A COMPARISON OF MORINGA PEREGRINA PLANT EXTRACTS WITH STANDARD ANTIBIOTIC AGAINST ENTEROBACTER HORMAECHEI AND STAPHYLOCOCCUS AUREUS

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Abstract

Pathogenic microbes are increasingly resistant to unnatural antibiotics made by humans, leading to the search for substitute drugs. In this study, Moringa (Moringa peregrina) was tested to see if it had antimicrobial effects on a few commonly occurring bacteria, specifically Staphylococcus aureus and Enterobacter hormaechei. During September 2022 to May 2023, this experiment was conducted at the Department of Science and Arts of the University of Nizwa in collaboration with the Laboratory of Microbiology at the University of Nizwa to determine whether ethanolic extract of Moringa peregrina leaves, roots, and seeds could be effective against Enterobacter hormaechei and Staphylococcus aureus bacteria. A well- diffusion method and minimum inhibitory concentration (MIC) were used to test the antibacterial activity of Ethanolic extract at different concentrations (100, 200, 400, 600, 800, and 1000 g / ml). A dose-dependent inhibitory effect was noted against the test microorganisms. Moringa peregrina leaves showed the greatest inhibition zone against S. aureus. Moringa leaves, roots and seeds were found to have antibacterial effects against gram positive bacteria (S. aureus) in this study. In comparison with roots and seeds, Moringa peregrina leaves have better potential against S. aureus compared with roots and seeds due to their composition of phytochemical compounds. There is a need for further research to isolate and identify the antimicrobial agent in M. peregrina seed oil. A deeper investigation should be conducted into the antibacterial agent dosages of these plant parts, which may be used by the pharmaceutical industry. In continuing studies, I suggested using both ethanol and methanol as solvents and utilizing a variety of bacterial species to get further contrasts.

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1. Introduction and Statememnt of the Problem

Traditional medicines have been based on plants for thousands of years. Historical records and ethnobotanical field studies highlight their importance in the traditional treatment of infectious diseases. Plants, on the other hand, make up only a small percentage of antibacterial drugs approved by the FDA today. The aim of this article is to describe the effects of Moringa peregrina against Staphylococcus aureus and Enterobacter hormaechei as a potential source of antibiotics. Plants have been utilized for millennia to flavor and preserve food, to treat health problems, and to prevent infections. Human networks have communicated information about their mending properties for hundreds of years (Sofowora, 2008; Evans, 2008). Antibiotic resistance has reached dangerously high levels worldwide. New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases. A loss of antibiotic effectiveness makes it increasingly difficult, and in some cases impossible, to treat infections such as pneumonia, tuberculosis, blood poisoning, gonorrhoea, and food-borne illnesses. As a consequence of widespread access to antibiotics, resistance to antibiotics is more likely to emerge and spread. Veterinary professionals and medical professionals frequently overprescribe antibiotics in nations without standardised treatment guidelines (WHO, 2012). Antibiotics, which were once commonly used to treat bacteria, no longer work on some of them. The bacteria that cause gonorrhoea (Neisseria gonorrhoeae) and Staphylococcus aureus (also known as MRSA) are two examples of organisms that are now almost always resistant to benzyl penicillin. These infections were typically treated with penicillin in the past. Antimicrobial resistance occurs when bacteria and fungi acquire the ability to resist drugs intended to kill them. This indicates that the germs remain viable and expand. Treatment of resistant infections can be challenging, and sometimes impossible (World Health Organization, 2016). A variety of infections are caused by Staphylococcus aureus, and antibiotics are used to treat them. Staphylococcal infections are treated with antibiotics that target major bacterial processes like cell wall synthesis, translation, transcription, and DNA synthesis. Despite this, protecting against anti-infection agents is a developing issue, and therapy failures are associated with huge costs. Drug target changes, enzymatic drug inactivation, increased efflux of antimicrobial compounds, and altered drug accessibility are some of the mechanisms through which antibiotic resistance develops (Boyle-Vavra and Daum., 2016). The Moringa Peregrina tree belongs to the Moringaceae family and is deciduous. In addition to its ability to withstand extreme aridity, it is the fastest-growing species of Moringa, reaching heights of 3 to 10 meters. Approximately 30–40 cm long, the leaves are alternate, obovate, and deciduous. When M. Peregrina leaves reach maturity, their leaflets fall off, leaving the leaf rachises naked. There are 18 to 30 cm long, heavily branched axillary inflorescences with axillary panicles. This plant has pentamerous, 10-15 mm long flowers, which are hermaphrodites and zygomorphic, and are pinkish white in color with white sepals. Root tubers develop during the seedling phase of M. Peregrina (Abd El-Wahab, 1995). Moringa peregrina seeds contain a high level of antioxidants. The various plant extracts were also tested for their anti-inflammatory and antioxidant properties in vitro indicate that Moringa peregrina seed oil may possess anticancer and cytotoxic properties against various cancer cell lines-referred to as a "miracle tree" for its antimicrobial, antiviral, cancer-fighting, antioxidant, and immune-modulating properties. Moringa peregrina was used in south Asia to treat gastrointestinal, haematological, cardiovascular, hepato-renal, and inflammation disorders. Moringa peregrina has also been used to treat diabetes and hypertension (Reddy et al., 2015).

Aim of the Study

Our main objective was to evaluate Moringa peregrina extracts' antibacterial activity against Staphylococcus aureus and Enterobacter hormaechei bacteria.

Significance of the Study

The purpose of this study is to find alternative antibiotics because most pathogens microbes have greater resistance to synthetic medical antibiotics due to their ability to adapt to external environments and other effects. Hence, scientists have tried to find other solutions to reduce pathogen resistance by using natural antibiotics that are extracted from plants. Compared to standard antibiotics, each of these components significantly inhibited bacterial growth. At this time, bacteria were able to change their genome and shape until they were able to resist various industrial and even natural antibiotics, as a result, human beings were unable to benefit from these antibiotics, and even with the advancement of science and scientists, it has been proven that repeated intake of antibiotics weakens and destroys human immunity. Different parts of Moringa peregrina have been shown to possess antibacterial properties against positive gram bacteria in the present study. In addition, there are indications that all parts of Moringa peregrina (seeds, leaves, roots) possess several different phytochemicals, giving them the strength to be an important medicinal and commercial plant.

Literature Review and Background of the Study

Moringa peregrina: The genus Moringa contains 13 types of species in tropical and subtropical regions, the only member of the Moringaceae family. There are many uses for the species Moringa peregrina, including traditional, industrial, nutritional, and therapeutic purposes. In traditional medicine, the plant's parts are used to treat a wide range of health problems, such as diabetes, wound healing, disinfection, muscle discomfort, slenderness, burns, labor pain, hypertension, malaria, stomach diseases, asthma, and skin concerns. The Moringa Peregrina also had cultural, spiritual, and religious ties to the native inhabitants of the Arabian Peninsula. Using M. peregrina plant parts, a number of pharmacological properties were assessed, including antioxidant, antimicrobial, anti-diabetic, anti-spasmodic, hypertension, hepatotoxicity, lipid lowering activity, anti-inflammatory, anti-cancer, and memory disorganise. Furthermore, several anti-trypanosomal, antioxidant, anthelmintic, anti-cancer, anti-hypertension, anti-diabetic, anti-infective, anti-allergic, and herbicidal compounds were identified (FAO, 2014). There is a Moringa species with the fastest growth among the rest (ABD EL-Wahab, 1995) which has a height of 3–10 meters and a greyish-green bark that adapts to high levels of aridity. The leaves have an obovate shape, alternate arrangement, and are deciduous. A distinctive characteristic of Moringa peregrina is its axillary inflorescence, which has an 18-30 cm long panicle and heavy branching. When the leaves developed, leaflets fall, went from the leaf rachises naked. Hermaphrodite, zygomorphic, pentamerous, and 10–15 mm long, the flowers have white sepals and a pinkish-white colour. The M. peregrina tree can produce over 1,000 pods per year, ranging from 20 to 40 centimetres in length. Each pod contains eight to fifteen trigonal ovoid seeds (Asharypuor et al., 2010). Another characteristic of Moringa Peregrina is the development of root tubers during the seedling phase (Munyanziza and Yongabi, 2007).

Figure 1: Seeds of Moringa Peregrina

Figure 2: Leaves of Moringa peregrina

Figure 3: Roots of Moringa peregrina

Distribution of Moringa peregrina

The native Arabian Peninsula Moringa Peregrina thrives in challenging environmental conditions (Bellostas et al., 2010). In Eastern India and tropical Africa, the plant is abundant (Sengupta and Gupta, 1970; Al-Kahtani, 1995; Ghahreman, 2010; Singh et al., 2013). The Moringa Peregrina is most prevalent in southern and northern Hijaz (Magadi, 1978) , where it is indigenous and domesticated. It is also flourishing in Baluchistan (Iran), Sistani, and the southeast of the country. Several of the plants are grown in Somalia, Syria, Yemen, and Palestine (Somali et al., 1984).

Traditional uses of Moringa Peregrina

Moringa's medicinal properties were first mentioned in Indian Vedic literature around 5000 years ago (Patwardhan, 2000). A leaf extract from Moringa Peregrina is used topically for the treatment of several diseases, including paralysis and skin rashes (Ghzanafar and Al-Sabahi, 1993). Convulsions and infantile paralysis are treated with pod oil in the northern part of Oman. In the Sultanate of Oman, its seeds are most commonly used to treat diabetes (Al-Kahtani, 1995; Reddy and others, 2015). Diabetes-related symptoms like high blood sugar and cholesterol are also treated with it on the Indian subcontinent. In Arab countries, young leaves of the Moringa Peregrina plant are commonly used as antioxidants and for wound healing. Additionally, the bark liquid is used to treat fever, headache, constipation, back and muscle pain, burns, labour pain, and constipation (Marwah et al., 2007). Seeds are used to treat abdominal pain and leaves to heal wounds. The roots and leaves of Moringa Peregrina are used to treat diabetes, hypertension, malaria, asthma, stomach problems, and placenta retention (Mekonnen et al., 1999). Scabs, itches, and freckles on the skin have traditionally been treated with this plant's oil (Al-Dhaheri, 2016). As well as their nutritional value, Moringa Peregrina has medicinal properties as well. Moringa Peregrina's young leaves can be used such as vegetables (Al-Dhaheri, 2016) . The seeds are blinded with other herbs and used as a food to combat malnutrition (MPCP, 2006). In India and Malawi (Fao, 1998; Elba tren et al., 2005; Afsharypuor et al., 2010), the mature seeds are eaten either fried or roasted. Additionally, Moringa Peregrina is one of the UAE's most important native trees due to its cultural, spiritual, and religious significance (Al-Dhaheri, 2016). Smoked meat (tan-our) is prepared by using the plant's leaves to flavor the meat. This is an age-old custom followed by the native people of the United Arab Emirates.

The medical uses of Moringa peregrina:

Moringa peregrina leaves are used as a poultice for wounds and headaches because they are purgative and stop bleeding. In addition to treating gastric ulcers and diarrhea, Moringa peregrina leaf tea is antibacterial and anti-inflammatory. The bark, leaves, and roots of M. peregrina are bitter and impactful, which is taken to advance assimilation (Hartwell JL, 1967). Most folk remedies for tumors use M. peregrina's roots, leaves, flowers, and seeds. The units are used to deworm, treat liver and spleen issues, and relieve joint pain. Cases are a great source of protein and fiber, so they can satisfy hunger and provide energy. A rubefacient or counter-aggravation is applied remotely using the juice of M. peregrina for dropsy. Additionally, the roots contain emmenagogues, expectorants, mild diuretics, stimulants in paralytic conditions, epilepsy, and hysteria, and bitter compounds that are lung and body tonics. Generally, M.peregrina flower juice can be beneficial for urinary tract problems. It also improves the quantity and quality of mother's milk. The seed oil regards the runs while likewise going about as a diuretic (Täckholm, 1974).

The phytochemical characteristics of different parts of Moringa peregrina

In addition to aggregated data on Moringa parts and bioactive compounds, the contents of these parts may provide health benefits. The most extensive

research on Moringa has been conducted on M. Oleifera, whose polyphenols and unsaturated fats have been assessed from different tree parts. As a result of its nutritional and pharmaceutical properties, M. peregrina has recently emerged as a promising medicinal plant in biochemistry research. Mohammad Pour et al., for instance, calculated the significant anticancer activity of M. peregrina roots extracted and dissolved in various solvents using gas chromatography and mass spectrometry (GC-MS) (Mohammadpour et al., 2019). Each part of Moringa peregrina has a different phytochemical to enhance their ability to be effective to microorganisms. Moringa peregrina roots contain alkaloids, tannins, glycosides, and flavonoids that are useful for medical purposes. Alkaloids may explain the plant's anti-inflammatory, antibacterial, antineoplastic, and palliative effects (Shariff and Pharam, 2001). The Moringa genus has high antioxidant activity due to its abundance of flavonoids. In this genus, glycosides and flavanols are the most abundant flavonoids. The most widely used flavonoids in this genus are rutin, quercetin, rhamnetin, kaempferol, apigenin, and myricetin. The purpose of enhancement research has been to find the most effective way to remove flavonoids from M. peregrina with the best return. Subcritical ethanol extraction yielded 26.7% more flavonoids than reflux extraction (Wang et al., 2017). Flavonoids and other phenolic compounds play an important role in antioxidant activity by preventing oxidative stress (M Rafieian- Kopaei, et al., 2013). Additionally, these compounds have active hydroxyl groups, and the seeds of this plant contain significant amounts of these compounds, which are more effective at neutralizing free radicals. It is possible to create new therapeutic drugs based on a variety of molecule structures found in medicinal plants. Flavonoids and other phenolic compounds in the plant may reduce cancer cases. Moringa species oil and olive oil have been found to have comparable therapeutic effects (G Khodarahmi, 2001). Plants are beneficial when they contain a wide variety of phytoconstituents and the significant gathering of bioactive mixtures present in the plants, like alkaloids, glycosides, flavonoids, proanthocyanidins, tannins, terpenoids, phenylpropanoids, tars, lignans, furocoumarines, naphthodianthrones, proteins, and peptides (Bernhoft, 2010). Glucosinolates are also abundant in all Moringa species. In this species, the most abundant glucosinolate is 4-O-(- L-rhamnopyranosyloxy)- benzyl glucosinolate (30), otherwise called glucomoringin (GMG). In addition, three isomers of 4-O-(-L-acetylrhamnopyrosyloxy)-benzyl glucosinolate (31-33) were distinguished in M. oleifera leaves, contingent upon the development and physiological properties of the leaves (Leone et al., 2015). Moreover, these compounds are important for human nutrition (Asgary, et al., 2011); glycosidic compounds suggest beneficial cardiovascular effects (H Khosravi-Boroujeni, et al., 2012). Tannins, such as the antioxidant compounds mentioned earlier, have also played a significant role in cancer prevention (N Amini-Sarteshnizi, et al., 2015).

The effects of Moringa peregrina against different pathogenic microorganisms:

Worldwide, irresistible illnesses are the leading cause of death. Currently, manufactured anti-microbials are widely used to prevent and treat a few irresistible infections. People are at risk from the unpredictable use of engineered anti-infection agents. as the disease-causing microbes develop multidrug resistance. Increasingly, scientists are turning to drugs derived from plants to treat infectious diseases, which are neither the least nor most toxic. In addition, it may assist in overcoming the development of multidrug resistance. Therefore, concentrates of M. peregrina were read up for antiviral, antibacterial, and antifungal exercises. ((Lin et al., 2018) Moringa peregrina is considered a marvel tree. Extracts and isolated compounds from Moringa peregrine have antimicrobial, antiviral, anticancer, antioxidant, and immunomodulatory properties. On the other hand, Moringa peregrina is generally used as a home cleaning specialist, manure, foliar supplement, green compost, gum, honey, sugar stick juice clarifier, and bio pesticide. For a few issues, the use of healing plants in people medicine has been a profoundly beneficial practice since human culture's birth. Humans only consume a small proportion of plants for food (Heinrich, 2000). Most of them are eventually used for medical purposes. A significant component of sickness treatment in rural regions is the use of healing plants. Almost all populations on the planet rely on natural medicine for their medical needs (Manandhar, 1994). In numerous regions worldwide, medicinal plants have been used extensively throughout human history (Heinrich, 2000). Increasing awareness of the harmful effects of conventional drugs has led to a growing interest in homeopathy and medicinal plants (Uprety et al., 2012).

Overview of Staphylococcus aureus

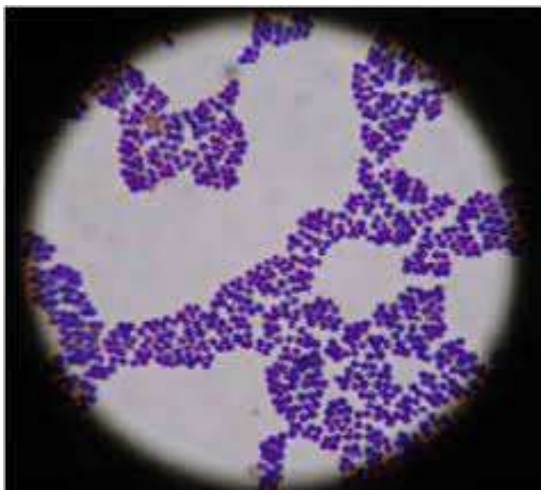
The major human pathogen Staphylococcus aureus causes a wide range of clinical manifestations. Multidrug-resistant strains like MRSA (Methicillin-Resistant Staphylococcus aureus) are making treating community- and hospital-acquired infections more challenging. Staphylococcus aureus can be found in the environment as well as in normal human. On the other hand, these bacteria can cause a variety of potentially life-threatening infections if they are allowed to enter the bloodstream or internal tissues Centers for Disease Control (CDC) and

Prevention. Community-associated methicillin-resistant Staphylococcus aureus skin infections. Most often, they are spread through direct contact.

Etiology of S. aureus

In general, Staphylococcus aureus clusters in the form of grape-like bunches and is a gram-positive microbe (stain purple by Gram stain). These organisms can grow in media with as little as 10% salt, and their colonies are often golden or yellow (aureus means golden or yellow). It is possible for these organisms to grow vigorously or anaerobically (optionally) at temperatures between 18 C and 40 C. Run of the mill biochemical recognizable proof tests incorporate catalase positive (all pathogenic Staphylococcus species), coagulase positive (to recognize Staphylococcus aureus from other Staphylococcus species), novobiocin delicate (to recognize from Staphylococcus saprophytic), and mannitol maturation positive (to recognize from Staphylococcus epidermidis) (Rasigade JP, Vandenesch F., 2014). In Methicillin-Resistant Staphylococcus aureus (MRSA), there is a mec quality on the bacterial chromosome, which is a part of the bigger Staphylococcal chromosomal tape mec district, presenting protection from different anti-toxins relying upon the SCCmec (Staphylococcal chromosomal mec) type. PBP-2a (penicillin-restricting protein 2a) is encoded by the mec quality. PBP-2a is a penicillin-restriction protein (PBP), a fundamental bacterial cell wall chemical, that catalyzes peptidoglycan synthesis. Due to its low affinity for binding to beta-lactams (and other penicillin-derived antibiotics), PBP-2A continues to catalyze the synthesis of bacterial cell walls even in the presence of numerous antibiotics. As a result, S. aureus strains that synthesize PBP-2A are resistant to a wide range of antibiotics. MRSA strains will quite often be impervious to methicillin, nafcillin, oxacillin, and cephalosporins. CDC stands for Centers for Disease Control and Prevention. Outbreaks of community-associated methicillin-resistant Staphylococcus aureus skin infections.

Figure 4: S. aureus under microscope



Epidemiology of S. aureus

People are the major repository of Staphylococcus aureus (including drug-resistant strains like Methicillin-Resistant Staphylococcus aureus) on their skin and mucous membranes. Approximately half of all adults are colonized, and around 15% carry S. aureus in the foremost nares. Several populations will have higher rates of S. aureus colonization (up to 80%), including medical care workers, diabetics, IV drug clients, hospitalized patients, and immunocompromised individual Staphylococcus S. aureus can be sent one individual to the next by direct contact or by fomites (Tong et al., 2015).

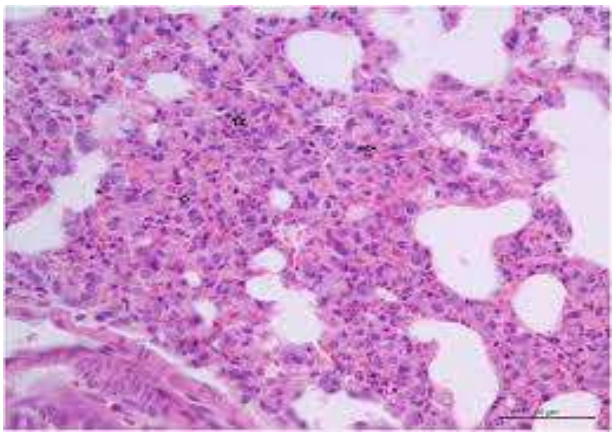
Pathogenicity of S. aureus

In most cases, S. aureus infections come from asymptomatic colonization or, less frequently, from infected fomites or transfers from other people (von Eiff C et al., 2001). There is a relationship between colonization of various body locales and obtrusive contamination (Desai et al., 2011) . Despite the fact that S. aureus can also colonize the intestine, the nares are typically thought to be the primary site of S. aureus colonization. Persistent colonization occurs in only 10 to 30 percent of the population, depending on the study. In general, colonization of various body parts is strongly correlated. The distribution that results from frequent touching and picking one's nose is thought to be the source of this correlation (Acton et al, 2009). In addition to humans, S.aureus can also be acquired from animals, especially in the livestock industry, where the spread of MRSA (LA-MRSA) associated with livestock has been a major concern. In humans, LA-MRSA strains are not a major cause of MRSA infections outside of that setting.

Overview of Enterobacter hormaechei

Enterobacteriaceae, which includes the genus Enterobacter, is mainly associated with illnesses related to healthcare. Enterobacter now has 22 species. However, not all species are considered dangerous to humans. A large number of nosocomial and less frequent community-acquired diseases are caused by Enterobacter species, including urinary tract infections, lung infections, soft tissue infections, osteomyelitis, and endocarditis. Another Enterobacter species can be found on human skin, in water, some foods, soil, and sewage, while another species can be found in the microflora of the gastrointestinal tract of animals (Davin-Regli et al., 1997). Enterobacter hormaechei (E. hormaechei) is an oxidase-negative gram-negative rod species first described in 1989 (Halda-Alija L., et al 2001). There are a variety of environmental niches where E.hormaechei can be found. Nosocomial infections are frequently associated with it (Davin-Regli A., et al 1997), and it rarely causes diseases in animals other than humans. Up to now, only two strains of E. hormaechei associated with illness in home-grown creatures have been accounted for: From a dead fox, one strain of E. hormaechei associated with uterine infection has been identified, and another strain from diarrheal piglets' excrement has been identified (Lu-Yao et al., 2017).

Figure 5: Enterobacter hormaechei under microscope.



In soil, water, and the human gastrointestinal tract, Enterobacter are commensal gram-negative bacteria. They are members of the Enterobacteriaceae family. These organisms aren't extremely dangerous to people, but they are starting to become infections, especially in immunocompromised patients in hospitals. These bacteria can be challenging to treat and are the root of many diseases because of their innate and acquired antibiotic resistance. (Lu-Yao LI et al., 2017). Most of the bacterial isolates found in clinical specimens belong to the Enterobacteriaceae family. The outer membrane of these bacteria contains lipopolysaccharides, which are a major component of lipid-A, which contributes to sepsis. The main trigger for the releasing of cytokines, the mediators of systemic inflammation and its side effects, is lipid-A, also known as endotoxin (Suay-García, 2019).

Enterobacter bacteremia

Intensive care units are most commonly affected by nosocomial (hospital-acquired) Enterobacter bacteremia. The two Enterobacter species that are most frequently connected to cases of Enterobacter bacteremia are Enterobacter Cloacae and Enterobacter hormaechei. (14-53%) A mixed bacteremia is typical. Although the exact point of entry into the bloodstream is frequently unknown, any diseased organ, central line, or arterial catheters may be the main cause of bacteremia. Symptoms of Enterobacter-induced bacteraemia were similar to those of gram-negative bacilli-induced bacteraemia.

Enterobacter pneumonia

Tracheobronchitis, pneumonia, lung abscess, and empyema are among the clinical signs of lower respiratory tract infections caused by Enterobacter. As with other respiratory pathogens, lower respiratory tract infections can lead to cancer, chronic obstructive pulmonary disease, diabetes, alcoholism, and neurologic problems. In the past, antibiotic therapy could have contributed to Enterobacter pneumonia. Enterobacter species frequently much causes ventilator-associated Pneumonia. Enterobacter species are the main culprits behind early post-lung transplant Pneumonia. Bacteria are typically transmitted through the donor.

2. Research Methods and Study Design

Using Moringa peregrina plant extracts as an antibacterial compared to standard antibiotics against Enterobacter hormanechei and Staphylococcus aureus bacteria, this chapter describes the materials and methods used for research on antibacterial activity, including the collection and processing of moringa peregrina parts, phytochemical tests, bacterial cultures, antibacterial tests, well-diffusion tests, and minimum inhibitory concentrations (MIC tests).

Figure 6: Moringa peregrina trees on Sumail's mountains

Figure 7: Moringa peregrina leaves, roots and seeds.

Moringa peregrina parts collection and processing

Moringa Peregrina leaves, roots, and seeds were collected from a nursery in Ad Dakhiliyah (Birkat Almouz and Samail) and Muscat. During the fruit production season, Moringa peregrina plants were collected and stored in a cool, dry shed. First, all parts were ground into powder, weighing 100 grams each (figure 3). As a first step, 400 ml of methanol (100%) was added to a 1L flask with 100 g of each powder sample from roots, seeds or leaves (figure 4). During approximately 4 days, the mixture was shaked and sonicate it every day on the bench. Tthe sample was filtered using cloth of lab coat and funnel (figure 7) according to (ibrahim et al., 2015) then the sample was and evaporated to remove extra methanol by using rotary evaporation (figure 8) and kept in clean inside laminar flow (Figure 9) until the extract was dried enough. Using the same steps as first deep, the sample was dept and kept in the beaker.

Figure 8: Weight 100g of leaves powder

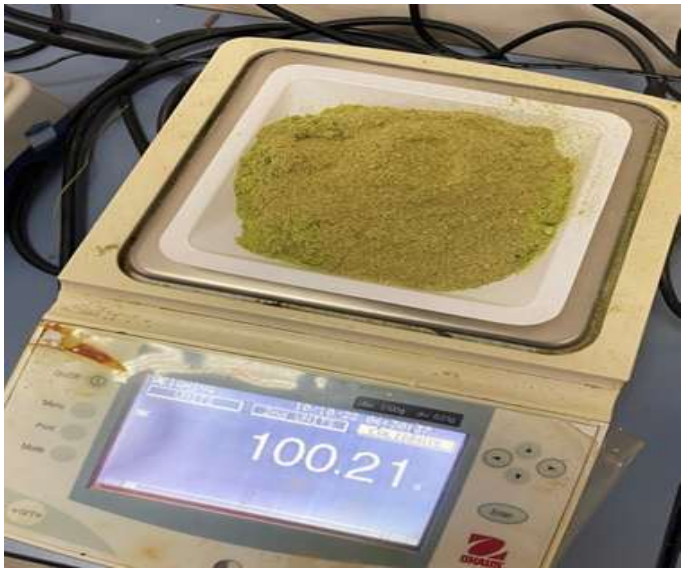
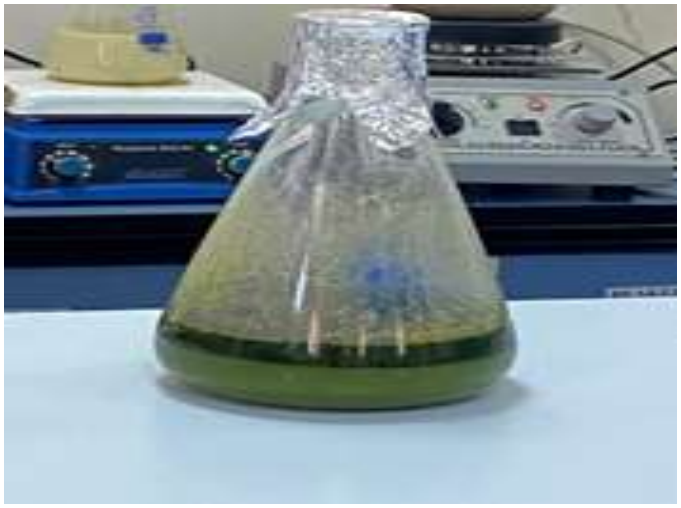


Figure 9: Methanol with 100g of leaves powder.



Figures 10 and 11: using of shaker and sonicator to mix the methanol and different parts of Moringa peregrina powder.

Figure 12: Filter the extract with piece of lab coat

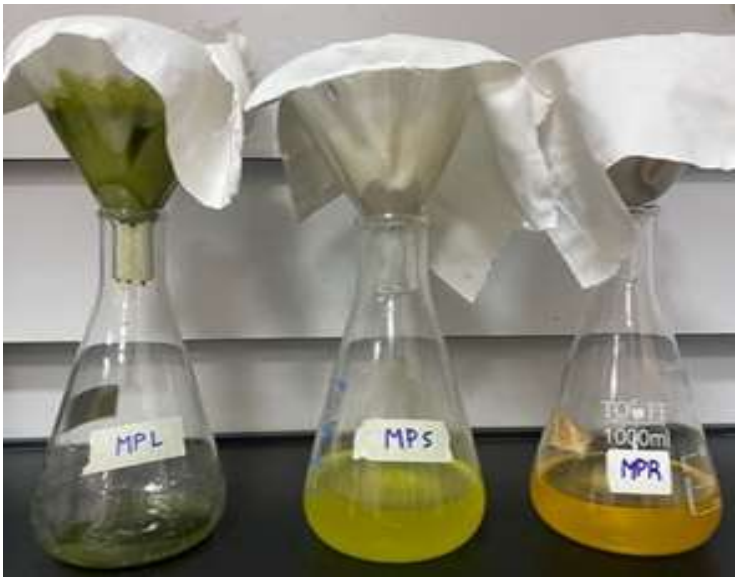


Figure 13: Using rotary evaporator.

Figure 14: The extracts placed in laminar flow.

Phytochemical assay

Different parts of Moringa peregrina (leaves, seeds, and roots) were subjected to phytochemical analysis tests to indicate which phenolic compounds were presented in Moringa peregrina' parts by several changes like, change of color , releasing gas and increasing or decreasing of Temperature directly once mixed the methanolic extract with the chemical compounds

Tannins test

In a ferric chloride test for tannins, 0.5g of methanolic extract was dissolved in 10ml of distilled water , filtered, and 10% ferric chloride added (Harbone, 2001).

Flavonoid test

Ammonium test was used with flavonoid phenolic compounds by adding 10 ml of ethyl acetate to 0.2g of extract then heated in water bath for 4 minutes and shaked in 1ml of diluted ammonium with 4ml of filtrate until the product layers were separated (Harbone, 2001 and Sofowora, 2005).

Saponnins test

Saponins compounds were tested by adding 20ml of water into 0.25g of extracted sample in a 100ml beaker, boiling and filtering, then diluting 5ml of filtrate with 20ml of distilled and shaking it vigorously (Khalil et al., 2013).

Phenolic compound test

In the ferric chloride test for phenolic compounds, 3 drops of 1% ferric chloride were added to 3ml of methanolic extract (Harbone, 2001; Khalil et al., 2013).

Bacterial cultures

The stock for bacterial cultures including Staphylococcus aureus (ATCC 29213), and Enterobacter hormaechei (ATCC 700323), were provided by the microbiology laboratory NMSRC, University of Nizwa, Oman. Bacterial strains were inoculated on nutrient agar (Liofilchem, Teramo, Italy) and incubated for 24 h at 28 °C. Immediate colony suspension was utilised to make the inoculum. Three to five morphology characteristics same to colonies were transferred with using a loop from fresh Nutrient agar in approximately 5ml from normal saline in capped test tube and then vortexes (Mohamed et al. , 2019). The suspension was altered to meet the 0.5 McFarland requirement for turbidity. The colonies were measured and expressed as colony-forming units per milliliter (1.5 108 CFU/mL) at the conclusion of incubation (Figure 10).

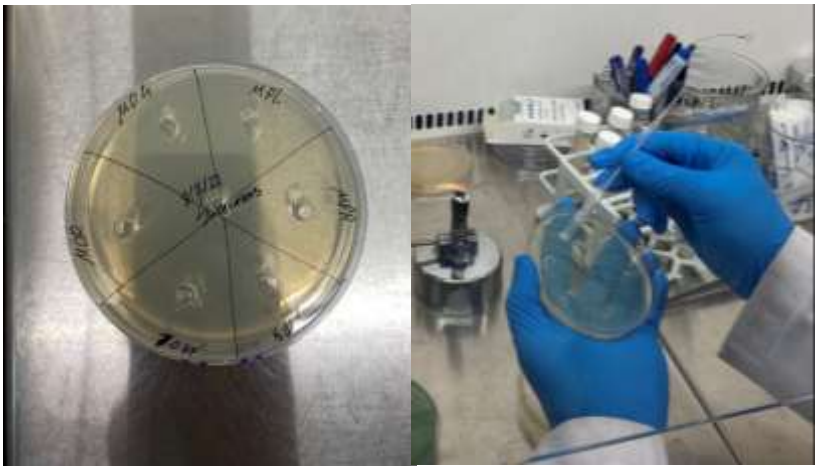
Figure 15: the bacteria culture of S. aureus.

Antibacterial assay

Well-diffusion method: In current research the Mueller Hinton agar media was used. 38 g of Mueller Hinton agarwas weighted and dissolved in 1000 ml of distilled water. The mixture was then mixed until it was completely dissolved. Then should was put it in auto cleave at 121°C for 15 minutes. Next, the media was pour into petri dish then was waited until it completely solidified. The antibacterial activity was evaluated using the agar diffusion method. To create a 1000 ppm solution, 0.25 g of each extract was homogenised in 1 mL of distilled water (D.H2O), and 30 L of each homogenised solution was obtained for additional analysis. Using a cotton swap, 0.5 McFarland suspension was inoculated on Muller-Hinton agar (MHA) plates (Liofilchem, Italy). Using a cork-borer, the medium was punched, and 30 L of the appropriate substance was added (Scott, 1989). The standard and the blank were subjected to punches (D.H2O). At 28 °C, both discs were incubated for 24 hours, and the inhibition zone around each disc was measured (Figure 11). E. hormaechei (ATCC 700323, Gram-negative bacteria for Moringa peregrina, Escherichia coli and S. aureus (ATCC 29213, Gram-positive bacteria) were used to test the

antibacterial activity of Moringa peregrina extracts according to (Dzotam et al., 2015). Ciprofloxacin was used as a standard for the S. aureus strain and Gentamicin was used for the E. hormaechei strain.

Figure 16: The well diffusion method



Minimum inhibitory concentration (MIC)'s method
Moringa peregrina leaves, roots, and seeds were serially diluted to determine the minimum inhibitory concentration (MIC). Each portion was prepared by serial dilution from the stock solutions. From our stock solutions with more dilution with 2 fold dilution factors were made (1/2, 1/4, 1/8, 1/16, 1/32, and 1/64). Each plate is filled with 30 LR of each solution after being inoculated with the exact type of bacteria and ensuring that the diffusion is well done in the plates. Then , plates incubated at 28 °C for 24 h for Enterobacter hormaechei and Staphylococcus aureus . then the clear zones were surrounded wells and discs served as indicators of the degree of sensitive of each the test microorganisms were to the crude extracts and the common antibiotics. The diameter of the inhibitory zones produced around each hole was measured in millimetres, and the inhibition was then quantified as the degree of sensitivity. For calculation the fowling equation was used to take the result of MIC in mg/mL from the final concentration that appeared with a zone of inhibition (Bibitha et al., 2002) .

$$MIC = D \times [C] = mg/mL$$

where D is the dilution value, and [C] is the starting concentration (stock**).**

3. Results and Dissuction

This chapter describes the main research results and compares the antibacterial activity of Moringa peregrina plant extracts with standard antibiotics against Enterobacter hormaechei and Staphylococcus aureus bacteria. It consists of collection and preparation of Moringa Peregrina plant parts, phytochemical screening of sequential extracts of Moringa Peregrina plant parts, Well-diffusion antibacterial assay, and Minimum inhibitory concentrations (MICs).

Collection and preparation of Moringa plant's parts
The Moringa Peregrina parts were collected from several nurseries, which are in Ad Dakhiliyah, Birkat Almouz, and Muscat thrives in challenging environments, and the extractions used were prepared according to the method The study was conducted using ethanol solvent and distilled water to ensure the results, which confirmed Moringa peregrina has antibacterial properties against several bacteria in its leaves, roots, and seeds.

Phytochemical screening of sequential extract of Moringa Peregrina plant parts.
Phytochemical screening of the sequential extract of Moringa Peregrina plant leaves, roots and seeds show the presence of various bioactive components which are phenol, alkaloid, flavonoids and tannin are the most prominent and the result of phytochemical tests presented in Table 1. There was a significant difference in phenolic compounds between the various desired parts of Moringa peregrina, based on their structure on their cell walls as well as their types. In prior studies (Cia et al., 2004) found that leaves of moringa in general has a high bioactive compound which consider as high anticancer and have high potential source in medical field , as shown by the current study research, leaves contain more phenolic compounds than seeds and roots. According to figure 17, phenolic compounds are tested with ferric acid and the results are seen in all desired parts of M. peregrina. The flavonoid compound results are shown in figure 18,19 for leaves, seeds, and roots of M. peregrina. Figure 20 shows a Saponins compound in just the root of M. Peregrina which was used as froth test. With tannins test in figure 21, the color immediately becomes darker than normal, so Moringa

Peregrina contains tannins. As the result showed the seeds of Moringa Peregrina have more bioactive compound (Phenolic compound) , as Guevara et al (1999) suggested that the antimicrobial activity of Moringa peregrina seed is due to the presence of an array of phytochemicals, but most importantly due to the activity of a short polypeptide named 4 (- a - L-rhamnosyloxy) benzyl isothiocyanate . As a result of disrupting cell membrane synthesis or synthesis of essential enzymes, this peptide may directly inhibit microorganism growth (Suarez et al., 2003).

Table 1. Phytochemical Screening of Extracts of M. peregrina (leaves, roots and seeds)

	MPL	MPR	MPS
Phenolic compound	-	+	++
Flavonoids	+	+	+
saponnins	+	-	-
Ferric acid	+	+	+

(MPL) Moringa peregrina leaves, (MPR) Moringa peregrina roots , (MPS) Moringa peregrina seeds

Figure 17: Ferric chloride test indicate for phenolic compound.

Figures 18 and 19: Ammonium test for flavonoids

Figure 20: Froth test indicates saponins compound.

Figure 21: The ferric chloride test indicates the presence of tannins

Well-diffusion antibacterial assay

In the well-diffusion test, Moringa Peregrina crude extract inhibited gram-negative bacteria (E. hormaechei) and gram-positive bacteria (Staphylococcus aureus) indicated an inhibition of S. aureus but there is no visible inhibition in E. hormaechei. Figure 6 shows an inhibition zone in S. aureus when use a different concentration of ethanolic extract. But in other hand, there is no effect against E. hormaechei (Figure7). Despite the fact that (Ibrahim et al., 2015) found that both positive and negative bacteria were effective against ethanolic extract of M. peregrina unlike with research result which are showed that S. aureus is gram positive bacteria had be more antibacterial than E. hormaechei which is gram negative bacteria. As my results showed, (Tajbakhsh et al., 2008, and 2011) found that the gram positive bacteria have more antibacterial effectiveness than gram negative bacteria. Also, (Nikaido.et al., 2008) reported that this difference in their susceptibility might be due to their outer membranes and lipopolysaccharide contents, which make them easier to penetrate than gram positive bacteria.

A zone of inhibition in S. aureus and E. hormaechei growth caused by antibacterial ethanolic extract of Moringa Peregrina roots, leaves, and seeds, as shown in table 1. Aureus against moringa peregrina higher than E. hormaechei. Moringa peregrina leaves are more effective than seeds and roots; however, roots have a slightly lower effect than leaves, and this result is likely similar to the result of(Ibrahim et al., 2015) who explained that the extract of root more antibacterial than leaf and seed . While Moringa Peregrina leaf and roots contain more chemicals than seeds, which makes them more toxic to bacteria, S. aureus bacteria as result showed is more sensitive to this chemicals. According to the results showed in Table 2, the measurement of inhibition zone in serial dilution for positive screening with S. aureus, as the result appear that there is only effect on growth with leaves of M. peregrina in about 7 mm in dilution factor 1/2 . Moringa peregrina leaves extracts were more effective than other parts.

Table 2: The screening of chemicals for S. aureus and E. hormaechei.

	Standard	**Blank D.H2O**	**MPS**	**MPL**	**MPR**
S. aureus	30 (NR. >20)	0	0	10	9
E. hormaechei	20 (NR. >15)	0	0	0	0

Figure 22: The inhibition zone of S. aureus with different concentration with different parts of Moringa peregrina and Moringa oleifera.

Figure 23: The inhibition zone of E. hormaechei with concentration of D.H2O with different moringa peregrina leaves, roots, and seeds.

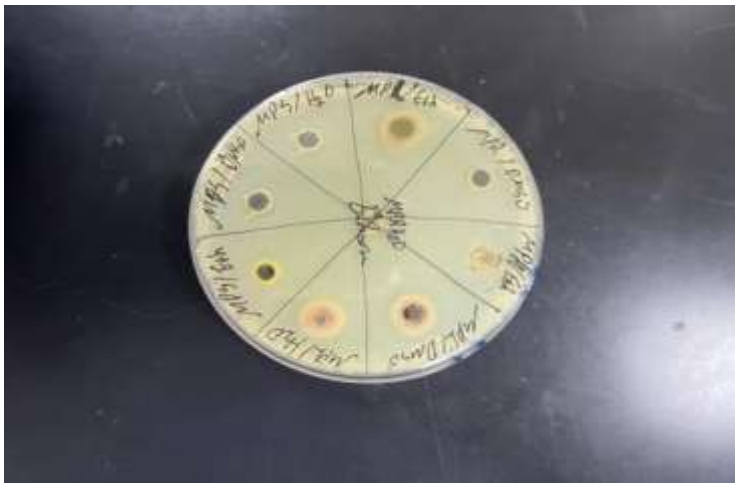


Table 3: Minimum inhibition concentration (MIC) activity of Moringa Peregrina roots, seeds and leaves extracts as assayed by well-diffusion method and compared to standard antibiotic Ciprofloxacin against S. aureus and Gentamicin against E. hormaechei.

Type of antibacterial substance	mg/ml	Staphylococcus aureus	E. hormaechei
MPL	1/2	7	0
	1/4	0	0
	1/8	0	0
	1/16	0	0
	1/32	0	0

	1/64	0	0
MPR	1/2	0	0
	1/4	0	0
	1/8	0	0
	1/16	0	0
	1/32	0	0
	1/64	0	0
Antibiotics	Ciprofloxacin	30 (NR. >20)	-
	gentamicin	-	20 (NR. >15)

An inhibition zone of 7 mm was observed in the leaf extracts of Moringa peregrina against S. aureus, but not against E. hormaechei, whereas root and seed extracts had no effect. Gram positive bacteria (S. aureus) were more sensitive than Gram-negative bacteria (E. hormaechei) (Table 1 and Fig 22 and 23). Overall, Gram-positive organisms were more susceptible to the extract from M. peregrina leaves compared to Gram-negative organisms. Moringa peregrina leaves extract showed the strongest activity against Staphylococcus aureus. Some previous studies have also reported higher susceptibility of Gram-positive bacteria to other extracts (Tajbakhsh et al., 2008, and 2011). According to such studies, gram-negative bacteria are less susceptible to antibacterial substances due to their outer membrane and lipopolysaccharide molecules, which provide a barrier against antimicrobial molecules' easy penetration. Tajbakhsh et al (2011) investigated the in vitro antibacterial activity of Moringa peregrina against Gram-positive bacteria. Moringa peregrina leaves have antibacterial properties. Singh et al (2011) investigated the antibacterial effect of alkaloid rich Moringa peregrina fractions obtained from different parts of the plant including the leaves, seeds, and roots. Other parts, such as pods and flowers, also showed antibacterial activity with the potential to inhibit antibiotic-resistant bacteria strains, according to their study. Stem and root extracts did not show a zone of inhibition against any of the tested bacteria (Singh et al., 2011).

Minimum inhibitory concentration (MIC)'

Table 4 Minimum inhibition concentration (MIC) activity of Moringa peregrina leaf, root and seed extracts as assayed by well-diffusion method and compared to different standard antibiotics against S. aureus pathogenic bacteria.

	MPL (mg/ml)	MPR (mg ml)
S. aureus	125.0	250.0

(MPR) Moringa peregrina root, (MPL) Moringa Peregrina leaf soaked against S. aureus

The other major test conducted to investigate the antibacterial effect of Moringa Peregrina ethanoic extracts was the Minimum inhibitory concentration. On the same two bacterial species, the MIC for Moringa Peregrina roots was higher than those for Moringa Peregrina leaves, which have stronger potential for 250 mg/ml (Ibrahim et al., 2015), who found that Moringa Peregrina leaves has higher MIC there is an effect with minimum inhibitory concentration in Moringa peregrina leaf and roots against S. aureus. Previously, similar methods have been used to study the effects of short-term application of ethanolic extracts from different parts of M. peregrina on two types of bacteria. Previous studies demonstrated high prevalence of active antibacterial substances in where they produced good results about this antibacterial in moringa peregrina which they found that the roots of Moringa peregrina have more effective than other parts and but there is an effect of leaves than seeds (Majali. et al., 2015). Based on the present study, leaves have more effective antibacterial properties than roots and seeds, and there is a greater effect on positive bacteria than on negative bacteria.

4. Conclusion and Recommendations

It can be concluded that Moringa peregrina leaves, roots, and seeds extracts have antibacterial properties; however, the seeds extract has a very weak antibacterial effect. When treated with ethanol extract of Moringa peregrina leaves, standard strains and clinical isolates showed varying degrees of antibacterial activity. In this study, ethanolic extracts of Moringa Peregrina were found to be more effective against Gram-positive bacteria (S. aureus). A special antibacterial characteristic of the plant under investigation may make it as effective as an antibacterial in the medical field. Therefore, this plant demonstrated intriguing biological activity, which might serve as a solid basis for more research into creating naturally occurring bioactive

chemicals. Further investigation is recommended for this attitude in order to determine whether it can be used in medicine. It is essential that further research is conducted to isolate and identify the antimicrobial agent found in M. peregrina seed oil. This plant part should be studied in-depth for antibacterial agents' dosages, which may be used in pharmaceuticals. To obtain stronger extracts, I recommended using both ethanol and methanol as solvents and using multiple strains of bacteria to obtain further differences in results.

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