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Antibacterial Activity of *Moringa oleifera* Plant Extracts in Comparison with Ciprofloxacin Antibiotic Against *Staphylococcus aureus*

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Abstract:

The current drug resistance in human pathogens is a result of the abuse of antibacterial drugs commonly used to treat diseases. Early human civilizations used *Moringa oleifera* extracts to treat illnesses and infections caused by food-borne bacteria such as *Staphylococcus aureus*. In order to calculate the antibacterial effect of *Moringa oleifera* against *Staphylococcus aureus*, methanolic extracts from its three parts were prepared. A photochemical analysis of the methanolic leaves, seeds, and roots extracts was performed when the extracts were ready for testing. We used well-diffusion methods to add the three extracts, and the ciprofloxacin antibiotic was used as the standard. From the stock solution, serial dilutions were made in order to calculate the minimum inhibitory concentration (MIC). In the phytochemical screening test, steroids, terpenoids, tannins, phenolic compounds, saponins, and flavonoids were most abundant in leaves extract, followed by seeds then roots extracts. *Moringa oleifera* seeds have the highest inhibition zone, which is about 10mm, followed by *Moringa oleifera* roots at 9mm, and *Moringa oleifera* leaves at 7mm. In comparison to the other two extracts, the MIC of methanolic extract from *Moringa oleifera* leaves was 250 mm, the highest concentration, with a MIC of 125 mm for roots and 62.50 mm for seeds. Methanolic extracts of Moringa seeds demonstrated antibacterial activity against *Staphylococcus aureus* in the present study. For further studies, it is suggested a deeper investigation to study the antibacterial agent dosages of these plant parts, which may be used by the pharmaceutical industry.

Keywords: *Antibacterial activity, Moringa oleifera, Ciprofloxacin, Staphylococcus aureus.*

Introduction

There have been a number of therapeutic compounds discovered from traditional medicinal plant anti-bacterial screening. For finding new biologically active compounds in the antibiotic field, random screening has proven to be the most effective method (Pavithra and Saravanan 2020). In this study, *Moringa oleifera* leaves, seeds, and roots are tested for their ability to inhibit *Staphylococcus aureus* growth and to be used as an antibiotic against this bacterium in comparison to Ciprofloxacin.

There are a number of phytoconstituents that contribute to the medicinal properties of plants, including phenols, alkaloids, flavonoids, tannins, terpenoids, and steroids. It is expected that the information will contribute to the discovery of novel biologically active chemicals that could be used as lead compounds in the food and pharmaceutical industries (Pavithra & Saravanan, 2020).

Moringa oleifera belongs to the Moringaceae family and is a tropical deciduous plant with long, pendulous fruits and seeds, as well as strong, tuberous roots, light-green leaves, and prolific flowering. Despite its widespread distribution in Madagascar, Madagascar, southwest and northwest Africa, and southwest Asia, northern India is its native region (Asensi et al., 2017).

Among its therapeutic benefits are asthma, epilepsy, eye and skin conditions, fever, and hemorrhoids. The herb has long been used to treat ailments including starvation. It thrives in arid and semi-arid regions and can tolerate extended droughts. The species tolerates soils with a pH range of 4.5 to 8, but neutral or slightly acidic soils are preferable. After just six months, it grows to 4 meters tall, reaching 10 meters in just 20 years (Padilla et al., 2017).

Proteins, sterols, tocopherols, and monounsaturated/saturated fatty acids are abundant in *Moringa oleifera* seeds. *Moringa oleifera* seed oils which can be utilized as a good alternative for non-hydrogenated oils since they are similar in fatty acid composition and physicochemical characteristics to their counterparts, as described in earlier studies (Leone et al., 2016).

There are several virulence factors present in *S. aureus*. In the presence of these elements, the organism is capable of infecting both humans and animals with a variety of illnesses. Infection, tissue invasion, sepsis, and toxin-mediated syndromes are all facilitated by virulence factors. This is what leads to staphylococcal infections that persist despite a strong host immune response (Kim et al., 2016).

It is well known that *Staphylococcus aureus* can develop antibiotic resistance. Most antibiotic-resistant strain infections occur in epidemic waves caused by a single or a small number of successful clones. Fluoroquinolone resistance in *Staphylococcus aureus* is one of the best examples of modern biological evolution. It has been reported that up to 89% of some *S. aureus* isolates are currently resistant to the antibiotic. The first reports of Ciprofloxacin resistance appeared not long after the drug was first used in clinical practice (Diekema et al., 2001).

In this study, the activity of *Moringa oleifera* leaves, seeds and roots extract will be tested against staphylococcus aureus.

Aim of the Study

This study compared *Moringa oleifera* leaves, seeds, and root extracts with Ciprofloxacin against *Staphylococcus aureus*.

Significance of the Study

This research could help in finding new alternatives and natural drugs instead of the current antibiotics, as the bacteria have become resistant to the antibiotic ciprofloxacin. This study is a preliminary investigation to cure diseases caused by *Staphylococcus aureus* that can harm the human body in several ways.

Literature Review

Moringa oleifera

The genus Moringa forms part of the Moringaceae family along with Anoma and Hyperanthera. The family is often referred to as "drumsticks" or "horseradishes." (Pharmacol, 2018). Moringaceae plants contain a wealth of phytochemicals that make them a living nutritive treasure. Seven times the vitamin C of oranges, nine times the protein of yoghurt, ten times the vitamin A of carrots, fifteen times the potassium of bananas, seventeen times the calcium of milk, and twenty-five times the iron of spinach (Rockwood et al., 2013).

Because *Moringa oleifera* is an edible tree, it has a variety of purposes. Its remarkably high nutritional content is another surprise. Figure 1

shows the seeds, roots and leaves of *Moringa oleifera* plant.

Figure 1. *Moringa oleifera* Seeds, Leaves and Roots

The History of *Moringa oleifera*

Moringa has been around since 150 B.C. Ancient kings and queens consumed moringa fruits and leaves to maintain healthy skin and mind. Indian warriors of the Maurian era were fed moringa leaf extract on the battlefield. Warriors were said to experience less stress and anguish during combat when they consumed the Elixir drink. "Alexander the Great" was overthrown by these valiant soldiers (Dhakar et al., 2011).

Plants have always been essential to human survival, regardless of location or age. As dietary, social, cultural, religious, and environmental, as well as human health, they were, are, and will continue to be beneficial (Dhakar et al., 2011).

Distribution and Availability of *Moringa oleifera*

Native to Asia, Africa, and Arabia are the 13 species that make up the genus Moringa (Olson, 2002). *M. oleifera*, sometimes known as Moringa, has so far become the most utilized and researched of all of these species (Leone et al., 2015). Throughout tropical and subtropical parts of the world, *Moringa oleifera* is widely grown. Ancient Romans, Greeks, and Egyptians used it. Several tropical parts of the world have seen widespread naturalization of the Moringa plant (Fahey, 2005) (figure 2).

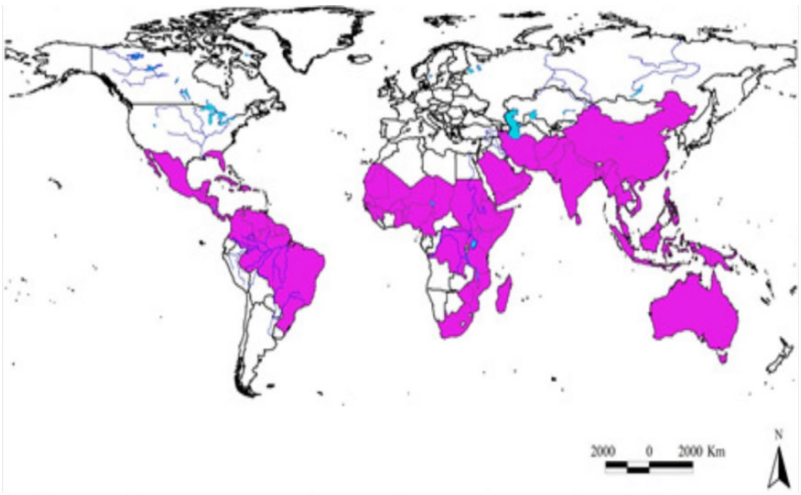

Figure 2. Countries where *Moringa oleifera* has been Recorded as Either Native or Naturalized

Source: Navie and Csurhes (2010)

It has been recorded in many regions of southern and eastern Asia. Naturalization has also been widespread in Africa, particularly sub-Saharan Africa. Moringa has become a native plant in both tropical North and South America. Moringa has also become naturalized on numerous Pacific islands. Because of its widespread naturalization, moringa might be viewed as a plant with a high level of adaptability (Navie and Csurhes, 2010).

Moringa can be regarded as a highly plastic plant with such naturalization in such various environments that it can thrive in hot, semi-arid climates with as little as 500 mm of yearly precipitation. With the help of its robust antioxidant system, it can tolerate moderate saline conditions, with just a minor drop in mineral quality. Moringa thrives in lowland agriculture generally, although it can also be grown at higher elevations (Gandji et al., 2018).

Traditional Uses of *Moringa oleifera*

Asian nations have used *M. oleifera* has as food or a folk remedy for centuries. The plant's edible portions are all nutritive. It has reportedly been used as a nutrition for many years in Africa, India, and Nicaragua during pregnancy and breast-feeding (Chirag et al., 2022).

The plant *Moringa oleifera* is a rich source of trace elements and minerals, as well as a variety of amino acids that are essential to human health. In terms of nutrition, it is the same as spirulina (Chirag et al., 2022).

Nigeria, Pakistan, the Philippines, Hawaii, India, and other Asian and African nations consume the leaves, fruits, blossoms, and immature pods of this tree. *M. oleifera* seeds are eaten raw in Malaysia, whilst young leaves are cooked into spices or used to salads or vegetable curries in other nations (Zahidul et al., 2021).

In addition, *Moringa oleifera* seeds exhibit strong cohesive and antibacterial qualities. They have historically been used to purify water in rural areas of India, the Philippines, Sudan, and Malawi as well as Asia and Africa. Since *Moringa oleifera* Lam and Moringa seed oil produce a large amount of oil, *M. oleifera* seeds are used to make biodiesel. As a result of the large amount of nutrients in it, it is also used as animal feed (Zahidul et al., 2021).

Medical Uses of *Moringa oleifera*

The common name "miracle tree" refers to *Moringa oleifera's* extraordinary healing abilities for a variety of ailments and diseases, including catarrhal affections, asthma, enlarged liver and spleen, deep-seated inflammation, and flu and other viral infections (Julia et al., 2015).

In earlier studies, some bioactive substances from various plant components had been discovered (Ajayi and Fadeyi 2015). According to Atef et al. (2019), *M. oleifera* included phenol and flavonoids with varied contents as a result of the various extraction techniques. According to their findings, the most effective extraction technique involved steeping followed by extraction with 70% ethanol. Adeyemi et al., (2021) conducted further studies demonstrating *Moringa oleifera's* antioxidant properties in vitro and in vivo.

The Phytochemical Characteristics of Different Parts of *Moringa oleifera*

The *Moringa oleifera* tree has special glucosinolates, flavonoids and phenolic acids, carotenoids, tocopherols, polyunsaturated fatty acids, incurably accessible minerals, and folate. In the stem, leaves, flowers, pods, and seeds of *M. oleifera*, (glucomoringin) predominates among glucosinolates, glucotropaeolin, however, is most noticeable in the roots (Amaglo et al., 2010).

The seeds and leaves have the most glucosinolates. Myrosinase, a naturally occurring plant enzyme, breaks down glucosinolates into isothiocyanates, nitriles, and thiocarbamates, all of which have potent hypotensive and spasmolytic properties (Anwar et al., 2007).

Moreover, Indian variants contain higher levels of quercetin and kaempferol than indigenous African samples. *Moringa oleifera's* strong antioxidant properties are due to its high polyphenol content. Seven *Moringa oleifera* cultivars from Pakistan have recently been

studied for their polyphenolic, nutritive, and antioxidant potential. In the hydromethanolic extracts of *Moringa* foliage, quercetin, apigenin, and kaempferol derivatives contributed 47.0, 20.9, and 30.0%, respectively, of the total flavonoids (on average) (Ramesh et al., 2016).

Carotenoids in the foliage, flowers, and immature pods (fruits) of different commercially farmed Indian cultivars of *Moringa oleifera* have been used to identify them. Leaf and immature pod (fruit) carotenoid contents (53.6 and 52.0%, respectively) are dominated by all-E-lutein (Ramesh et al., 2016).

Moringa oleifera leaves also contain omega-3 and omega-6 polyunsaturated fatty acids in the form of -linolenic acid and linoleic acid. With 16–18% of the total fatty acids in the *Moringa* leaves, palmitic acid is the most prevalent saturated fatty acid. In comparison with leaves, immature pods and flowers contain lower levels of polyunsaturated fatty acids (PUFA) and a higher concentration of monounsaturated fatty acids (Saini et al., 2014d).

Seeds and seed oil contain more oleic, palmitoleic, stearic, and arachidic acids than oleic, linoleic, and linolenic acids. Other than linoleic acid, this seed oil has the same fatty acid composition as olive oil. Solvent-assisted extraction with chloroform and methanol in a 3:1 ratio at 100 °C is considered to yield the highest yield of oil from seeds. Due to the residue of these harmful compounds, it is not recommended to consume oil extracted with these solvents (Machado et al., 2015).

Moringa oleifera tissues contain potassium (K), calcium (Ca), and magnesium (Mg). Plant vegetative parts and immature pods contain the most K, but leaves and seeds contain significant amounts of Ca and Mg, respectively. Additionally, Moringa oleifera contains a large amount of iron (Fe). In a bioavailability study on a rat model for treating iron deficiency, *Moringa* leaf Fe was superior to ferric citrate (Amaglo et al., 2010).

The Effects of Moringa oleifera against Different Pathogenic Microorganisms

Moringa oleifera leaf extracts showed variable degrees of antibacterial activity against a variety of microbes, according to (Latifa and Muneera, 2016). In the study, the extract killed the harmful bacteria more effectively than conventional antibiotics. There was a greater likelihood of finding antimicrobial activity in chloroform extracts of the same plants than in petroleum ether extracts. New antibiotic compounds may be found in the plant.

In spite of widespread belief that *Moringa oleifera* is a miracle tree and traditional treatment for many diseases, few studies have been published on the effectiveness of *Moringa oleifera* extract as a natural food preservative with an antimicrobial effect, particularly against foodborne pathogens, that have been published. Adding *Moringa oleifera* leaves extract to ground beef exhibits powerful antibacterial activity against *E. coli*, *Salmonella enterica serovar Typhimurium*, and *Staphylococcus aureus*, three food-borne pathogens.

In a study by Forrit and Saskia, 2022, the extracts were found to have weak antibacterial activity against gram-negative bacteria. There was no indication of inhibition for *Staphyloccus aureus*. When examined by disk diffusion, whole seed extracts showed the greatest efficacy compared to dehusked seed extract. Aqueous extracts had marginally worse antibacterial activity than cold methanol extracts.

A number of clinical bacterial isolates were tested for the antibacterial efficacy of *Moringa oleifera* leaf extracts, and the ethanol extract was found to perform better than the aqueous extract. It has been shown that *Moringa oleifera* can prevent infections caused by the pathogenic bacterium isolates studied (Kingsley et al., 2021).

Moringa oleifera might be an alternative source of antimicrobial compounds to combat pathogenic bacteria, according to the results. More research is needed to determine the best concentration and application method for various bacteria.

Staphylococcus aureus

In gram-positive bacteria, the shape of the *S. aureus* cells is spherical. After gram staining, they

look like clusters of grapes under a light microscope. A scanning electron microscope can reveal cells with a smooth surface and a roughly spherical shape. The name staphylococcus comes from the Greek word staphyle, which means "berry and bunch of grapes." The diameter of the cells varies from 0.5 to 1.0 M. Transmission electron microscopy can reveal cells with robust cell walls, an easily discernible cytoplasmic membrane, and amorphous cytoplasm (Gnanamani et al., 2017) (figure 3).

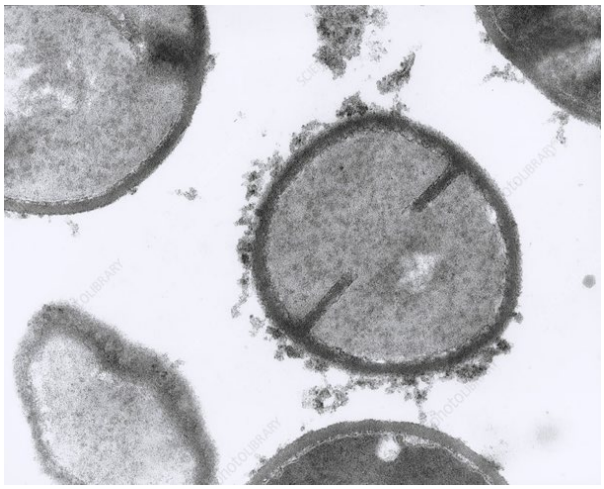

Figure 3. TEM of *Staphylococcus aureus* Bacteria

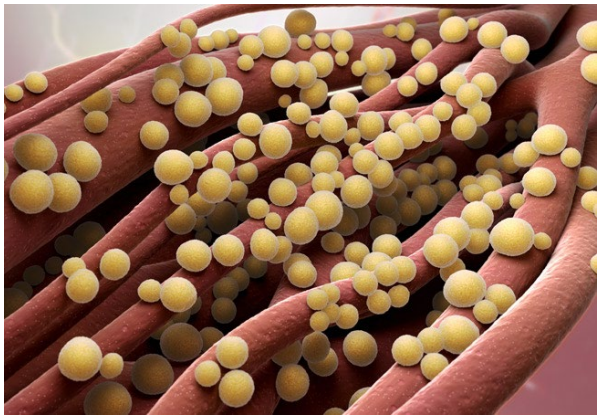

Figure 4. Serious Cause of Bloodstream Infection Associated with Significant Morbidity and Mortality Caused by *S.aureus*

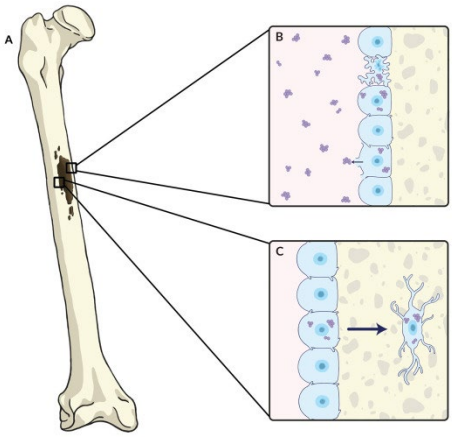

Figure 5. An Umbrella Term for Inflammation Usually Due to *S.aureus* Infection of Bone and/or Joints

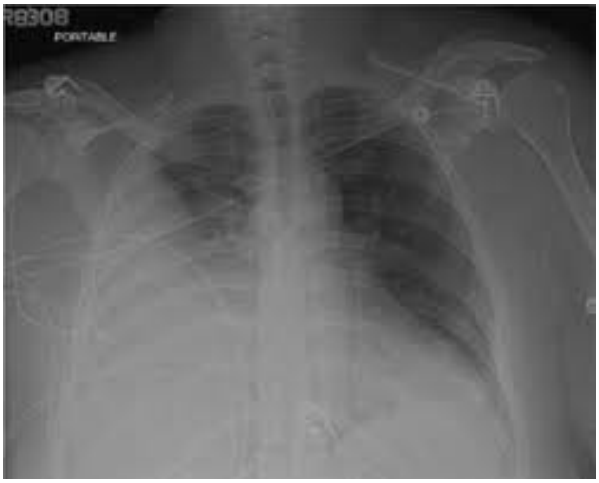

Figure 6. Iinflammation of the Lung Parenchyma Caused by *S.aureus*

S. aureus is a major cause of human infections. Clinical *S. aureus* infections can be classified as nosocomial or community infections. Clinical symptoms, medication susceptibility, and genetic makeup of *S. aureus* strains that are causing them are all different between these two categories. Most *S. aureus* infections are nosocomial, and they have long been a major cause of morbidity and mortality in hospitals. In spite of this, *S. aureus* infections are becoming more common. Some of the important clinical *S. aureus* infections include bacteremia (figure 4) infectious endocarditis, skin and soft tissue

infections, osteoarticular infections (figure 5), and pleuropulmonary infections (figure 6) (Gnanamani et al., 2017).

As shown in figure 7, *S. aureus* infection involves five stages. These include toxinosis, metastatic infections, systemic spread and/or sepsis, local infections, colonization, and local infections. During the carrier stage, the bacterium can remain in the anterior nares for weeks or months without infecting anyone. Several factors can cause the transition from colonization to infection, including prolonged hospitalization, immune suppression, operations, use of invasive medical equipment, and persistent metabolic abnormalities. A localized skin abscess develops when the organism is introduced into the skin from a site of carriage. A variety of clinical signs and symptoms may result, including carbuncles, cellulitis, impetigo bullosa, or wound infections. A bacterium can cause sepsis if it gets into the bloodstream and spreads to other organs throughout the body (Gnanamani et al., 2017).

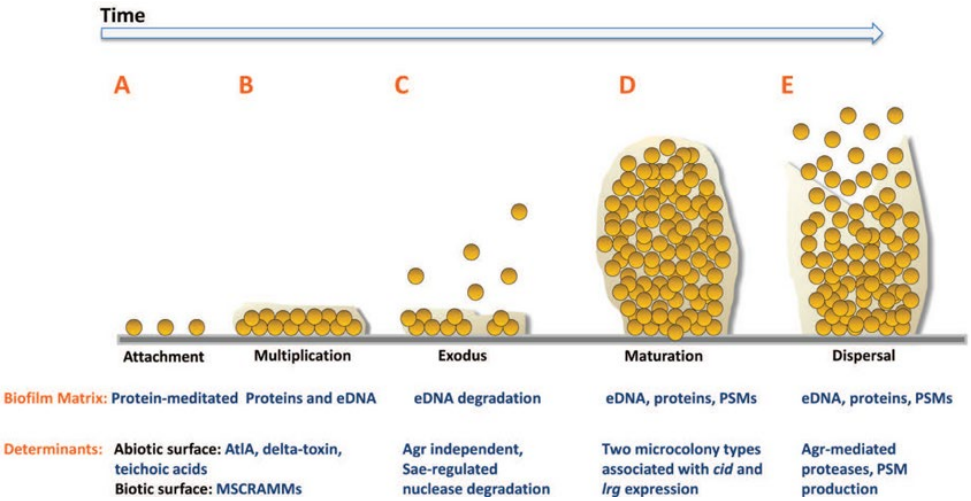

Figure 7. Model of *Staphylococcus aureus* Biofilm Development

The effects of *M. oleifera* Against *S. aureus*

Moringa oleifera's entire plant has an antimicrobial effect. According to a previous study (Zaffer et al., 2014), aqueous extract of *M. oleifera* bark presented high activity against *S. aureus*, while (Devi et al., 2011) demonstrated the antibacterial properties of methanolic *M. oleifera* leaf extract against the bacteria *S. aureus*.

(Devendra et al. 2011) demonstrated that chloroform *Moringa oleifera* leaf extract inhibits the growth of *S. aureus*, with halos of inhibition of 6.2 and 6.0 mm. (Moyo et al., 2012) used water extract of *M. oleifera* and found no antimicrobial activity against *S. aureus*.

Different levels of antibacterial activity were present in the studied microorganisms when *Moringa oleifera* extracts were used. Methanol and ethanol extracts of the same plant had a higher potential to detect antibacterial activity than water extracts did. New antibiotic compounds may be sourced from *Moringa oleifera*. Leaf extract also exhibits significant antioxidant activity in addition to its antibacterial properties. As a result, plants can serve as good sources of antioxidants for therapeutic purposes, such as preventing aging and other diseases caused by free radicals (Kumar et al., 2011).

The broadest spectrum of antibacterial activity was shown by *Moringa oleifera* leaf ethanol (MLE) extract on bacterial isolates. A few people use *Moringa oleifera* to prevent and treat malnutrition. There is evidence that phytochemicals are present in *M. oleifera* leaves (Amabye et al., 2016).

According to a study published by Maurya et al. (2014), gram-negative and gram-positive infections, such as *Klebsiella pneumonia*, *Staphylococcus aureus*, *Escherichia coli*, and

Staphylococcus saprophyticus, may be reduced by *M. oleifera* methanolic leaf extract.

Research Methods and Study Design

This section describes the materials and methods used in the process of extraction three extracts from *Moringa oleifera* tree leaves, seeds, and roots, which include *Moringa oleifera* parts collection and processing, phytochemical assay, bacterial culture, well diffusion method, and minimum inhibitory concentration (MIC)'s method.

Moringa oleifera Parts Collection and Processing

Moringa oleifera is a tree that is native to the Indian subcontinent, but it is now widely cultivated in many tropical and subtropical regions around the world (Satish Patil et Al., 2022). *Moringa oleifera* plants were collected during the fruit production season from Plant nursery which is located in Birkat Al Mouz, Ad dakhiliyah, Oman (figure 9). The samples were collected and directly brought to the laboratory.

Figure 8. *Moringa oleifera* tree

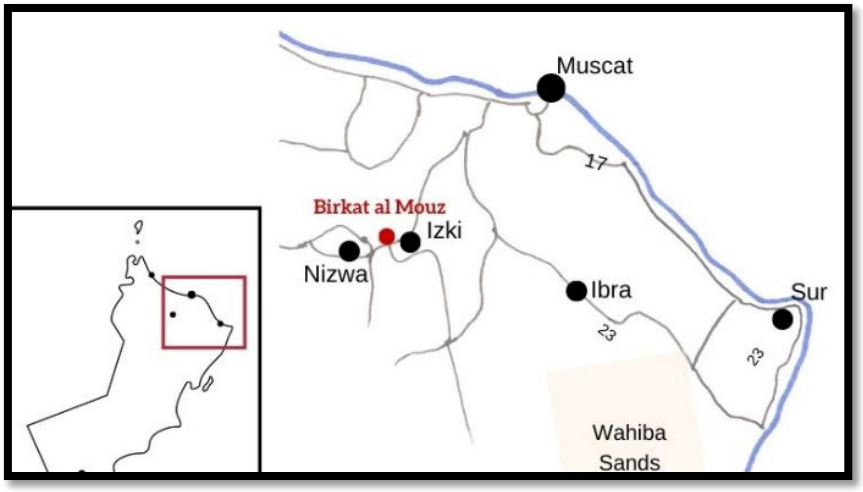

Figure 9. Locations of Birkat Al Mouz, Ad dakhiliyah, Oman

All parts of *Moringa oleifera* tree (leaves, seeds, and roots) were converted into powder by grinding it, then 100g of each one was separated and labeled in a special flask. Initially, powders were added to flasks and 400 ml of methanol was added to each flask (Figure 10). For four to five days, samples were kept on the bench and shaken daily (Figure 11) and sonicated daily (Figure 12). The three samples were filtered after four to five days. Pieces of clothing were used

instead of filter paper for proper filtering of the samples (Figure 13). The methanol from the filtered solution was evaporated with a rotary evaporator (Figure 14), then the extracts were stored in a clean beaker inside the fume hood to ensure that all methanol had been evaporated (Figure 15). Hanaa Elgamily et al. (2016) repeated the first filtration process by adding 100% methanol to the remnants of the previous filtration.

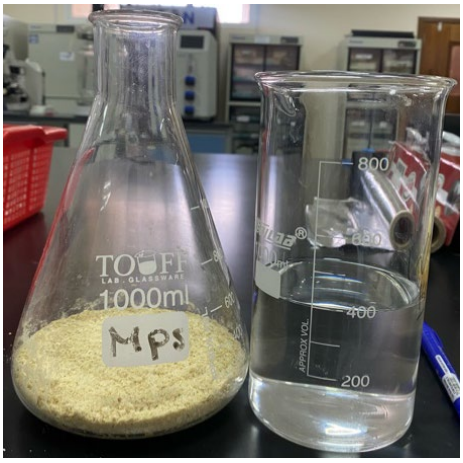

Figure 10. Process of Adding 400ml of Methanol to the Powder of Three Parts

Figure 11. Shaking Process

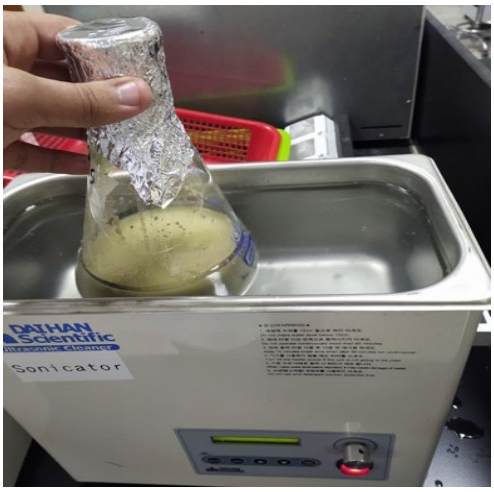

Figure 12. Sonication process

Figure 13. Sonication process

Figure 14. Evaporation Process of Methanol by Rotary Evaporator

Figure 15. Extracts Inside the Fume Hood

Phytochemical Assay

A variety of *Moringa oleifera* parts were subjected to phytochemical analysis.

Tannins

In the ferric chloride test, 0.5 grams of methanolic extract were dissolved in 10 ml of water and then filtered. After that, 10% chloride was added to the filtered material (Harbone, 2001).

Alkaloid

In a conical flask diluted in methanol, 0.2 g of methanolic extract was mixed with 20 ml of sulphuric acid using Dragendorff's test. Then the mixture was filtered and 2 drops of Dragendorff's reagent were added (Ghani, 1998; Harbone, 2001).

Flavonoid

In the ammonium test, 0.2 ml of the extract was mixed with 10 ml of ethyl acetate and heated for four minutes in a water bath. The mixture was cooled followed by filtration (Harbone, 2001; Sofowora, 2005).

Saponnins

Froth test was used, as 0.25 g of the extract was added to 20 ml of water in a 100 ml beaker, followed by boiling then filtering the mixture. 5 ml of filtrate was diluted by adding it to 20 ml of d H2O and gently shaking it (Harbone, 2001; Khalil et al., 2013; Sofowora, 2005).

Steroids/ Triterpenoids

In Liebermann-Burchardt tests, chloroform was mixed with a 1 ml methanolic extract, then allowed to cool. Following by adding 1-3 drops of concentrated sulphuric acid, then shaking it and after that allowing it to stand (Khalil et al., 2013).

Phenolic compounds

Using ferric chloride, 3 ml of methanolic extract was mixed with 1-3 drops of concentrated sulfuric acid, followed by shaking and allowing to stand (Harbone, 2001; Khalil et al., 2013).

Terpenoids

As part of the Salkowski's test, 2 ml of chloroform was used to dissolve 2 ml of the extract, then the extract was allowed to dry. After adding 2 ml of sulphuric acid to the extract, it was boiled for 2 minutes (Kadhim and Al-Shammaa, 2014; Khalil et al., 2013).

Bactria Cultures

A microbiology lab at the University of Nizwa obtained *S.aureus* (ATCC 29213). The bacteria were inculcated in nutrient agar plates and incubated for one day at 28 C. The direct colony suspension method was used to transfer 3-5 similar colonies from fresh NA into a test tube containing 5 ml of normal saline, then the tube was vortexed. To adjust the suspension, the sample must have a turbidity of 0.5 McFarland. Finally, the tube was incubated and after that the colonies were counted and expressed as colony-forming units per milliliter (1.5 × 108 CFU/mL) (figure 16) (Pengov, 2010).

Figure 16. *S. aureus* Broth

Antibacterial Assay

The Mueller Hinton agar medium was prepared by mixing 38g of Mueller Hinton agar powder with 1000ml of distilled water. A magnetic stirrer was used to ensure that all components had dissolved completely. An autoclave was used for 15 minutes at 121 degrees Celsius to sterilize the solution. The solution was poured into a labeled Petri dish then allowed it to solidify before storing it in refrigerator.

Well-diffusion Method

The antibacterial activity was evaluated using the agar diffusions method. 30 ul from each homogenized solution was obtained for further analysis after 0.25 g from each extract was homogenized in 1 mL of distilled water (D.H2O) to create a 1000 ppm solution. On Muller-Hinton agar (MHA) plates (Liofilchem, Italy), the suspension of 0.5 McFarland was inoculated in a constant zigzag pattern using a cotton swap (figure 17). A cork-bor er was used to punch the medium (figure 18), and 30 ul of the appropriate substance was then added. Punches were created to subject the blank and the standard (D.H2O) (Prasad1 and Elumalai 2011).

Both were incubated for 24 hours at 28 °C, and the results were determined by measuring the inhibition zone surrounding the discs in millimeters. The antibacterial efficacy of *Moringa peregrina* and *Moringa oleifera* extracts was examined using *S. aureus* (ATCC 29213, Gram-positive bacteria). For the *S. aureus* strain, ciprofloxacin was employed as a standard.

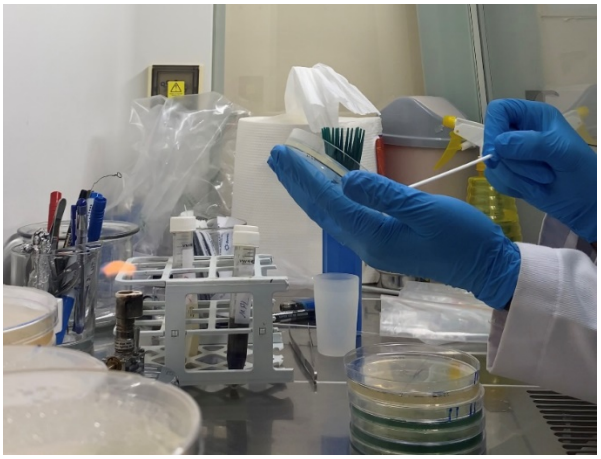

Figure 17. Inoculation of *S. aureus* by Cotton Swap

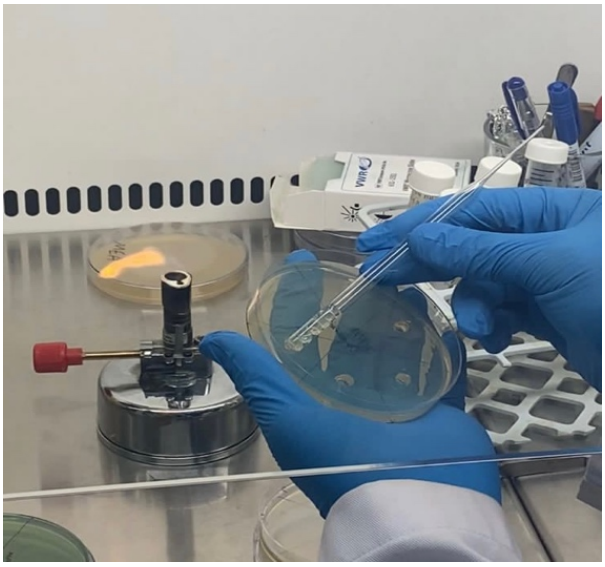

Figure 18. Using of Cork-Borer to Punch the Medium

Minimum Inhibitory Concentration (MIC)'s Method

To determine the minimum inhibitory concentration, serial dilutions were made from stock solutions. From the stock solution, dilutions (12, 14, 1/8, 1/16, 1/32, and /64) were prepared. *S.aureus* was inculcated in plates with

the made of several wells on it. The wells were filled with approximately 30 uL of samples. Incubating the bacteria at 28 C for 24 hours. After that, the sensitivity test was measured using a ruler for the clear zone formed around the wells. Arévalo-Hijar et al. (2018) used equation (1) to determine MIC in mg/mL.

$$MIC = D \times [C] = mg/mL \quad (1)$$

Where:
D = dilution value,
C = starting concentration (stock)

Results and Discussion

This section describes the main research results and discusses the effects of *Moringa oleifera* extract (seed, leaves, and roots) on *Staphylococcus aureus*. Moringa plant parts are collected and prepared, phytochemical screening is performed on sequential extracts of *Moringa oleifera* plant parts, and well-diffusion antibacterial assay.

Collection and Preparation of Moringa Plant's Part

The collection of the *Moringa oleifera* parts had been done from a plant nursery located in Birkat Al Mouz, Ad Dakhiliyah, Oman, and the extractions used were prepared according to the method for preparing *Moringa oleifera* methanolic leaves, seeds, and roots extract that could ensure a proper process of extractions that would then be used in growth inhibitions of Gram positive bacteria (*Staphylococcus aureus*).

Similar methods have been used in previous experiments with the testing of *Moringa oleifera* against several food born bacteria including *S.aureus* (Shaymaa et al., 2021) where they produced good results by collecting seeds and leaves for this study from plantation in Al-Diwaniyah city, Iraq. Biologically, the plant materials were recognized by the College of Science at the University of Baghdad. After being washed, dried, and crushed in a grinder, the leaves were stored at 4°C for further analysis. To shell the seeds, a mortar and pestle were used. Separately crushed to a fine powder, the husk and kernel were kept at 4oC for additional study.

In the study mentioned above, methanolic extract was prepared using Soxhelt equipment. In a thimble, 350 cc of 70% methanol was added to 100 grams of *Moringa oleifera* leaves, and the mixture was kept at 40 to 60°C for six hours. To remove the methanol, the solution was filtered with Whitman No. 1 filter paper and evaporated to dryness under vacuum at 40 °C by a rotary evaporator. The extract was then kept in amber glass vials at 4 °C until it was tested.

Another previous study found similar results with the use of methanol. Emmanuel et al. (2014) used a stoppered glass container, 100g of the blended mixture of husk and seeds was added first, followed by 300 ml of each of the three solvents – distilled methanol, ethyl acetate, and water. In the end, the mixture was left to extract for three days before being filtered. Using a water bath, whole seeds were concentrated in methanol, ethyl acetate, and aqueous extracts.

Rao et al. (2011) focused on the antibacterial properties of methanolic extracts of *Moringa oleifera*. Aerial plant material was gathered in May 2008 from the Andhra University campus in Visakhapatnam and air-dried before being extracted with methanol. The dried extract was redissolved in methanol and filtered after creating a 100 mg/ml solution. Using the well diffusion technique, the extract's antibacterial activity was assessed against specific oral bacteria, including *Staphylococcus aureus*, which showed the main result.

For the above-mentioned scientific paper, a similar method was used to prepare the methanolic *Moringa oleifera* extract.

Phytochemical Screening of Sequential Extract of *M. oleifera* Plant Parts

Phytochemical screening of the sequential extract of *M. oleifera* plant leaves, roots, and seeds shows the presence of various bioactive components such as steroids (Figure 19), terpenoids (Figure 20), tannins (Figure 21), phenolic compounds (Figure 22), saponins (Figure 23) and flavonoids (Figure 24), which are the most prominent and the result of phytochemical screening is presented in Table 1.

Table 1. Phytochemical Screening of Extracts of *M. oleifera* (Leaves, Roots and Seeds)

	Steroids	Terpenoids	Tannins	Phenolic compounds	Saponnins	Flavonoids
MOL	+++	+++	-	+++	++	+++
MOR	+++	+++	+++	-	-	+++
MOS	++	++	++	++	-	+++

Note: MOL refers to *Moringa oleifera* leaves, MOR for *Moringa oleifera* roots and MOS for *Moringa oleifera* seeds

Steroids Test

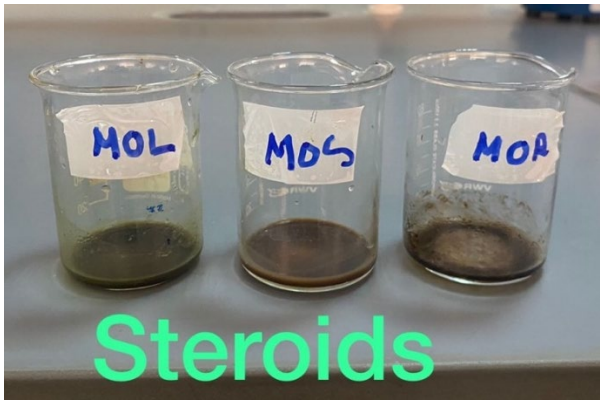

Figure 19. Liebermann-Burchardt Test Used for the Detection of Steroids
Note: MOL = *Moringa oleifera* leaves, MOS = *Moringa oleifera* seeds, MOR = *Moringa oleifera* roots

Tannins

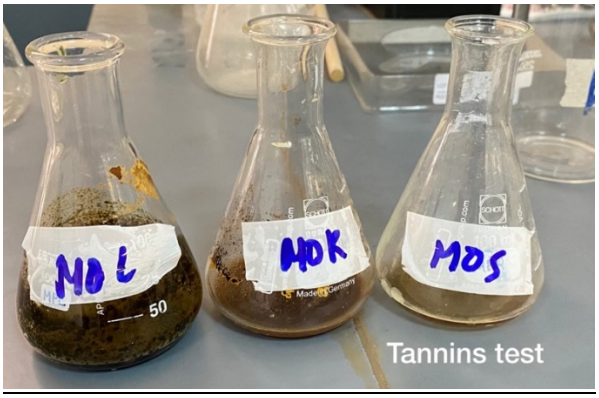

Figure 21. Ferric Chloride Test is Used to Determine the Presence of Phenols
Note: MOL = *Moringa oleifera* leaves, MOS = *Moringa oleifera* seeds, MOR = *Moringa oleifera* roots

Terpenoids

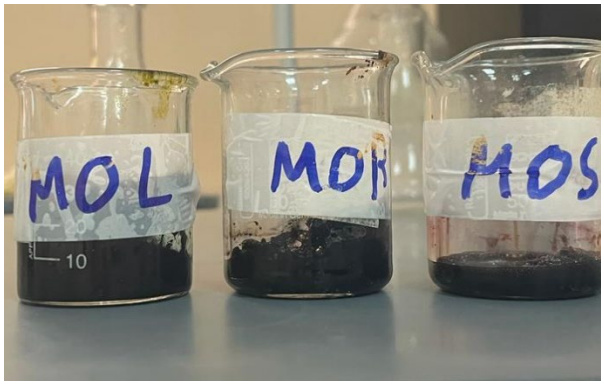

Figure 20. Salkowski's Test was Used to Detect Terpenoids
Note: MOL = *Moringa oleifera* leaves, MOS = *Moringa oleifera* seeds, MOR = *Moringa oleifera* roots

Phenolic compounds

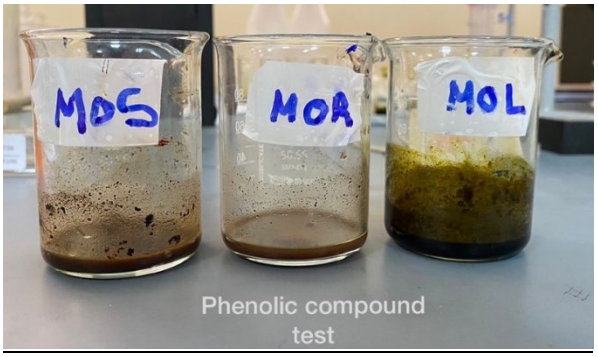

Figure 22. Ferric Chloride Test Used to Determine the Presence of Phenols
Note: MOL = *Moringa oleifera* leaves, MOS = *Moringa oleifera* seeds, MOR = *Moringa oleifera* roots

Saponnins

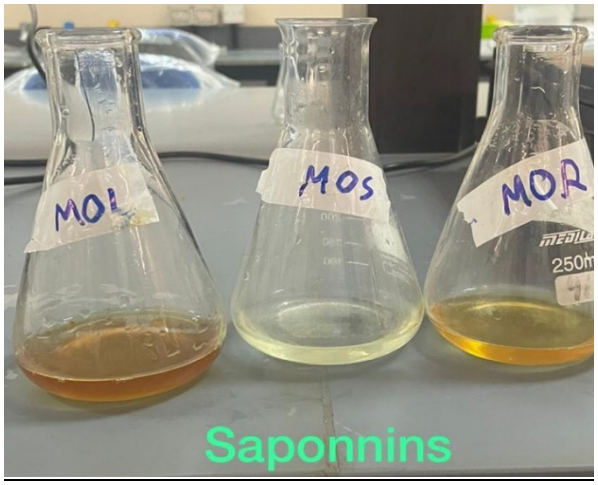

Figure 23. Froth Test for Saponins (Frothing Test)

Note: MOL = *Moringa oleifera* leaves, MOS = *Moringa oleifera* seeds, MOR = *Moringa oleifera* roots

Flavonoids

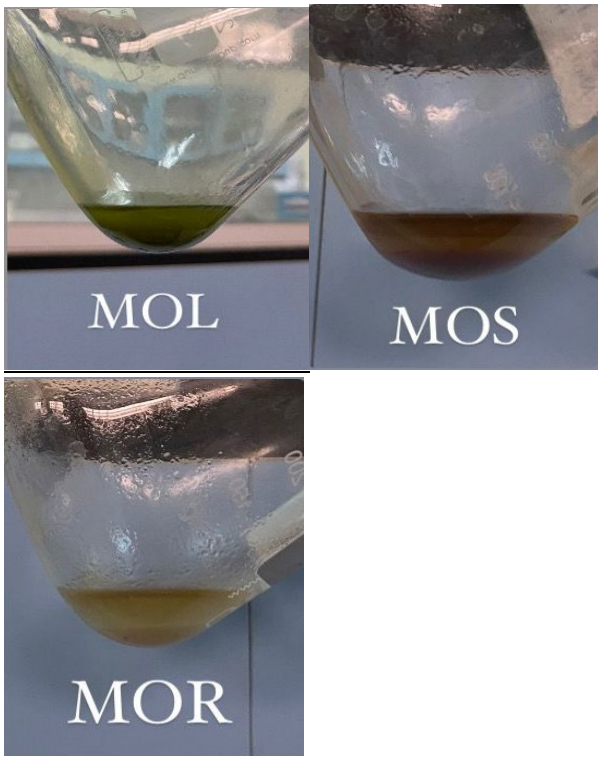

Figure 24. Ammonium Test Used to Detect Flavonoids

Note: MOL = *Moringa oleifera* leaves, MOS = *Moringa oleifera* seeds, MOR = *Moringa oleifera* roots

They all have good antioxidant properties and antibacterial properties against *Staphylococcus aureus*. Table 4 shows the proof of the appearance of these photochemicals in three methanolic extracts (MOL, MOS, and MOR).

In a previous study (Satinder and Tapan, 2018), several phytochemicals were detected using a qualitative phytochemical analysis of *M. oleifera* extracts. Phytochemicals found in *Moringa oleifera* extracts include tannins, flavonoids, glycosides, terpenoids, phenols, and other phytochemicals that give the plant its antibacterial properties.

Ishwor pathak et al. (2020) also did the process of identifying the major class of chemical compounds found in plant extracts is known as phytochemical screening. The freshly generated crude extracts were put through conventional protocols for phytochemical screening (Harborne, 1998). The color reaction with various reagents was used to identify the various phytochemicals that were present in the extracts.

The previous research mentioned above several phytoconstituents were found in the methanol and hexane extracts of *M. oleifera*, according to a phytochemical screening. Both plants' methanol extracts contained alkaloids, avonoids, carbohydrates, terpenoids, polyphenols, glycosides, and coumarins. All extracts included saponin, but none contained volatile oils, quinines, or phytosterols.

S. aureus's cell membrane (figure 25) contains peptidoglycan; this layer is made of a water-soluble polymer, which makes it simpler in favor of polar antibacterial substances like phenolic substances to adhere (Sunarti Sunarti et al., 2022). This explains why *S. aureus* was affected by *Moringa oleifera* extract.

According to Sunarti et al., 2022, terpenoids are secondary metabolites found in Moringa leaves that prevent bacterial growth. This is in line with Retnowati's theory (M. Mazzoniet al., 2021), to which secondary metabolites like terpenoids might reduce bacterial activity. Terpenoid compounds' antibacterial properties work by damaging membranes with the help of lipophilic molecules. Terpenoids have the ability to interact with the porins (transmembrane proteins) that make up the

outer membrane of the bacterial cell wall, forming strong polymeric bonds and damaging the porin. By reducing the permeability of the bacterial cell wall, the bacterial cell becomes starved of nutrients, stops growing, or even dies.

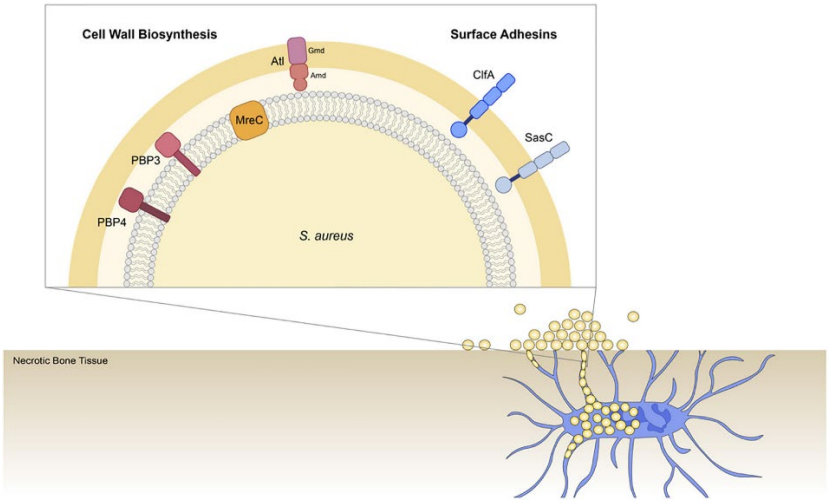

Figure 25. Cell membrane of Staphylococcus aureus

Flavonoids, alkaloids, and phenols can also inhibit the action of bacteria. Plants use the glycoside group, which includes saponin compounds, to store carbohydrates and protect themselves against pests. By lowering the surface tension of the bacterial cell wall, saponin compounds increase the permeability of cell leakage, causing the release of intracellular compounds. Flavonoids perform as antioxidants that can prevent body cells from oxidizing (Sunarti et al., 2022).

Well-diffusion antibacterial assay and Minimum inhibitory concentration (MIC)'s

Table 1 shows that *Moringa oleifera* methanolic crude extract from three parts of the tree (seeds, roots, and leaves) inhibits gram-positive bacteria (*S. aureus*).

Methanolic extracts showed the widest zone of inhibition against *S. aureus* bacteria with 250. Muhammad et al. (2016) determined the MIC of an aqueous *M. oleifera* extract against *S. aureus* to be 6.25 µg/ml which was similar to this research results.

Table.2 Minimum Inhibition Concentration (MIC) Activity of *Moringa oleifera* Methanolic Extracts as Assayed by Well-Diffusion Method and Compared to Ciprofloxacin as a Standard Antibiotic Against *S. aureus* Bacteria

	MOS	MOL	MOR
S. aureus	62.50	250.0	125.0

Malhotra and Tapan (2018) use the agar well diffusion method, the antibacterial activity of ethanolic extract was examined against gram-positive organisms (*S. aureus*). *Moringa oleifera* ethanolic extract demonstrated antibacterial activity. *S. aureus* and *E. coli* both had a 25 mm maximal zone of inhibition.

A similar study found that ethanol leaf extracts were sensitive to *S. aureus* at concentrations of 200 mg/ml, as reported by (Bukar et al., 2010) and (Nepolean et al., 2019). However, (Arzai 2008) reported that S. aureus showed no antimicrobial action at a dosage of 125 mg/ml, but activity at a higher concentration of 250 mg/ml.

According to Table 2, the minimum inhibitory concentration after 24 hours was determined by serial dilution. In comparison with the other two extracts, the MIC of methanolic extract from

Moringa oleifera leaves was 250 mm, followed by 125 mm for *Moringa oleifera* roots and 62.50 mm for seeds. This is also comparable to the pepper established by Jayant et al. (2022) which confirmed that *Moringa oleifera* demonstrated an antibacterial effect against *Staphylococcus aureus* in low concentrations (about 500 µg/ml) especially the *Moringa oleifera* leaves extract.

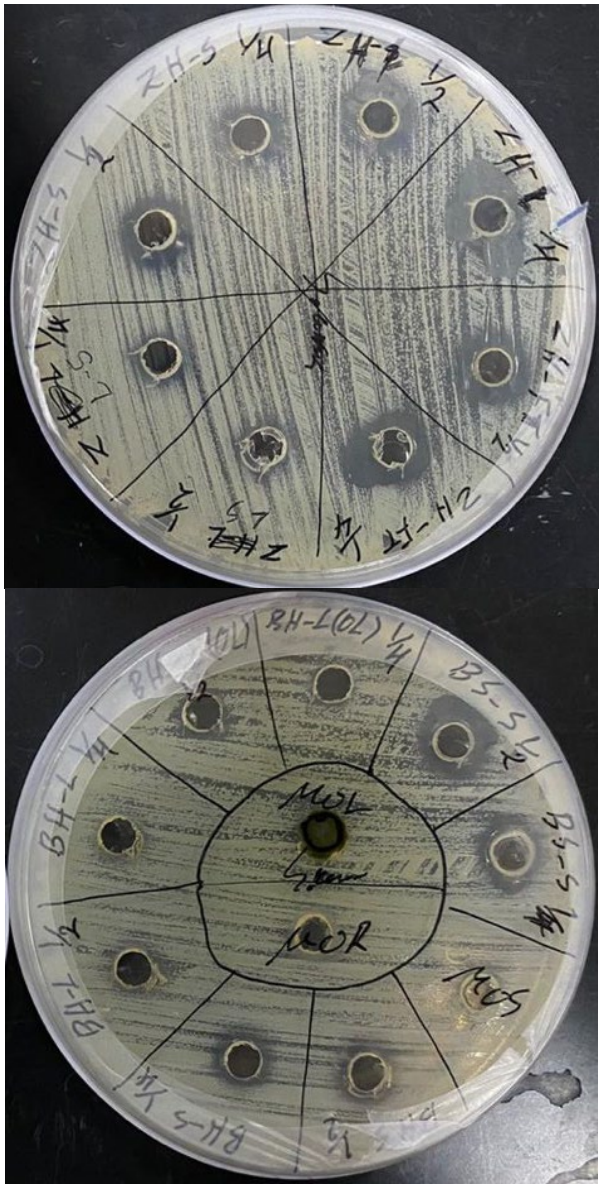

Figure 26. The Inhibitory Effect of Methanolic Crude Extract of *Moringa oleifera* Extracts Against *S. aureus.* Compared to Ciprofloxacin Standard Antibiotics

Table 3. Inhibition Zone Measurement in Serial Dilution for Positive in Screening with *S. aureus*

S. aureus	1/2	1/4	1/8	1/16	1/32	1/64
MOS	9	8	0	0	0	0
MOL	0	0	0	0	0	0
MOR	7	0	0	0	0	0

The three extracts of *Moringa oleifera* has the ability to inhibit the growth of *S.aureus* (Hanaa Elgamily et all., 2016) , in the paper mentioned the inhibition zone of leaves extracts was about 19.25 mm, roots extract was about 9.25mm and finally the seeds extract was 3.25 mm. This research reached a close result, as table 3 shows the inhibition zone of three different extracts when the solvent is a distilled water measured in mm, so that result shows that the methanolic extract of Moringa seeds has the highest inhibition zone, followed by *Moringa oleifera* roots by 9mm, and finally *Moringa oleifera* leaves by 7mm.

Gram-positive bacteria were generally susceptible to *Moringa oleifera* extract. The strongest activity (MIC of 250 mm of *Moringa oleifera* leaves extract) was reported against *Staphylococcus aureus*. Gram-positive bacteria are also susceptible to other extracts in previous studies (Gamal Enan et al., 2020). Researchers studied the antibacterial activity of Moringa seeds and leaves extract against *Staphylococcus aureus*. Against *S. aureus*, inhibition zones reached 47–50 mm, *Moringa oleifera* seeds extract demonstrated the highest inhibitory activity, whereas *Moringa oleifera* leaves extract had a poor inhibitory activity of just 15 mm. In this study they also found that as IZD. In vitro tests against *S. aureus* by *Moringa oleifera* seed methanol extract reached 34-50 mm; and 0–10 mm; 0–18 mm; and 0–20 mm by *Moringa oleifera* seed extract; *Moringa oleifera* seed methanol extract, respectively, water demonstrated the better extraction solvent than either ethanol or methanol. A disc diffusion experiment using Muller Hinton agar was used to determine the MIC value of *Moringa oleifera* seed extract since it had the strongest inhibitory activity against *S. aureus*. In this study, *Moringa seeds* extract is crucial for inhibiting the *S. aureus* bacterium and inhibiting it using a natural substance either by alone or in conjunction with medicines has great promise.

Table 4. Screening of Chemicals (mm) for *S. aureus*

	Standard Ciprofloxacine	Blank D.H2O	MOS	MOL	MOR
S. aureus	30 (NR.>15)	0	10	7	9

Note: NR = Normal range.

Conclusions and Recommendations

Moringa oleifera is relatively safer than synthetic alternatives. Furthermore, it is a rich source of bioactive chemicals that can be used to treat a variety of disorders. *Moringa oleifera* inhibits *Staphylococcus aureus* and other gram-positive bacteria. Extracts of the plant contain steroids, terpenoids, tannins, phenolic compounds, saponins, and flavonoids with antibacterial properties. *Moringa oleifera* also contains antibacterial peptides. The ethanolic extract of *Moringa oleifera* seed extract reduces liver lipid peroxides as well as the antihypertensive substances thiocarbamate and isothiocyanate glycosids. Methanolic extracts from *Moringa oleifera* leaves had a MIC of 250 mm, the highest concentration among the three extracts, with roots and seeds having MICs of 125 mm and 62.50 mm, respectively.

This study compares *Moringa oleifera* extract with ciprofloxacin, a standard antibiotic, to determine whether *Moringa oleifera* extract can inhibit the growth of gram positive bacteria. Based on the results above, *Moringa oleifera* extract inhibits *Staphylococcus aureus* growth like ciprofloxacin.

Moringa oleifera seeds, leaves, and root extracts have antibacterial properties; however, *Moringa oleifera* leaves exhibit extremely weak antibacterial activity. To obtain greater effectiveness against pathogenic bacteria, it is suggested to mix the three extracts from the three parts of the *Moringa oleifera* tree.

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