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(RESEARCH ARTICLE)

Comparative study between *Moringa peregrina* plant extracts and a standard antibiotic against *Candida albicans*

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Abstract

Candida albicans is a common fungus that can infect people, especially those who have compromised immune systems. In the face of declining efficacy of conventional antibiotics for treating fungus infections, natural plant-based remedies are gaining popularity. *Moringa peregrina* has long been used for its medicinal benefits, particularly for healing fungus infections. *M. peregrina* extracts were evaluated for their antifungal activity against *C. albicans* in vitro. A comparison was made between the antifungal susceptibility of plant extracts and the antifungal susceptibility of a common antifungal drug (amphotericin B). The ethanolic extract of *M. peregrina* had the strongest antifungal activity against *C. albicans* with a minimum inhibitory concentration (MIC) of 12.50 mg/ml. In a similar way to the MICs of common antibiotics such as Amphotericin B, *M. peregrina*'s antifungal properties have been attributed to flavonoids, alkaloids, and tannins. As a natural antifungal agent, *M. peregrina* may be useful in treating *C. albicans* infections. More research is needed to determine the safety and effectiveness of *M. peregrina* in human clinical trials. Considering the information currently available, *M. peregrina* is recommended as a potential natural antifungal agent for the treatment of *C. albicans* infections. In order to determine the optimal dosage, method of administration, and potential adverse effects of *M. peregrina* as an antifungal agent, more research is still needed.

Keywords: *Moringa peregrina*; *Candida albicans*; Antibiotic; Minimum inhibitory concentration.

1. Introduction

1.1. Introduction and statement of the problem

The health effects of fungal infection can be severe, especially for people with compromised immune systems or respiratory issues. The exposure to fungus spores can cause life-threatening infections, allergic reactions, and respiratory symptoms such as coughing and wheezing (Pitt et al. 2009). *Candida albicans* is a form of yeast commonly found in the mouth, genitalia, and digestive system of humans. Although *C. albicans* is often harmless, it can occasionally cause infections, particularly in people with compromised immune systems or those who recently received antibiotics. Candidiasis, oral thrush, and genital candidiasis are some of the manifestations of *Candida albicans* infections. Oral thrush causes white patches on the tongue and interior of the mouth, while vaginal candidiasis causes itching, burning, and discharge. When *C. albicans* enters the bloodstream, it can lead to dangerous infections in vital organs like the heart, kidneys, and brain (Pappas et al. 2016). *Candida albicans* infections are typically treated with antifungal medications. It is important to consider the intensity and location of the infection, as well as the patient's general health, when choosing an antifungal medication. *Candida albicans* infections are most commonly treated with the following antifungal medications: Azoles; Fluconazole, itraconazole, and voriconazole prevent the production of ergosterol, which is

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essential for fungus cells. Polyenes: like amphotericin B, these medicines attach to ergosterol in the fungal cell membrane, damaging it and killing the fungus. Echinocandins: These medications, such as *caspofungin*, *micafungin*, and anidulafungin, prevent beta-glucan from forming, a crucial component of fungal cells. In addition to antifungal medications, *Candida albicans* infections may be treated by addressing underlying risk factors such as immunosuppression and diabetes. Drug-resistant fungal infections have brought to light the need for novel antifungal medications. *Moringa peregrina*, a natural plant-based alternative, is being researched as a novel antifungal agent for treating *Candida albicans* infections (Pappas et al.2016). *Moringa peregrina* is native to the Middle East and other parts of the world. It is sometimes referred to as desert moringa or wild moringa. Medicinal benefits of the plant, such as its ability to heal fungus infections, have been known for centuries. In recent studies, *Moringa peregrina* has been shown to possess antifungal properties, making it a potential candidate for the creation of new antifungal medications. Several fungus species, including *Candida albicans*, Aspergillus fumigatus, and Cryptococcus neoformans, are resistant to extracts from *Moringa peregrina*. *Moringa peregrina* has antifungal properties because it contains flavonoids, alkaloids, and tannins. *Moringa peregrina* was examined in a 2017 article published in the Journal of Ethnopharmacology for its antifungal properties. An extract of *Moringa peregrina* was found to reduce the fungal burden in a mouse model of candidiasis and to inhibit Candida species growth in vitro. *Moringa peregrina* has the potential to be used as a natural antifungal medication to treat candidiasis, according to the study. An extract of *Moringa peregrina* decreased the fungal burden in a mouse model of dermatophytosis and inhibited dermatophyte growth in vitro.

Aim of the study

The main objective of the current study is to investigate the antifungal activity of *Moringa peregrina* leaves , seeds and roots extracts on *Candida albicans* fungus.

1.2. Significant of the study

With *Candida albicans* being a widespread fungus that can cause a variety of diseases, especially in people with compromised immune systems or those who have received antibiotic therapy, the antifungal activity of *Moringa peregrina* against *Candida albicans* is significant. The development of novel antifungal medicines is crucial for enhancing treatment outcomes and lowering the danger of medication resistance since candida infections can be challenging to cure. The development of new antifungal medicines is especially important due to concerns about treatment resistance in fungal diseases. As antifungal medications lose their effectiveness against fungi, new treatments are urgently needed. Since *Moringa peregrina* has substantial antifungal activity against *Candida albicans*, it may have potential as a natural antifungal agent for treating candidiasis and other fungal infections. Research is needed to fully understand the mechanisms of action of *Moringa peregrina* and to establish its safety and efficacy in humans.

2. Literature review

2.1. Moringa peregrine

2.1.1. Distribution and Availability of Moringa peregrina

Moringa peregrina belongs to the *Moringaceae* family. With a height of 5–15 m, a diameter of 20–40 cm, and grayish–green bark, it grows quite quickly. There are many leaflets on its 20–70 cm-long leaves that fall off as the leaf ages. Blooms (10-15 mm in length) are typically pink to yellowish white, bisexual, and exhibit traits that encourage insect pollination, such as being large, showy, lightly perfumed, and zygomorphic. Fruiting lasts up to three months, while flowering occurs from March to April. 5–15 triangular, ovoid seeds with a rough coating are found inside the fruit, which measures 10–25 x 1–1.5 cm. Hegazy et al. (2008) reported that fruit set is extremely low, ranging between 0.05 and 0.07%, while blooming buds wither between 40 and 50%. In spite of thousands of flowers appearing during the flowering season, peregrina trees yield only a small number of seeds. As early as the seedling stage, Munyanziza and Yongabi (2007) reported that the root of the tree develops into a tuber.

2.1.2. Botanical Description of Moringa peregrina

Young seedlings have enormous underground tubers with broad leaves. As the plant ages, its leaflets become narrower and more widely spaced, while its leaves become longer. Adult trees produce mature leaves with a full complement of small leaflets before dropping them. Even so, the tree still has exposed leaf axes, giving it a wispy appearance like Tamarix species. Naked leaves are alternate, 15–40 cm long, 2-pinnate, and have 2–5 pairs of pinnae. They are opposite or alternate, obovate, oblanceolate, or spatulate, 3–20(–35) mm, 2–10(–13) mm, with rounded or notched bases and grey or waxy green leaflets. There are 2.5 cm wide flowers with a wonderful scent that are produced in profusion in axillary, drooping panicles that are 10-25 cm long. They have yellow polka dots at the base and are white or cream in

color. There are five reflexes on the linear-lanceolate sepals. All five petals are reflexed and slender-spatulate except for the lowest. These petals surround the five stamens and five staminodes. An oblong, somewhat triangular fruit with a beak that is glabrous, dehiscent, and has three valves measuring (10-) 32–39 cm (1-) 1.5–1.7 cm. Seeds are brown, 10- to 12-mm globular to trigonous .

Figure 1 *M. peregrina* seeds

Figure 2 *M. peregrina* leaves

2.1.3. History of Moringa peregrina

As far back as 150 BC, Moringa plants have been around. "Moringa" is derived from the Tamil or Malayalam word "murunggi" or "muringa". Indian, Greek, and Egyptian civilizations have used moringa for thousands of years for a variety of purposes, according to historical evidence. They ate Moringa leaves and fruits to maintain their skin health and mental fitness. The ancient Maurian soldiers of India were fed Moringa leaf extracts on the battlefield since it was thought that the concoction would soothe their pain and tension from fighting. Additionally, the beverage gives soldiers extra energy during fighting. For producing perfume and protecting their skin, ancient Greek, Roman, and Egyptian civilizations highly valued edible oil derived from the seeds of Moringa. Ben oil has been used by the Egyptians since the middle and old dynasties (3000–2000 BC).

2.1.4. Characteristics of Moringa peregrina

Moringa is the only plant in the family Moringaceae that can be found in tropical and subtropical climates. Among them is the species *Moringa peregrina*, which serves a variety of conventional, practical, dietary, and therapeutic purposes. It is used in traditional medicine to treat a variety of health problems, such as diabetes, wound healing, disinfection, muscular aches, slenderness, burns, labor pain, hypertension, malaria, stomach illnesses, asthma, skin troubles, and the removal of a stuck placenta. Aside from its medicinal properties, *Moringa peregrina* shared spiritual, cultural, and religious ties with the Arabian Peninsula's indigenous people. A number of pharmacological properties, including antioxidant, antimicrobial, antidiabetic, antispasmodic, hypertension, hepatotoxicity, cholesterol-lowering activity, anti-inflammatory, anti-cancer, and memory disorganization, were evaluated using *M. peregrina* plant parts. There were also a few active substances extracted, identified, and asserted to have anti-cancer, anti-hypertension, anti-diabetic, anti-infective, anti-allergic, and herbicidal properties.

2.1.5. Description of Moringa peregrina

The *Moringa peregrina* is a deciduous tree with big leaves and thin, pendulous branches. The tree blooms twice a year, in spring and fall. The five-petaled flowers are white, crimson, or pink, with red or pink streaks. Throughout the year, its peculiar fruits can be seen hanging from its branches. Fruits are elongate, cylindrical, and up to 30 cm long with deep longitudinal grooves. They split into three valves when they are fully ripe, releasing a substantial white seed known as the behen-nut.

2.1.6. Traditional uses of Moringa peregrina

Indian Vedic literature was the first to describe moringa and its therapeutic properties around 5000 years ago. In folk medicine, *Moringa peregrina* leaf extract is applied topically to treat paralysis and skin rashes. In northern Oman, pod oil is used to treat infantile paralysis and convulsions (Miller and Morris, 1988). The Sultanate of Oman uses its seeds most frequently to treat diabetes (Reddy et al., 2015). It is also used successfully on the Indian subcontinent for diabetes-related symptoms such as hyperlipidemia and hyperglycemia. The young leaves of *Moringa peregrina* are traditionally used as antioxidants and wound healers in Arab folk medicine. Apart from disinfecting (Marwah et al., 2007), the bark juice is also used to treat burns, slimness, fever, headaches, constipation, back and muscle pains, and labor discomfort.

Nawash and AlHorani (2011) state that the leaves heal wounds, while the seeds treat abdominal pain (Van der Vossen and Mkamilo, 2007). Diabetes, stomach diseases, asthma, hypertension, and malaria can all be treated with *M. peregrina*'s roots and leaves and water (Mekonnen et al., 1999). Scabies, itches, and freckles have traditionally been treated with this plant's oil (Al-Dhaheri, 2016). Furthermore, *Moringa peregrina* has important nutritional value. Young leaves of *Moringa peregrina* can be used as a vegetable (Al-Dhaheri, 2016). The immature seeds are consumed in India, while the adult seeds are consumed either roasted or fried in Malawi. Traditional herbal medicine uses the seeds of the plant to treat malnutrition in combination with other herbs (MPCP, 2006). Additionally, *Moringa peregrina* is a significant native tree in the UAE due to its connections to culture, spirituality, and religion. The plant's leaves are used to flavor smoked meat (tanour) in the region. This custom is still practiced by the local population of the UAE (Al-Dhaheri, 2016).

2.1.7. Morphology of Moringa peregrina

A thin, deciduous shrub or tree with an oval crown can grow up to 10 meters tall. Compound tri-pinnate leaves of *Moringa peregrina* are fluffy, pale green, and 30–60 cm long. Each leaflet measures 1.3–2.5 cm and 0.3–0.6 cm wide. The leaves are simple, petiolate, early deciduous, and glabrous on both surfaces. Blades are ovate-oblanceolate, with an entire border and obtuse, occasionally mucronate apex. The flowers of *Moringa peregrina* are fragrant, 2.5 cm in diameter, white, creamy, pinkish to pale, and borne in sprays with five stamens. In *M. peregrina*, the pendulous, triangular, ridged fruits or pods are brown in color. Within the pith of the pod, which splits into three halves, there are roughly twenty to twenty-five trigonous seeds. There are nine ribs on each pod, and both ends taper. Dark brown *M. peregrina* seeds with three papery wings.

2.1.8. Phytochemical Composition and Biological Activities of Moringa peregrina

Ben oil, made from *Moringa peregrina* seeds, is its main by-product. It dates back to antiquity that the Bible and ancient Egyptian scriptures mention the use of oil. The oil is used in cosmetics, medicinal, and cookery. Small machinery in Yemen is lubricated using oil. In Sudan, for example, the seeds are utilized as a coagulant to cleanse water. Yemen and southern Sudan use the leaves of *Moringa peregrina* as animal feed. The seeds are used in traditional medicine in Sudan and the Middle East. The oil can be used to treat abdominal pain. The young tubers of the plant are consumed in Yemen and Oman. The plant is raised as an ornamental plant in Saudi Arabia and the Middle East. Wood is harvested for fuel in the southern Sinai, but supplies are limited. About half of a *Moringa peregrina* seed is made up of oil. It is comparable to the oil made from Moringa oleifera Lam seeds. The oil's approximate fatty acid breakdown is as follows: oleic acid 71%, palmitic acid 9%, stearic acid 4%, arachidic acid 2%, behenic acid 2%, and gadoleic acid 2%. The oil contains the sterols campesterol, stigmasterol, and sitosterol, as well as tocopherols and -tocopherols. Seeds filter water by coagulating scattered particles [Somali et al.1984; Tsakis J. (1998); Moustafa et al.1998]. Biodiesel, food, medicine, and water purification are some of the applications of *Moringa peregrina* (Osman et al. 2012). There is a high oil content in the seed kernel, ranging from 42 to 54 percent (Afsharypuor et al. 2010). Mortinga oil contains high levels of oleic acid (>73%) and negligibly low levels of polyunsaturated fatty acids. Increasing antibiotic resistance and the emergence of new strains of disease-causing bacteria have highlighted the need for new, secure antibacterial medicines. *Moringa peregrina* has antimicrobial properties in several parts. They work either by killing the pathogen (bactericidal) or by inhibiting its growth (bacteriostatic). Antibiotics generally have the following effects. Recently, n-hexane was used to fractionate *Moringa peregrina* aerial portions, and apigenin, amyrin, and sitosterol-3-O-D-glucoside were evaluated against several bacteria and fungi. Compared to conventional antibiotics, each of these ingredients had a significant antibacterial inhibitory effect (Hussein et al. 2017).

2.1.9. Cultivation of Moringa peregrina

The plant is native to the dry tropics. Young seedlings form a large tuber and have broad leaves. During the dry season, young plants frequently produce only herbaceous growth before withering back to the ground. After the tuber reaches a certain size, the growth above ground continues to grow but turns woody. The first fruits are produced three years after planting. Both seeds and cuttings grow rapidly; with enough moisture, the plant can reach a height of 3 to 4 meters per year. After harvesting, pollarding or trimming is advised to encourage branching. Because the tree is kept at a moderate height, pod production is increased, and harvesting is easier. Martin et al. (1998) estimate that a single tree can produce 1,000 seedpods per year.

2.1.10. Uses of Moringa peregrina in medical care

There are a number of traditional, nutritional, commercial, and therapeutic uses for the peregrine. The plant is used in folk medicine in Arab nations to treat fevers, muscle aches, asthma, and wounds (Abdelraouf and Samira, 2020).

2.1.11. Candida albicans fungus

About 70% of people, including approximately 75% of women, have *Candida albicans* as a benign commensal in their gastrointestinal and genitourinary tracts (Ruhnke and Maschmeyer, 2002). It becomes an opportunistic pathogen for immunocompromised patients, some people with weak immune systems, and even healthy individuals. The infection caused by *Candida albicans* is called candidiasis. Depending on how severe the condition is, candidiasis can be divided into two groups. Infections of the mucosa fall into the first category. The most well-known mucosal illness is thrush, which is characterized by white spots on the membranes. The epithelial cells of the vaginal, oropharyngeal, or gastrointestinal tract are typically affected by these infections. In addition, some women get recurrent Vulvo Vaginal Candidiasis (RVVC), which is a recurrence of Vulvo Vaginal Candidiasis (VVC).It results in fatal and life-threatening systemic infections in extremely ill patients, with a mortality rate of about 30% (Sexton et al. 2007). HIV-positive patients, transplant recipients, chemotherapy patients, and low birth weight babies are frequently infected with systemic Candida. *C. albicans* continues to be a significant infectious fungus agent, even though some non-albicans species, including *C. glabrata, C. krusei, C. dubliniensis, C. parapsilosis, and C. tropicalis*, have been isolated from infected individuals. The eminent Greek physician Hippocrates discovered a microbial infection in 400 BC and called it "thrush," which is caused by *Candida albicans*. Early research were mostly focused on the discovery of *C. albicans* strains, and they did not begin to be examined like other model organisms until the twentieth century

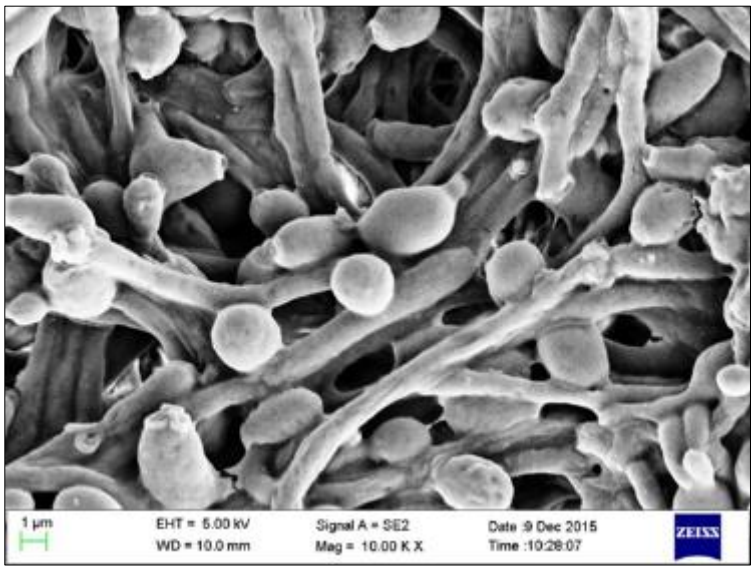


Figure 3 *Candida albicans*

2.1.12. Pathogenicity of Candida albicans

The pathogenicity of *Candida albicans* is mainly determined by two variables. One is the host's immune system, and another is the pathogen's virulence factors. Numerous laboratories have discovered numerous virulence factors of this significant disease during the past three decades, and they have been linked to the pathogenesis. According to Naglik et al. (2003), the adhesion to the host cell, secretion of hydrolytic enzymes, dimorphic phenotype (yeast to filamentous form or hyphae), phenotypic switching, modulation of the host's immune system, and formation of biofilm on biotic and abiotic surfaces are all examples of microbial factors that contribute to pathogenicity. In order for Candida infections to colonize and establish, adhesion is believed to be required. *Candida albicans* can attach to a wide range of surfaces, including endothelium cells, inert implants, extracellular matrix, and epithelial cells. There are a number of mechanisms used for adhesion, many of which have been extensively investigated in the literature (Pendrak and Klotz, 1995). An adhesive molecule is comprised of Als1p-Als7p and Asl9p, Hwp1p, Int1p, Mnt1p, and several others. Besides other secreted hydrolytic enzymes, *Candida albicans* also secretes SAPs (secreted aspartyl proteinases), which are thought to play a role in candidiasis pathogenesis. In addition to being secreted in vivo during infection, these SAP enzymes may break down a variety of critical host proteins, and mutant strains have decreased virulence, all researchers have concluded that these SAPs play a role in pathogenicity (Schaller et al., 2005). *Candida albicans* secretes phospholipases A, B, and C, which may contribute to its virulence. These enzymes contribute to host cell damage, adhesion, and penetration, according to Calderone (2002).*Candida albicans*' ability to build biofilm on biotic and abiotic surfaces is another crucial characteristic, and it is believed to play a significant role in pathogenicity. A significant factor contributing to *Candida albicans*' pathogenicity is thought to be phenotypic flipping. There are a variety of colony forms associated with this disease, including rough, smooth, irregularly wrinkled, fuzzy, star, hat, and stippled. There has been

much research on the white-opaque switching characteristic of the clinical isolate WO-1. Furthermore, *Candida albicans* can form filamentous hyphae or pseudohyphae as well as unicellular budding yeast. As a whole, *Candida albicans* can exhibit plasticity in phenotypic or morphological states, contributing significantly to its pathogenicity; however, much more research is needed to fully understand the relationship between various morphogenetic states and their contribution to pathogenesis (Anaul et al. 2012).

2.1.13. The effects of Moringa peregrina against Candida albicans fungus

The seed oil of *Moringa peregrina* exhibited antibacterial properties (fungi, bacteria). *Candida albicans* has a MIC (minimum inhibitory concentration) of 5.70 mg/mL, while Candida glabrata has one of 3.25 mg/mL.Conventional antibiotics were only effective against a small number of germ species despite their extensive use. There is no effect of netilmycin or AMCA (7-amino-4-methylcoumarin-3-acetic acid) on Candida species demonstrates this. However, Lalas et al. (2012) found that only pathogenic fungi were resistant to intraconazole and 5-flucytocine.

In various solvents, *Moringa peregrina* extracts suppressed Phytophthora infestans, *Candida albicans*, Aspergillus brasiliensis, and Pencilliumdigilatum. In vitro aqueous extracts were more effective against Pencilliumdigitatum than positive controls and ex vitro aqueous extracts. Despite its weaker anti-*Candida albicans* activity. In vitro and ex vivo, methanolic extracts were more effective against Aspergillus brasiliensis and Phytophthora infestans than the positive control. The vitro methanolic extract was more effective against *Candida albicans* than the positive control. Both ex vitro and in vitro extracts against Aspergillus brasiliensis were found to be more effective than positive controls, ethanolic extract being more effective than the positive control (Linda et al. 2018).

In recent years, scientific evidence has confirmed the nutritional and therapeutic benefits of Moringa, which were previously understood by ancient civilizations. There are abundant antioxidants and antimicrobial components in the plant, as well as micronutrients that are effective at controlling human and plant pathogenic bacteria, fungi, and insects (Senthilkumar et al., 2018). A thermostable chitin-binding protein from *M. peregrina* has been found to have antifungal activity against Fusarium solani, F. oxysporum, Colletotrichum musae, and C. gloesporioides. Alternaria alternate and A. brassicola are inhibited by two additional chitin-binding peptides found in *M. peregrina*, but not by *C. lunata, Rhizoctonia solani, Verticillium dahilae, or Aspergillus niger.*

Aqueous extracts of moringa leaves can inhibit *Candida albicans*, Candida glabrata, and C. tropicalis, as well as Aspergillus niger, A. flavus, Alternaria species, Fusarium species, R. stolonifer, and Penicillium species. A high potency extract inhibited fungi less than 10 mm, while a moderate potency extract inhibited fungi between 10 and 9 mm, according to Latifa and Muneera (2016).

Moringa oleifera and *Moringa peregrine* leaf extracts both demonstrated antibacterial efficacy against gram-positive and gram-negative bacteria and fungi that were locally grown in Egypt. Although most serial extracts have antibacterial properties, each has a unique action against a particular bacterial class.

3. Materials and methods

This Chapter describes the materials and methods used in antifungal activity of *Moringa peregrina* plant extracts in comparison with standard antibiotics against *Candida albicans* fungus which include *Moringa peregrina* parts collection and processing, phytochemical assay, fungal cultures , antifungal assay, well-diffusion method and minimum inhibitory concentration (MIC)'s method.

Figure 4 *Moringa peregrina* tree

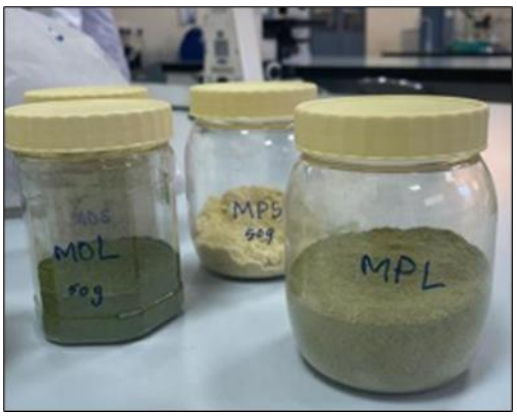
Figure 5 *Moringa peregrina* leaves

Figure 6 *Moringa peregrina* seeds

Figure 7 *Moringa peregrina* roots

3.1. *Moringa peregrina* parts collection and processing

Plants of the *Moringa peregrina* species were gathered during the time when fruits were produced and kept in a cold, dry shed. 100g of fresh *Moringa peregrina* leaves, seeds, and roots were shade-dried at room temperature (32–35 °C). (Fig. 8)



(MPL: *Moringa peregrina* leaves, MPS: *Moringa peregrina* seeds)

Figure 8 Dried samples of *Moringa peregrina* parts

Using a mill and pestle, the dried leaves, seeds, and roots were ground into a powder (figure 8). In 500 ml conical flasks with 100 ml of methanol, 100 g of powdered leaves, seeds, and roots were separately extracted (figure 10). Rubber corks were used to seal the conical flasks, which were then shaken at 120 revolutions per minute for 30 minutes and left to stand at room temperature for 24 hours while gently shaking (figure 11). Using Whatman no. 1 filter paper that was sterile, the extracts were separated and filtered.

Figure 9 Powdered samples

Figure 10 Methanolic extracts

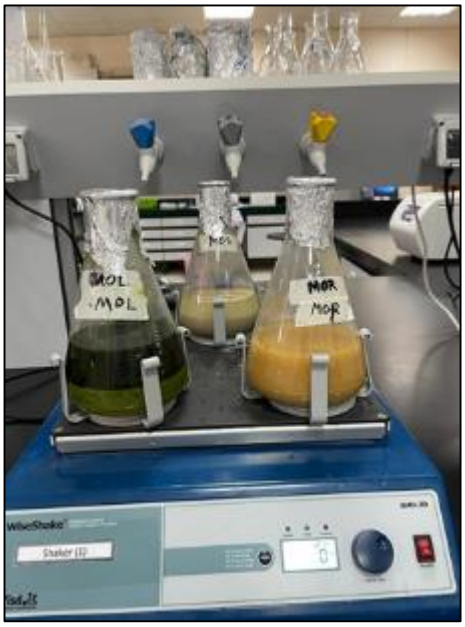


Figure 11 Shaking

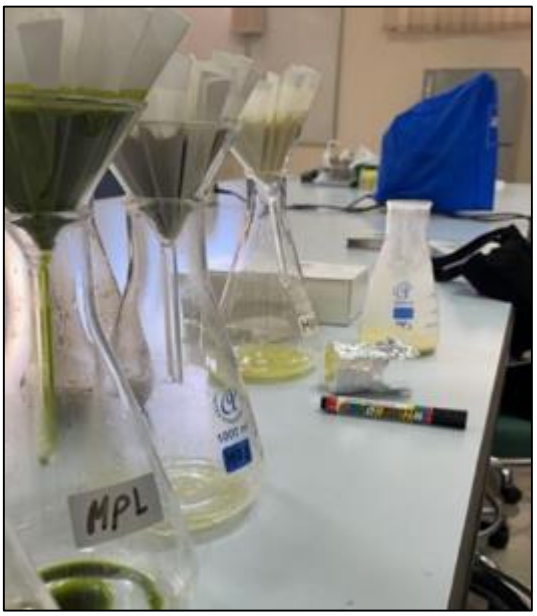


Figure 12 Filtration

Figure 13 Evaporation

Figure 14 Samples after evaporating

(MPR; Moringa peregrina roots, MOS: moringa olivera seeds)

3.2. Phytochemical assay

The leaves, seeds, and roots of *Moringa peregrina* were subjected to phytochemical analysis of the extract for qualitative detection of phenolic component, flavonoids, tannins, ferric acid, and saponins.

3.2.1. Flavonoids (test with ammonium)

10ml of distilled water and 3ml of each extract were combined, then the mixture was agitated. 10% NaOH solution was added in a volume of 1ml to the mixture (Sofowora, 2005).

3.2.2. Frothing test for Saponins

A test tube was filled with 3ml of each extract and 2ml of distilled water to dilute it. The mixture was vigorously shaken. (Harbone, 2001;Khalil et al., 2013;Sofowora, 2005).

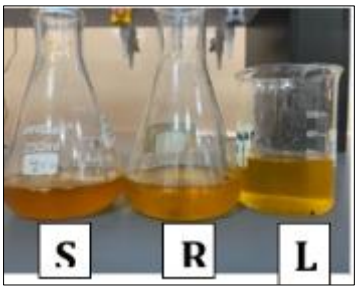
3.2.3. Ferric chloride test for tannins

Each extract was cooked gently for 2 minutes in a separate test tube with 2ml of the mixture before cooling. To each extract, 3 drops of a ferric chloride solution were added (Harbone, 2001).

3.2.4. Steroids (Salkowski Test)

In a different test tube, 1 ml of each extract was mixed with 5 drops of concentrated H2SO4 (Khalil et al., 2013).

Figure 15 Ferric acid



(S: seeds, R:roots, L: leaves of *Moringa peregrina*)

Figure 16 Phenolic compound)

Figure 17 Flavoniods

Figure18 Saponins

3.3. Fungal cultures

Freshly sub-cultured fungi were cultivated for 24-48 hours at 28 °C on sterile potato dextrose agar (PDA) (Liofilchem, Italy). After being rinsed in sterile normal saline and having their turbidity adjusted to a McFarland standard equivalent of 0.5, the resultant cells produced 1 106 CFU/mL colonies.

3.4. Antifungal assay

3.4.1. Well-diffusion method

Each extract's antifungal effectiveness was tested against *Candida albicans* (ATCC 14053) using the MPS, MPL and MPR, extracts. The gold standard for *C. albicans* was amphotericin B. The antibacterial efficacy of plant extracts was assessed using the diffusion method. Plant extract residues were separately diluted in D.H2O at concentration of 0.25 g/mL. In

Petri dishes, modified Mueller-Hilton agar (MHA), 2% glucose, and 5 g of methylene blue/mL were added. There were 30 mL of microbial suspension placed into each punch. 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 allow the plant extract to diffuse, all Petri plates were kept at room temperature for an hour. They were then incubated at 28 °C for 24 to 48 hours. Results of the incubation were observed (Pinal et al.2014).

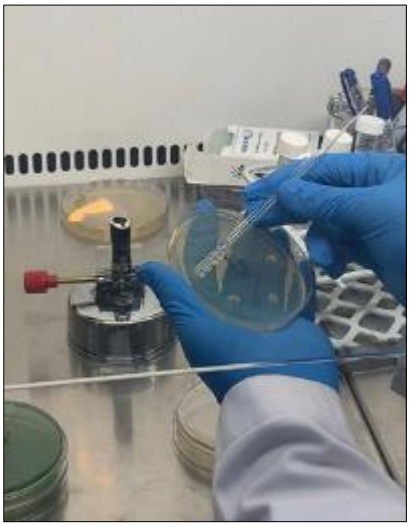


Figure 19 well – diffusion method

3.4.2. Minimum inhibitory concentration (MIC)'s method

To determine the MIC, crude extracts of *M. peregrina* were serially diluted from the stock solution of each fraction. From the stock solution, additional dilutions of 2 fold were made (1/2, 1/4, 1/8, 1/16, 1/32, and 1/64). Microorganisms were used to inoculate the plates, and each plate had 30 L of solutions in each well. At 28°C, plates were incubated for 48 hours. Indicators of sensitivity to conventional antibiotics and crude extracts were clear zones surrounding wells and discs. The diameter of the inhibitory zones produced around each hole was measured in millimeters, and the degree of inhibition was then quantified. Equation (3) was used in the computation to determine the MIC in mg/mL for the final concentration that showed an inhibitory zone.

MIC is defined as: MIC= [C] x D = mg/mL (3),

where [C] is the initial concentration (stock) and [D] is the dilution value. (Raheela et al. 2008).

4. Results and discussion

This chapter describes the main research results and discuss the finding on antifungal activity of *M. peregrina* plant extracts in comparison with standard antibiotics against *C. albicans* fungus, This comprises collection and preparation of *M. peregrina* plant's part, phytochemical screening of sequential extract of *Moringa peregrina* plant parts ,well-diffusion antifungal assay and minimum inhibitory concentration (MIC).

4.1. Collection and preparation of *Moringa* plant's part

The *M. peregrina* plant parts were collected from various locations in Oman, particularly the mountains, where the plant grows abundantly. In order to ensure the quality of the parts, methanolic extraction was used, *M. peregrina* plant parts were sequentially extracted for phytochemical analysis using minimum inhibitory concentrations (MICs), and well-diffusion antifungal assays.

4.2. Phytochemical screening of sequential extract of *M. peregrina* plant parts

According to Table 1, most of these bioactive components are present in *M. peregrina* leaf extracts. Sequential extracts of *Moringa peregrina* leaves, roots, and seeds contain phenols, alkaloids, flavonoids, and tannins. To produce the desired medicinal effects, plant extracts often combine two or more chemicals. Preliminary phytochemical quantification is crucial for connecting biological activity. It may also aid in identifying specific classes of secondary metabolites in the

future. Phytochemical components of a hydro alcoholic extract made from dried leaves of *M. peregrina* were examined by Ullah et al. (2015).At different concentrations, preliminary quantification analyses revealed the presence of alkaloids, tannins, phenolics, and saponins.

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

	MPL	**MPR**	**MPS**
Phenolic compound	-	+	++
Flavoniods	+	+	+
saponnins	+	-	-
Ferric acid	+	+	+

(MPL: *M. peregrina* leaves , MPR : *M. peregrina* roots, MPS: *M. peregrina* seeds)

Figure 21 Phenolic compound (S; seeds, R: roots ,L:leaves)

Figure 22 Flavonoids

Figure 23 Saponins

Plant phytochemicals are recognized as physiologically active substances that cause a variety of pharmacological behaviors (Ronghui et al. 2014). Some of these plant secondary metabolites are preferable to synthetic antioxidants due to safety concerns. Bioactive secondary metabolites have been shown to lower the risk and progression of diseases such as cancer, cardiovascular disease, neurological disease, etc. by scavenging free radicals through a variety of biological pathways (Ansari et al. 2013). Numerous reports available on phenolic compounds have demonstrated their usefulness in exhibiting potential biological activities such as antioxidant, antidiabetic, hepatoprotective, anti-inflammatory, antimicrobial, anticancer etc (Kumar and pandey 2013) (Sulaiman et al. 2011).

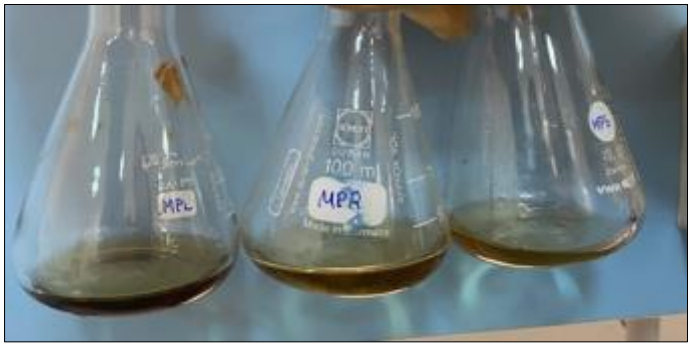


Figure 24 Ferric acid

The reduced characteristics of phenolic compounds, which enable them to serve as metal chelators, absorb and neutralize free radicals, are primarily responsible for their antioxidant activity (Mishra et al. 2010). Among plant secondary metabolites, flavonoids and tannins are thought to be the most promising polyphenolic chemicals (Tomczyk et al. 2010). Therefore, based on the phytochemical screening results, phenol, alkaloid, flavonoids, and tannin are the most prominent bioactive components found in the sequential extract of *Moringa peregrina* plant leaves, roots, and seeds.

4.3. Well-diffusion antifungal assay

The well-diffusion test for *M. peregrina* (root, leave and seed) extract on *C. albicans* indicated an inhibition of this fungus. (Table 2 and Figure 5).

Similar techniques were employed in earlier studies with (Mohamed et al. 2019), and they had positive outcomes. However, it was discovered that both *M. peregrina* and *M. Oleifera's* serial leaf extracts have antibacterial properties against fungus (*Candida albicans*), gram-positive bacteria, and gram-negative bacteria. Antimicrobial activity of ethanol extract of leaves, seed coat and endosperm of *M. peregrina* were studied by agar well diffusion assay against bacterial (*Bacillus subtilis, Micrococcus luteus, S. aureus, E. coli, P. aeruginosa, and K. pneumonia*) and fungal strains (*Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger*, and *C. albicans*; Hajar and Gumgumjee, 2014). In that investigation, *M. peregrina*'s leaf extract showed effective antibacterial activity.

Table 2 Minimum inhibition concentration (MIC) activity of *M. peregrina* extracts against *C. albicans* in mg/ml.

	MPL	**MPR**	**MPS**
C. albican	125.0	-	-

(MPL: *M. peregrina* leaves, MPR: *M. peregrina* roots, ,MPS: *M. peregrina* seeds)

The minimal inhibitory concentration after 48 hours, as determined by repeated dilution, is shown in Table 2. Extract from *M. peregrine* leaves had a 125.0mm MI. Tests of *C. albicans* on extracts from *M. peregrina* leaves revealed greater sensitivity than those on extracts from the plant's roots and seeds (Table 2 and Fig. 5).

The results usually showed that extracts from *M. peregrina* leaves were effective against the *C. albicans* fungus. The diameter of the inhibition zone (DIZ), which was measured in millimeters and ranged from 18mm to 21mm, was significantly larger in the methanol extract of *M. peregrina* leaves, which showed prominent activity against standard strains (Khalid et al., 2023).

The antimicrobial potential of *M. peregrina* seed oil was investigated using the disk diffusion method and the determination of minimum inhibitory concentrations against Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella pneumoniae, *Candida albicans*, C. tropicalis, and C. glabrata. The actions and common antibiotics were contrasted. The outcomes indicated that the oil was successful in killing every tested germ. The most resilient bacterial and fungi strain was found to be C. glabrata. According to Lalas et al. (2012), the MIC values of the microorganisms were 3.35, 3.50, 4.95, 4.38, 4.80, 4.30, 5.70, 3.30, and 3.25 mg/ml.

4.4. Minimum inhibitory concentration (MIC)

Table 3 Inhibition zone measurement in serial dilution for positive in screening (MPL) with *C. albicans*

MPL	**½**	**¼**	**1/8**	**1/16**	**1/32**	**1/64**
C.albicans	8	0	0	0	0	0

(MPL: *M. peregrina* leaves,)

The results show that the effect on *C. albicans* growth comes from *M. peregrina* leave extract in a dilution factor of 1/2 which was about 8 mm in comparison with (1/4, 1/8, 1/16,1/32,1/64), which were all 0 mm. Table 3 shows the measurement of inhibition zone in serial dilution for positive in screening with *C. albicans*. This study discovered that the *M. peregrine* plant has anti-*microbial* on properties *C.albicans* These results are consistent with those of Abdel-Rahman et al. (2010).

The current study's findings indicate that the extraction of *M. peregrina* leaves contains antifungal properties that can combat *Candida albicans*.

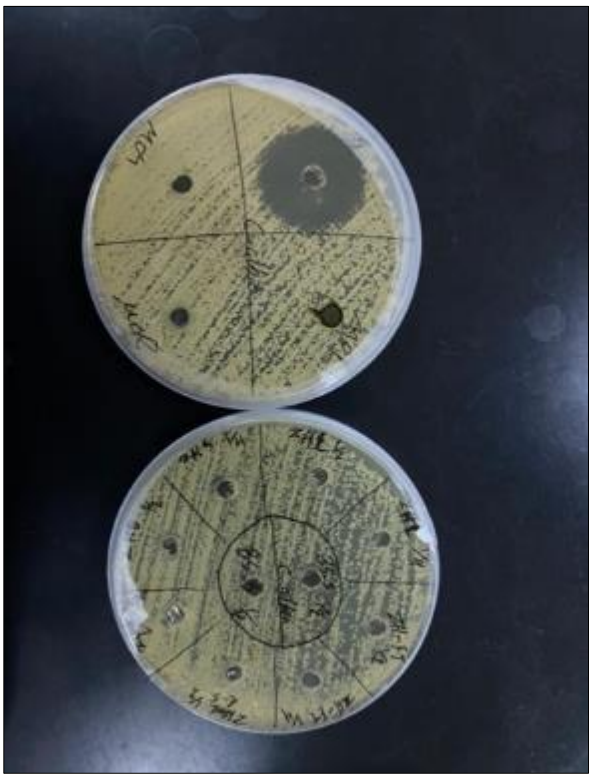


Figure 25 The inhibitory effect of methanolic crude extract of MPL, MPS and MPR Compared to Amphotericin B antibiotics and *C. albicans*.(MPL: *M. peregrina* leaves, MPS: *M. peregrina* seeds, MPR: *M. peregrina* roots)

M. peregrina has a strong potential as an antibacterial medicinal herb, according to Linda et al. (2018). By preventing the growth of the following fungi—Aspergillus digallium, *C. albicans*, Aspergillus Brasiliense's, and Phytophthora infectants—in vitro extract demonstrated antifungal activity. In terms of demonstrating the existence of antifungal activity in *M. peregrina* extracts, the results of the current study are comparable to those of the previous study.

The ethanolic leaf extract shown effective antifungal activity against the studied fungal strains, according to Annadurai et al. (2018): 24.67 mm *C. albicans*.

The current study looked at how the methanolic extracts from various *M. peregrina* sections affected the antifungal activity against the *C. albicans* fungus over a short period of time. According to Khalid et al. (2023), the MIC (minimum inhibitory concentration) value for *Escherichia coli*, *S. saprophyticus*, *Neisseria gonorrhoeae*, and *Enterococcus spp*. was approximately 12.50 mg/ml for *M. peregrine* leaves extract. For *P. mirabilis, K. pneumoniae,* and *C. albicans*, it was 25 mg/ml, but the same extract showed a MIC value of > 6.25 mg/ml for isolates of *P. aeruginosa*. The MBC of *M. peregrina*'s leaves methanol extract against isolates ranged from 6.25 to 25 mg/ml. 25 mg/ml inhibited clinical isolates of *C. albicans*. According to Abdel-Rahman et al. (2010), the presence of terepenoids, -amyrin, -amyrin, -sitosterol, and other compounds that have been shown to be antimicrobial agents may be the cause of this action.

5. Conclusion

In this study, methanol extract of *M. peregrina* leaves demonstrated varying degrees of antifungal efficacy against *C. albicans*. In comparison to *Moringa peregrina* root and moringa peregrine seed extracts, methanol extracts of *Moringa peregrine* leaves were found to be more efficient against the tested fungus. The screening *Candida albicans* used in this investigation revealed varying degrees of sensitivity to methanol extracts of *Moringa peregrina*. On the other hand, the methanol extract from the leaves of the *Moringa peregrina* reduced the growth of *C. albicans*. This research revealed that the *C. albicans* fungus is extremely sensitive to a methanol extract of *M. peregrina* leaves. As a result, this plant demonstrated intriguing biological activity and could serve as a solid foundation for its selection for more research to create new naturally occurring bioactive chemicals. It is advised that further research be studied on this mindset to see whether it may be used to the medical area.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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