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Antifungal Activity of *Moringa peregrina* Plant Extracts Against *Candida kruzei*

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Abstract:

Commercial antibacterial drugs commonly used to treat diseases have led to the current drug resistance in humans. Early human civilizations used *Moringa peregrina* extracts against a variety of illnesses and infections caused by food. *M. Peregrina* grows well in a variety of harsh conditions, including high temperatures, limited water supply, and nutrient-deficient soils. There is something remarkable about this plant's resilience and ability to survive in challenging environments. To conserve water and withstand extreme drought conditions, it has evolved unique adaptations. *M. peregrina*'s deciduous leaves allow it to shed its leaves during dry periods to reduce water loss through transpiration. As a result of this adaptation, it is able to endure prolonged periods of water scarcity. A serious infection can be caused by *Candida kruzei*, an opportunistic fungal pathogen that is especially dangerous to immuno-compromised individuals, and the increasing drug-resistance of several *Candida* strains have necessitated the search for alternative to standard anti-fungal agents to which resistance has grown. The objective of this study was to investigate the antifungal effect of *M. peregrina* ethanolic extract derived from its leaves, seeds, and roots against *C. kruzei*. The phytochemical screening of *M. peregrina* extracts were performed using qualitative determination whilst the antifungal activity of methanol and water extracts of leaves, seeds, and roots was performed using the agar diffusion method. The results of the phytochemical analysis demonstrated the presence of phenolic compounds, steroids, flavonoids, tannins, and saponins. The studied extracts displayed various degrees of antifungal activities against *C. kruzei*. The extract of the leaves was active against *C. kruzei* with recorded minimal inhibitory concentration (MIC) of 10mg/ml. There is a need for further research to isolate and identify the antimicrobial agent in different parts of *M. peregrina*. A deeper investigation should be conducted into the antibacterial agent dosages of these plant parts, which may then be used by the pharmaceutical industry.

Keywords: *drug resistance, antifungal activity, Moringa peregrina extract, pathogens.*

Introduction and Statement of the Problem

The *M. peregrina* plant belongs to the *Moringaceae* family and has been traditionally used in a variety of medicinal practices for centuries. The compounds it contains have been discovered to possess antifungal properties, among other potential therapeutic applications.

In immuno-compromised individuals, *Candida kruzei* can cause severe infections. It is an opportunistic fungal pathogen. As drug-resistant *Candida* strains have become more prevalent, alternative antifungal agents have been explored. In this regard, *M. peregrina* has emerged as a potential natural source of antifungal compounds.

Several studies have investigated the antimicrobial properties of *M. peregrina* leaves. *M. peregrina* leaf extracts have been tested against various *Candida* species, including *C. kruzei*. According to Mahomoodally et al. (2018), *M. peregrina* leaf ethanolic extract demonstrated significant antifungal activity against *C. kruzei*, as shown by the inhibition of fungal growth and the reduction of colony forming units. Leaf extracts have been reported to possess antifungal properties due to their high content of phenolic compounds, flavonoids, and alkaloids.

Additionally, the seeds of *M. peregrina* have been investigated for their antifungal properties. Studies have shown that *M. peregrina* seed extracts inhibit *Candida* species, including *C. kruzei*. According to Oliveira et al. (2019), *M. peregrina* seed ethanolic extract demonstrated significant antifungal activity against *C. kruzei*, with a notable decrease in fungal growth. Bioactive compounds such as phenolic acids, flavonoids, and saponins were credited with the antifungal effect. The compounds disrupt the integrity of the fungal cell membrane, causing the cell to die.

M. peregrina roots have also been studied for their antifungal properties. Da Silva et al. (2020) investigated the antifungal activity of *M. peregrina* root ethanolic extract against *Candida* species, including *C. kruzei*. *M. peregrina* root extract inhibited fungal growth significantly, highlighting its potential as an antifungal agent. According to the study, bioactive compounds like alkaloids and terpenoids, which have antimicrobial properties, could be responsible for the antifungal activity.

M. peregrina's antifungal activity against *C. kruzei* is attributed to its diverse bioactive compounds, including phenolic compounds, flavonoids, alkaloids, phenolic acids, saponins, and terpenoids. Fungal growth and proliferation are inhibited by these compounds, which interfere with fungal cell structure and metabolism. Further research is needed to identify specific active compounds responsible for *M. peregrina*'s antifungal activity against *C. kruzei*.

The leaves, seeds, and roots of *M. peregrina* have shown promising antifungal activity against *C. kruzei*. These plant parts contain diverse bioactive compounds that make them potential sources of antifungal agents. Researchers should explore the mechanisms of action, evaluate efficacy in vivo and clinical settings, and determine the safety profiles of *M. peregrina* extracts as antifungal treatments. *M. peregrina*'s antifungal potential could offer new therapeutic options for treating *Candida* infections, including drug-resistant strains, and address antifungal treatment concerns.

Aim of the Study

The purpose of this study is to investigate the antifungal effects of *M. peregrina* plant grown in the environment and condition of Oman, by conducting ethanolic extract derived from its leaves, seeds, and roots against *C. kruzei*. As part of the study, *M. peregrina* will be evaluated as a natural therapeutic agent against *C. kruzei* infections, contributing to the development of alternative treatments for drug-resistant fungal pathogens.

Significance of the Study

M. peregrina's potential as a natural antifungal agent against *C. kruzei*. Traditional medicinal systems have used *M. peregrina* for centuries, suggesting its therapeutic potential. The antifungal properties of *M. peregrina* ethanolic extract can provide valuable insights into its effectiveness against *C. kruzei* and contribute to the development of alternative treatments for drug-resistant *Candida* infections.

Literature Review / Background of the Study

Moringa peregrina

History of *Moringa peregrina*

Known as "wild moringa" or "desert moringa," *M. peregrina* has a long history interconnected with ancient civilizations and traditional medicinal practices. Known for its resilience and adaptability to arid environments, this plant is a valuable resource in regions with limited water and resources (Senthilkumar et al., 2018). Ancient populations in Arabian Peninsula, North Africa, and other arid regions have used *M. peregrina* since ancient times. In addition to providing valuable sustenance and medicinal benefits, the plant thrived in harsh desert conditions. The therapeutic properties of *M. peregrina* have been recognized throughout history. Many traditional healing systems, including Ayurveda and traditional Arabic medicine, have employed leaves, seeds, and roots of the plant for their medicinal properties. It was commonly used to treat ailments such as digestive disorders, skin conditions, respiratory problems, and as a general tonic to promote overall well-being. Its historical significance extends beyond its medicinal uses (Senthilkumar et al., 2018). Additionally, the plant has been used as a source of nutrition, especially during times of scarcity. In times of hardship, *M. peregrina*'s leaves and seeds have provided essential vitamins, minerals, and proteins to communities. Furthermore, *M. peregrina* holds cultural and religious significance in some regions. A deep connection between the plant and local communities is reflected in its incorporation into traditional rituals, culinary practices, and folklore. The *M. peregrina* has received comparatively less attention in modern research and commercial cultivation than its close relative, the *M. oleifera*. Recent efforts have been made to explore *M. peregrina*'s potential as a valuable resource for food security, sustainable agriculture, and as a source of natural products with medicinal and nutritional benefits (Farahat and Gaertner, 2019).

Characteristics of *Moringa peregrina*

A small to medium-sized tree, the plant typically grows to a height of about 5 to 10 meters. Its trunk is relatively thick and gnarled, demonstrating its resilience against harsh weather. *M. peregrina*'s bark is rough and rugged, providing protection and insulation against the intense heat and aridity of its habitat (Dadamouny, 2009). A notable physical characteristic of *M. peregrina* is its deciduous nature. During the dry season, it sheds its leaves, conserving water and reducing transpiration loss. As a result of this adaptation, the plant can survive long periods of drought in arid environments. *M. peregrina* has compound, fern-like leaves with multiple leaflets arranged in a pinnate pattern. The leaflets are elongated and slender, with a deep green color. Despite their delicate appearance, the leaves are remarkably resilient and can withstand extreme heat and high solar radiation. *M. peregrina*'s flowers are small, white, and fragrant. Bees and butterflies are attracted to them during the dry season. Elongated, thin, cylindrical seed pods follow the flowers. Pods contain numerous seeds surrounded by fibrous pulp (Vaknin and Mishal, 2021). The roots of *M. peregrina* are well adapted to its arid environment. They are long, strong, and capable of penetrating deep into the soil to reach underground water sources. By utilizing available resources efficiently, the plant can withstand periods of water scarcity.

Figure 1. *M. peregrina* leaves and seeds

Nutritional value of *Moringa peregrina*

A *M. peregrina* plant's leaves are its most nutrient-dense part. Their vitamins, minerals, and antioxidants provide a significant nutritional boost. Vitamin C, vitamin A, vitamin E, and various B-complex vitamins are abundant in the leaves, supporting health and well-being. *M. peregrina* leaves are also an abundant source of calcium, iron, potassium, and magnesium (Patil et al., 2022). The body needs these minerals for bone health, oxygen transport, muscle function, and electrolyte balance. Moreover, *M. peregrina* leaves contain all the essential amino acids that the human body needs. The plant is therefore a valuable source of protein, especially in regions where animal protein is scarce. Additionally, *M. peregrina* leaves contain antioxidants. In addition to flavonoids, polyphenols, and beta-carotene, they contain antioxidants that protect the body against oxidative stress. A plant's nutritional value extends beyond its leaves (Al-Harthi et al., 2022). Monounsaturated and polyunsaturated fatty acids are abundant in *M. peregrina* seeds. They are beneficial for cardiovascular health, brain function, and vitamin absorption to digest these fats. The nutritional value of *M. peregrina* makes it a valuable resource for combating malnutrition and promoting food security in regions with limited resources. Its leaves and seeds can be consumed fresh, cooked, or dried to retain their nutritional content and are used in a variety of culinary dishes. Despite *M. peregrina*'s nutritional benefits, its precise composition can vary depending on soil conditions, climate, and plant maturity.

Uses of *Moringa peregrina* in Medical Care

M. peregrina is traditionally used in medicine for its anti-inflammatory properties. A plant extract has been used to treat inflammation-related conditions such as arthritis, rheumatism, and joint pain. *M. peregrina* contains anti-inflammatory compounds that help reduce swelling, pain, and discomfort. In addition, *M. peregrina* has demonstrated antimicrobial activity against a variety of pathogens (Balogun, Toheeb A., et al., 2021), extracts of this plant have been shown to be effective against bacteria, fungi, and parasites. The antimicrobial properties of the plant make it a potential natural remedy for treating skin infections, respiratory tract infections, and gastrointestinal infections. The phenolic compounds and flavonoids in *M. peregrina* also possess antioxidant properties. Cells are protected from damage by these antioxidants by neutralizing harmful free radicals. *M. peregrina* may prevent and manage chronic diseases, such as cardiovascular diseases, cancer, and neurodegenerative disorders, by combating oxidative stress. Additionally, *M. peregrina* has been traditionally used for its potential anti-diabetic properties. People with diabetes or at risk of developing the disease may benefit from it since it regulates blood sugar levels and improves insulin sensitivity. There is

however a need for further research to understand the mechanisms underlying its antidiabetic effects and determine its efficacy and safety in clinical settings (Senthilkumar et al., 2018). A natural analgesic is another notable medical application of *M. peregrina*. Plant extracts have shown pain-relieving properties, offering a natural alternative to synthetic analgesics without the adverse effects.

Despite *M. peregrina*'s potential medical uses, further research is needed to validate its therapeutic effects, standardize its formulations, and determine its long-term safety. Furthermore, clinical trials are required to evaluate its efficacy in humans and potential interactions with medications.

Candida kruzei Fungus

Chocolate is manufactured using a kind of fungus known as budding yeast called *C. kruzei*. A nosocomial pathogen called *C. kruzei* mainly affects immuno-compromised individuals and patients with haematological malignancies. Fluconazole, a common antifungal drug, is naturally resistant to it. As a preventative measure, fluconazole should not be used since *C.kruzei* is more frequently discovered in individuals who have previously been exposed to the drug, according to contradictory research. Compared to the more prevalent *C. albicans*, *C. kruzei* fungemia has ahigher mortality rate. Pichia kudriavzevii is the teleomorph name, while *C. kruzei* is the anamorph name (Al Aboody and Mickymaray 2020). In 2021, the International Commission on Taxonomy of Fungi (ICTF) and the Nomenclature Committee for Fungi (NCF) recommend changing the standard name to Pichia kudriavzevii. *Clostridium kruzei* is described as living on mucosal surfaces in the earliest accounts of the pathogen in humans. In recent years, it has emerged as a major infectious agent with symptoms like fungaemia, uveitis, arthritis, and septicemia. In a nosocomial (hospital) setting, the majority of these frequently affect critically ill patients. Human immunodeficiency virus disease and the widespread use of azithromycin to treat staph infections are both responsible for a significant rise in *C. kruzei* infections. Due to the widespread prevalence of yeast drug resistance, this is certainly relevant. Experimental studies have shown that *C. kruzei* becomes less vitriolic than *C. albicans* in terms of its ability to attach to epithelial and synthetic surfaces, as well as its ability to produce proteolytic enzymes and hydrolytic enzymes. The truth that *C. kruzei* differs significantly from other bacterial or viral *Candida* species in terms of structural and insulin resistance functionalities, as well as in its behavior toward host defenses and strengthening (d'Enfert et al., 2021).

Through the use of the most recent metabolomic techniques, a better understanding of the pathotypes of this yeast may facilitate the official inquiry of the aetiology and disease pathogenesis of *C. kruzei* infections. There are approximately 150 species of asporogenic yeast in the genus *Candida*. More than 80% of clinical *Candida* isolates are from *C. albicans*, *C. tropicalis*, and *C. glabrata*, but others, including *C. kruzei*, *C. parapsilosis*, *C. guilliermondii*, and *C. kefyr*, are occasionally identified and are thought to be less harmful. Since the early 20th century, their therapeutic significance has been recognized, and current statistics show that > 30% of nosocomial *Candida* infections are caused by species other than *Candida albicans*. A typhus patient was first found to have *Candida* yeasts in buccal aphthae by Langenbeck in 1839, but the idea that *C. kruzei* could cause sickness was first proposed by Castellani over 75 years later. It has been widely accepted since then that this bacterium is a warm-blooded animal commensal with a low pathogenicity and virulence. It has only been in the past two to three decades that reports of *C. kruzei* as a human disease have dramatically increased. For instance, more than 65 publications have been published since 1960 that link *C. kruzei* to the aetiology of human illness (Sharma, and Aggarwal, 2013). There is no doubt that there has been an increase in *C. kruzei* infections throughout this time, even though this may be partially attributable to raising consciousness about the lifeform and advancements in laboratory detection techniques. This review highlights the characteristics that make C *kruzei* unique from other members of the genus and its

epidemiology, clinical symptoms, and pathogenicity. Infections caused by fungi, such as candidiasis, may be superficial or deep-rooted. *Candida* genus members cause illness. It is associated with high rates of morbidity and mortality, especially in immunocompromised patients (Gómez-Gaviria and Mora-Montes., 2020).

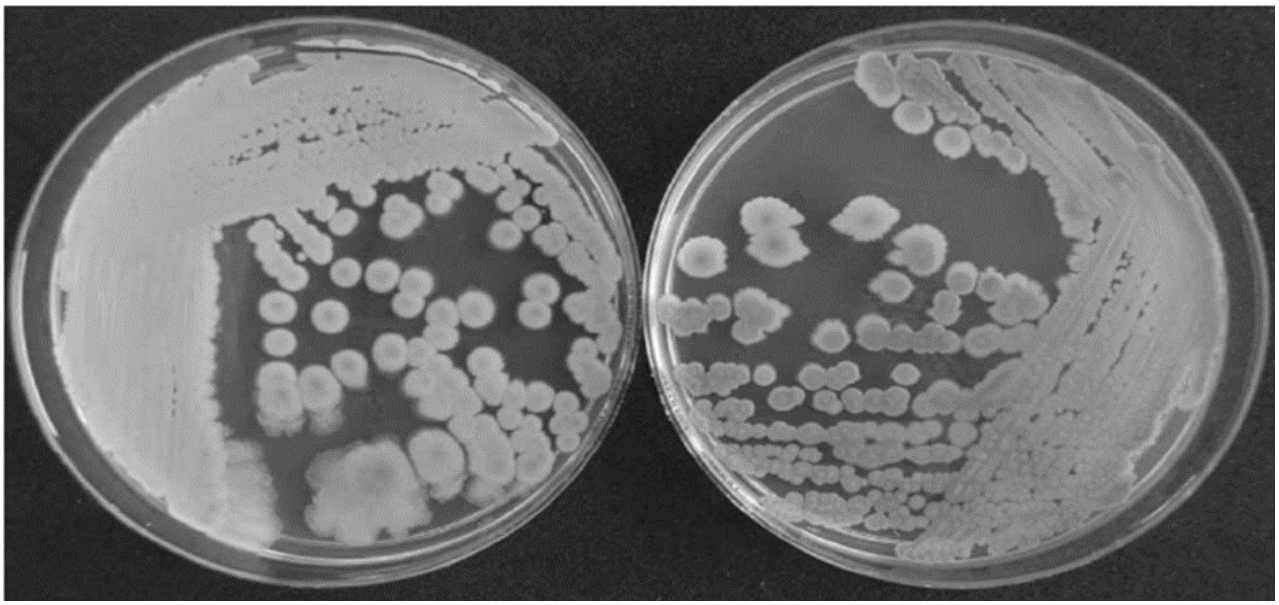

Figure 2. *C. kruzei* Fungus

Effect of *Moringa peregrina* Extracts Against *Candida kruzei* Fungus

M. peregrina extracts have been shown to inhibit *C. kruzei* growth in research studies. Flavonoids, alkaloids, and phenolic compounds found in the plant's extracts, particularly those from its leaves, have demonstrated antifungal activity (Senthilkumar et al., 2018). *C. kruzei* is inhibited from causing infection by these compounds by interfering with its growth and replication. Additionally, *M. peregrina* extracts combined with antifungal drugs commonly used to treat *Candida* infections have shown synergistic effects. Consequently, the plant's compounds may enhance conventional antifungal therapies, reducing the dosage of antifungal drugs necessary and minimizing the risk of drug resistance. *M. peregrina*'s antifungal activity against *C. kruzei* can be attributed to several mechanisms. The bioactive compounds present in the plant extracts disrupt the fungal cell membrane, resulting in cell death. Furthermore, these compounds may inhibit the growth of the fungus by interfering with enzymatic activity and DNA replication. Although the antifungal activity of *M. peregrina* against *C. kruzei* is promising, more research is needed to identify the specific compounds responsible for the antifungal effects and to elucidate the underlying mechanisms of action (Niazi et al., 2023). For the treatment of *C. kruzei* infections in humans, in vivo studies and clinical trials are necessary to determine the efficacy, safety, and optimal dosage of *M. peregrina* extracts.

Research Methods and Study Design

This section describes the materials and methods used to evaluate the antifungal effects of methanol extracts from *M. peregrina* leaves, roots, and seeds against *C. kruzei* fungus. which include *M. peregrina* parts collection and processing, phytochemical assay, bacterial culture, well diffusion method, and minimum inhibitory concentration (MIC)'s method.

Figure 3. *M. peregrina* Tree

Moringa peregrina Parts Collection and Processing

M. peregrina parts were collected from a nursery in Nizwa, Sultanate of Oman. and the preparation of the extractions used was done following the method described below.The different plant parts were assembled and dried using an oven, then made into a fine powder using a blender and mortar and pestle. Then 100g of each of the different parts was separated and labeled in a special flask, and 400 ml of diluted methanol was added and mixed with the fine powder (figure 8). After that, the samples were placed in a shaker (figure 9) and kept on the bench for four to five days. The solution was then subjected to a sonication process (figure 10) daily for four to five days Next the samples were filtered using pieces of clothing after 4-5 days (figure 11). Then the samples were subjected to a rotary evaporator to evaporate the methanol from the filtered solution (figure 12). Finally, the resulting solution was filtered and extracted for each part of the *M. peregrina* plant in a clean beaker inside the fume hood to ensure that all the methanol was evaporated (figure 13). This ensured that the extraction of each part of the *M. peregrina* was ready to be used in the tests of antifungal and antibacterial activity. El-Kamali and Awad El-Karim (2008) described similar techniques in their investigations.

Figure 4. *M. peregrina* leaves, roots, and seeds

One liter (1000 milliliters) of hot, sterile, distilled water was added with precisely 100 g of the plant powder and left to steep for 24 hours at room temperature. Sigma Aldrish, Inc. in the USA's What-man No.2 filter paper was used to filter the mother liquor. The extract was placed in a flask in a freezer until thoroughly dried after 48 hours. The dried plant extract was then removed??broken apart from the flask using a spatula. Weighing the residue allowed us to calculate the percentage yield. The aqueous waste (2 g) was dissolved in a sterile solution of distilled water (20 ml) at the time of testing (con. 100 mcg/ml) and stored in the refrigerator until employed.

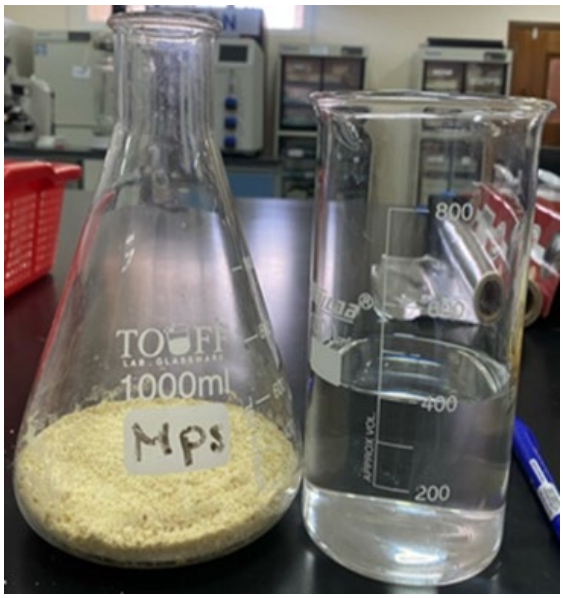

Figure 5. Process of Adding 400ml of Methanol to the Powder of Three Parts

Figure 6. Shaking Process

Figure 7. Sonication Process

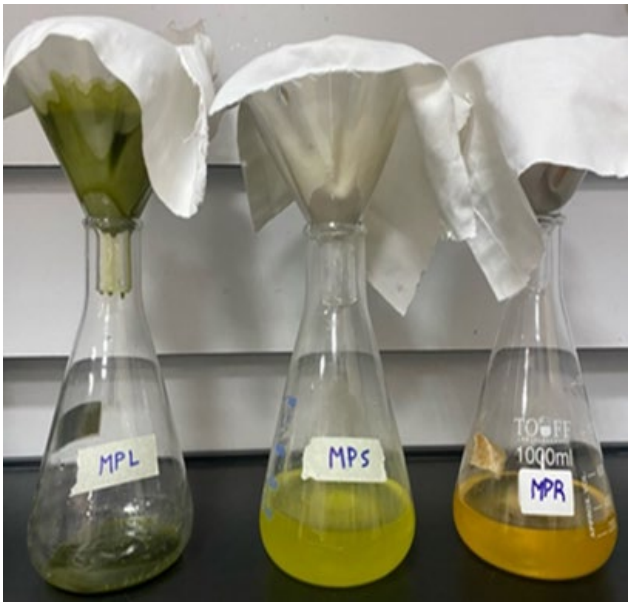

Figure 8. Filtration Process

Figure 9. Evaporation Process of Methanol by Rotary Evaporator

Figure 10. Extracts Inside the Fume Hood

Phytochemical Assay

Different parts of *M. peregrina* (leaves, seeds, and roots) were subjected to phytochemical analysis.

Tannins

A ferric chloride test was used, as 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

Dragendorff's test was used: 0.2 g of methanolic extract was mixed with 20 ml of sulphuric acid in a conical flask diluted in10 ml methanol. Then the mixture was filtered and 2 drops of Dragendorff's reagent were added (Ghani, 1998; Harbone, 2001).

Flavonoids

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

Saponins

The froth test was used: 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 distilled water and gently shaking it (Harbone, 2001; Khalil et al., 2013; Sofowora, 2005).

Steroids/ Triterpenoids

Liebermann-Burchardt tests were used: 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

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

Terpenoids

The Salkowski test was used: as 2 ml of chloroform was used to dissolve 2 ml of the extract following by allowing the extract to dry. The extract was then boiled for 2 minutes after adding 2ml of sulphuric acid (Kadhim and Al- Shammaa, 2014; Khalil et al., 2013).

Fungal Cultures

Candida kruzei (ATCC 6258) was obtained from the microbiology laboratory, NMSRC, University of Nizwa, Oman. Freshly cultured fungal isolates were incubated for 48 hours at 28 °C on sterile potato dextrose agar (PDA) (Liofilchem, Italy). After being cleaned in sterile normal saline and having their turbidity adjusted

to a McFarland standard equal to 0.5, the resulting cells produced colonies with 1×10^6 CFU/mL.

Antibacterial Assay

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

Well-Diffusion Method

Each extract's (MPS, MPL and MPR) antifungal efficacy against *C. kruzei* was assessed. Amphotericin B, a widely used antifungal agent/medication, was used as the standard.

Figure 11. Inoculation of *C. kruzei* by Cotton Swap

The antimicrobial efficacy of the plant preparations was assessed using the diffusion method. Plant extract residues were separately dissolved in distilled water at a specified concentration of 0.25 g/ml. In Petri plates, modified Mueller-Hilton agar (MHA), 2% glucose, and 5 µg of methylene blue/mL were added. 30 µL of the microbial suspension was/were put into each bunch. As positive and negative controls, 30 µL of amphotericin B and 30 µL of the solvent (without plant extract) were added to the modified MHA plates, respectively. To enable the plant extract to diffuse, all Petri plates were kept at room temperature for an hour. They were then incubated at 28 °C for 48 hours (Figures 11 and 12) and the results of incubation tracked. A similar method was used by Abd-Ulgadir (2023), in which the inoculum *C. albicans* was grown on Sabouraud's Dextrose Agar (SDA).

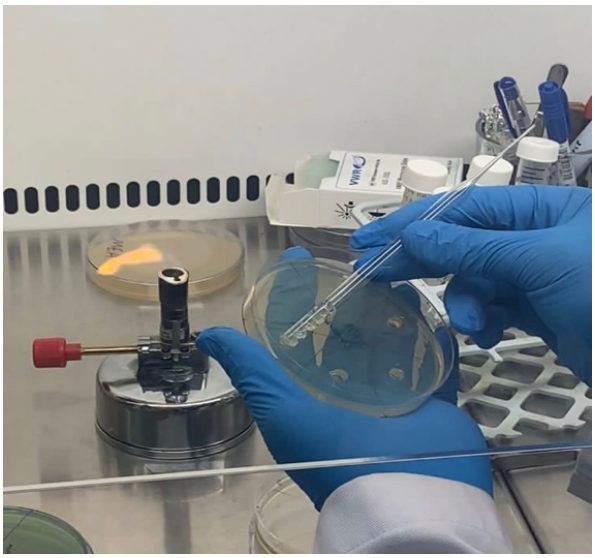

Figure 12. Using a Cork-Borer to Punch the Medium

Minimum Inhibitory Concentration (MIC)'s Method

Serial dilutions from the standard solution of each fraction were performed to ascertain the MIC of the crude extracts of *M. peregrine*. Additional dilutions with a 2-fold dilution factor were made from the standard solution (1/2, 1/4, 1/8, 1/16, 1/32, and 1/64). Microorganism-infected plates were used. and on each plate, several wells were created, and 30µL of solutions were placed inside each well. For 48 hours for the yeasts, plates were kept at 28 °C. Clear zones around wells and discs served as indicators of the test microorganisms' susceptibility to standard antifungal drug and crude extracts. The diameter of the inhibitory zones created around each hole was measured in millimeters, and the inhibition

was then expressed as the degree of sensitivity. Equation (1) was used in the computation to determine the MIC in mg/ml for the final concentration that showed a zone of inhibition (ZOI).

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

where [C] is the initial concentration (stock) and D is the dilution value.

Results and Discussion

This section describes the main research results and discusses *M. peregrina*'s leaves, roots, and seeds antifungal activity against *C. kruzei*, as well as comparing the performance of *M. peregrina* extracts relative to the antifungal Amphotericin B.

Phytochemical Screening of Sequential Extract of *Moringa peregrina* Plant Parts

According to the results, *M. peregrina* leaves inhibited the growth of *C. kruzei*, as shown in Table 1. The inhibition zone was about 10 mm.,which comprises (Shah et al., 2013; Lalas et al., 2012, and Al Ashaal et al.,2010), who have demonstrated the biological activities of *M. peregrina* and found that the extracted oils of *M. peregrina* have antibacterial and antifungal activities. The results found antibacterial activities against two gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923), and *S. epidermidis* (ATCC 12228), and four gram-negative ones: Pseudomonas aeroginosa (ATCC 27853), Escherichia coli (ATCC 25922), *Enterobacter cloacae* (ATCC 13047) and *Klebsiella pneumonia* (ATCC 13883), as well as three human pathogenic fungi *Candida albicans* (ATCC 10231), *C. tropicalis* (ATCC 13801) and *C. glabrata* (ATCC 28838). The oil proved effective against all of the tested microorganisms. Also, a recent study by Abd-Ulgadir (2023)) found that the antifungal activity of 100 mg/ml of leaves methanol extract of *M. peregrina* showed high antifungal activity towards the standard strain of *Candida albicans*. The phytochemical screening of the sequential extracts of *M. peregrina* plant leaves, roots, and seeds shows the presence of various bioactive components of which phenol, alkaloid, and flavonoids are the most prominent. The results of the phytochemical tests are presented in Table 1.

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

	MPL (*M. peregrina* leaves.)	MPR (*M. peregrina* root)	MPS (*M. peregrina* seed)
Phenolic compound	-	++	+++
Flavoniods	+++	++	-
Saponins	+++	++	-

Note: ++ Medium concentration, + Low concentration, - zero concentration; MPL - *M. peregrina* leaves, MPR - *M. peregrina* roots, MPS - *M. peregrina* seeds

Phenolic compounds:

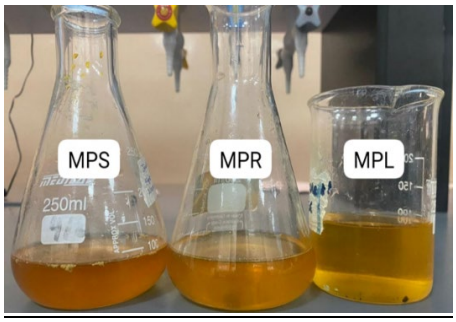

Figure 13. Ferric Chloride Test Used to Determine the Presence of Phenols
Note: MPL; *M. peregrina* leaves, MPS; *M. peregrina* seeds, MPR; *M. peregrina* roots

Saponins:

Figure 15. Froth Test for Saponins (Frothing Test)
Note: MPL; *M. peregrina* leaves, MPS; *M. peregrina* seeds, MPR; *M. peregrina* roots

Flavonoids:

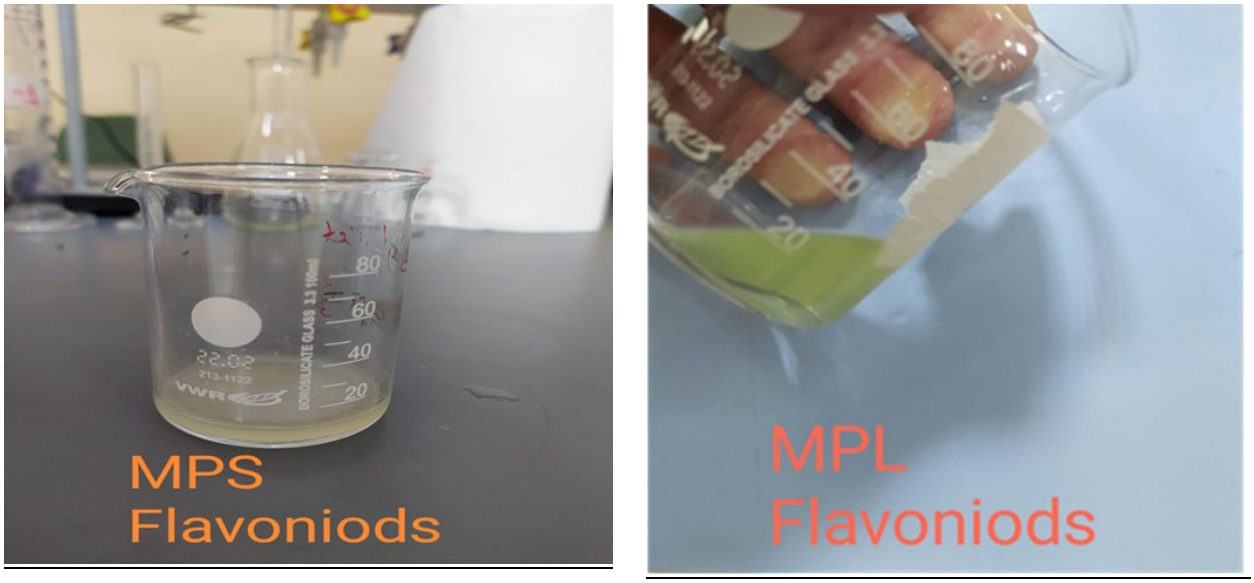

Figure 14. Ammonium Test Used to Detect Flavonoids.
Note: MPL; *M. peregrina* leaves, MPS; *M. peregrina* seeds, MPR; *M. peregrina* roots.

Well-Diffusion Antimicrobial Assay and Minimum Inhibitory Concentration (MIC)

The well-diffusion test for *M. peregrina* leaves, roots, and seeds crude extract on emerging fungal nosocomial pathogen (*C. kruzei*.) indicated inhibition of all tested bacteria except *Klebsiella* spp. (Table 3).

However, in antimicrobial screening, only one of the three extracts gave positive results: MPL extracts produced significant inhibitory results for *C. kruzei* growth, with 10 mm of ZOI clearly seen/measured around the disc (Table 2).

Table 2. Screening the ZOI of Chemical Extracts Against *C. kruzei* in mm

	Standard Amphotericin B	Blank D.H2O	MPS	MPL	MPR
C. kruzei	17 (NR.>14)	0	0	10	0

Note: NR; Normal range, MPS; *M. peregrina* seed, MPR; *M. peregrina* root, MPL; *M. peregrina* leaves.

These findings generally indicated that *C. kruzei* organisms are more susceptible to the *M. peregrina* leaf extract. Further testing by serial dilution was carried out to indicate the MIC. ½ of MPL solution appeared as the last well with an inhibition zone. Concentration of ½ is equal to 125.0 mg/ml, therefore, 125 mg/ml is the MIC. The strongest activity of MPL against *C. kruzei* was recorded at 10mg/ml of leaf extract . Recent research by Abd-Ulgadir (2023) stated that ZOI of the antifungal activity for methanol extract of *M. peregrina* leaves on clinical isolates of *Candida albicans* was 16.56±1.62 mm which was lower than the activity of Candizole 5 mg/ml. *M. peregrina* leaf extract showed varied MIC values towards clinical isolates that ranged from 6.25 mg/ml to 25 mg/ml, while the sample of the same isolates was from 25 mg/ml to 50 mg/ml. Finally, the methanol extract of *M. peregrina* was found to be effective against both the standard strains and clinical isolates compared to the aqueous extract.

Table 3. Determination of the Minimum Inhibition Concentration (MIC) Activity of Moringa Peregrina Leaves Extracts as Assayed by Well-Diffusion Method

MPL	1/2	1/4	1/8	1/16	1/32	1/64
C. kruzei	8	0	0	0	0	0

Note: MPL; *M. peregrina* leaves

Table 4. Minimum Inhibition Concentration (MIC) of *M. peregrina* extracts against *C. kruzei* (mg/mL).

Fungi	MPL
C. kruzei	125.0

Note: MPL; *M. peregrina* leaves

Conclusions and Recommendations

It can be concluded that *M. peregrina* leaf extracts have significant antifungal properties, has been widely demonstrated in various research studies. Alkaloids, flavonoids, phenolic compounds, and essential oils in the plant contribute to its potent antifungal properties. The effectiveness of *M. peregrina* extracts and isolated compounds against a range of fungal pathogens, including *Candida*, highlights its potential as a natural alternative for the treatment and prevention of fungal infections. In addition to its antifungal properties, *M. peregrina* has several other benefits. The product is natural with low toxicity, making it a safer alternative to synthetic antifungal drugs. Extracts of *M. peregrina* are easily accessible, low-cost, and sustainably sourced. The current study results under the conditions and the environment of Oman, have confirmed the previous studies findings. Therefore, it is a promising candidate for further exploration and development as a therapeutic agent against fungal infections. *M. peregrina* has promising antifungal properties, but further research is required. In the future, studies should focus on optimizing extraction methods, identifying and characterizing the active compounds responsible for antifungal activity, and evaluating the efficacy of *M. peregrina*-based formulations or products. By harnessing the bioactive compounds present in this plant, researchers and pharmaceutical companies can work towards developing effective and safe treatments for fungal infections, addressing the growing concerns of drug resistance and limited treatment options. This finding, however, is recommended for further study to see if it can be applied to medicine.

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