and effectively break it apart and dissolve it (Sargen, 2019). It may be more effective in extracting the more water-miscible compounds, such as glycosides, alkaloids, terpenoids, saponins, and carbohydrates (Kumar et al., 2023).

A more concentrated extraction may have been better as well. As seen in the MIC assay (see Results section), the more concentrated the plant extract in nutrient broth was with the antibiotic, the more bacterial growth was inhibited. I would also like to perform a different extraction of the *Momordica charantia* plant parts. Extraction methods such as steam distillation may extract more antibiotic compounds from this plant than maceration extraction. In steam distillation, the plant material within an apparatus is subjected to heat without being soaked in a solvent (Uddin et al., 2023). This results in steam, which allows the breakdown or release of aromatic compounds or essential oils (Uddin et al., 2023).

I would like to see if the location or time I harvested the *Momordia charantia* plant affected the concentration of its antimicrobial properties. This plant is native to the tropics, including East Africa, South America, Asia, the Caribbean, India, and Southeast Asia. However, this plant was harvested in Greensboro, North Carolina. The difference in the environment may factor in the concentration of biological activity in the plant. I harvested the *Momordica charantia* plant when traditionally eaten (see section 5.2.1). There may be a difference in antibiotic activity when the plant is harvested since the stages

are significantly different (see section 5.2.1). In a study looking at the different stages of the crude extract of plant *C. argentea,* the phytochemical constituents and antioxidant properties were investigated (Adegbaju et al., 2020). The study found quantitative differences in the concentrations of phytochemical compounds and antioxidant capacities at different seasons and times of growth (Adegbaju et al., 2020). The best period to harvest the plant for its biological activities is during its flowering stage.

My experiment has also faced a couple of limitations as well. Only three trials were done in triplicate with the ethanol extractions of *Momordica charantia,* and two were done in triplicate for the methanol extracts of the plant. I would have more reliable results if I could perform more trials, but time was limited. Another limitation was the amount of *Momordica charantia* plant material since I only harvested one plant. This has resulted in limited plant material where the fruit had the most supply. Since only one plant was harvested, I can not generalize the population of *Momordica charantia* plants. The concentrations of bioactive compounds may vary depending on the plant harvested. My last limitation includes the plate reader for the MIC assays being unable to work. This has resulted in finding conventional ways to visualize bacterial growth, such as streaking an agar plate (see Methods and Materials). If the plate reader had worked, I would have had more accurate results in determining how much bacteria grew in the MIC assay plates.

This research helps provide more information to those who consume the parts of *Momordica charantia*. Even though minimal antimicrobial activity was observed with the methanol and ethanol extracts of *Momordica charantia*, the results contribute to the plant's research. Phytochemicals make up plants and, when ingested, may impact the composition of the gut microflora by either encouraging or limiting the growth of microbial communities in the gut (Santhiravel et al., 2022). These bioactive compounds can benefit the gut's natural flora due to low absorption and long-time retention in the intestines (Santhiravel et al., 2022). It is seen that the presence of bioactive compounds exhibits prebiotic activities due to their promotion of the abundance of favorable bacteria and the suppression of harmful bacteria (Santhiravel et al., 2022).

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