

The biological activity of *Moringa oleifera* seeds extracts against *Candida* yeast

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Annotation: This study was conducted at the Open Educational College Science Laboratory from February to May 2026 to evaluate and compare the efficacy of alcoholic and aqueous extracts of *Moringa oleifera* seeds as biological agents two *Candida* species: *Candida albicans* and *Candida glabrata*. The results showed that both extracts (alcoholic and aqueous) The alcoholic extract resulted in an inhibition zone diameter of 14.944 mm for *Candida albicans* compared to 11.378 mm for *Candida gilberata*. The aqueous extract resulted in an inhibition zone diameter of 8.672 mm for *Candida albicans* and 6.911 mm for *Candida gilberata* exhibited biological activity that inhibited *Candida* yeast growth, with the alcoholic extract demonstrating significantly superior efficacy at all tested concentrations. The *Candida* strains also showed varying sensitivity to the extracts, with *Candida albicans* exhibiting greater sensitivity compared to *Candida glabrata*, which displayed higher resistance. GC-MS analysis of the alcoholic extract of *Moringa* seeds revealed the presence of biologically active compounds, such as phytosterols, long-chain fatty acids, and squalene, which are likely responsible for the antifungal activity, in addition to their antioxidant and anti-inflammatory properties. These results indicate that *Moringa oleifera* seeds possess antifungal biological activity, making them a potential natural source of antifungal compounds, particularly against certain strains of *Candida*.

Keywords: *Candida glabrata*, *Candida albicans*, GC-MS analysis.

Introduction

Invasive fungal infections caused by several pathogens such as *Ascremonium spp.*, *Candidiasis albicans*, *Aspergillus fumigatus* and *Mucorales spp.* constitute a global health threat. Consequently, the World Health Organization (WHO) included these fungi in its list of highest priority in 2022, due to their high infection rates, increasing resistance to antifungal drugs, and severe impact on immunocompromised groups [1]

This list, which ranks fungal pathogens based on research and development needs and their impact on public health, is the first systematic global assessment. Among the fungi classified as "critical" are *Candida albicans*, *Aspergillus fumigatus*, and *Candida auris*,—microorganisms of increasing concern due to their ability to cause severe, high-morbidity infections in immunocompromised patients and the difficulty in treating them resulting from their growing drug resistance [2].

Moringa oleifera is a fast-growing, drought-resistant tree native to the Indian subcontinent, but now widely cultivated in tropical and subtropical regions. Its versatility makes it an important medicinal plant, with virtually all its parts—leaves, seeds, pods, bark, flowers, and roots—used in traditional and modern food, medicinal, agricultural, and cosmetic applications. Agriculturally, its ability to thrive in minimal soil requirements and its tolerance for prolonged drought make it a valuable plant for promoting sustainable agriculture in areas suffering from water scarcity, climate variability, and land degradation [3]

Moringa oleifera (MOL), popularly known as the moringa tree or "miracle tree," is attracting increasing attention in scientific research due to its diverse chemical composition, rich in active compounds, and its numerous medicinal properties. These properties include antioxidant, anti-inflammatory, and anticancer activities, along with antimicrobial activity against fungi, bacteria, and viruses. The antifungal activity is attributed to low molecular weight peptides, which act as key plant defense mechanisms. These peptides interact molecularly with fungal cell walls (such as chitin) or enhance membrane permeability, thereby inhibiting fungal cell growth [4], [5].

One of the significant challenges facing humans is fungal infections, particularly in immunocompromised patients, as they pose a threat to human health. This has highlighted the need for effective antifungal agents. These agents target specific biological mechanisms within fungi, each class of which operates through distinct chemical and biological pathways to inhibit fungal growth and reproduction. These mechanisms include cell wall synthesis, DNA synthesis, sterol production, and cell membrane functions. Some major obstacles include high drug resistance, a narrow therapeutic spectrum, and associated toxicity. Therefore, developing antifungal agents derived from medicinal plants is imperative [6].

The search for safe and effective natural compounds from plant sources is emerging as a key strategic direction for antifungal drug development [7].

Moringa is known to be rich in biologically active compounds, but more specific studies are needed for its antifungal effects [8].

Therefore, this research primarily aims to evaluate and compare the antifungal efficacy of aqueous and alcoholic extracts from the seeds of the *Moringa oleifera* plant against *Candida* yeast, with the

goal of revealing the possibility of using these extracts as a natural source for treating or supporting the treatment of fungal infections caused by these yeasts.

Materials and Methods:

Plant Collection

Moringa oleifera, morphologically and molecularly identified, was obtained from a home garden in Karbala Governorate [9].

The dried pods were collected, the seeds extracted, and then air-dried. The seeds were then ground in an electric mill to prepare the alcoholic extract.

Extraction using a Soxhlet apparatus (hot method): Two hundred grams of plant material were soaked in hexane for forty-eight hours to extract the oil and fats. The plant was then immersed in a 70% ethanol solution in a Soxhlet apparatus to prepare it for extraction. The extraction process continued until completion. To remove any impurities or sediment, the extract was filtered using filter paper after collection. After a few hours, the mixture was allowed to cool before being concentrated by evaporation under reduced pressure in a rotary evaporator. The resulting concentrated crude extracts were then weighed and prepared for the next stages, where 5 ml of the concentrated alcoholic extract was taken for analysis using gas chromatography-mass spectrometry (GC-MS) [10].

Fungal Samples

The fungus used in the study

The 15 fungal isolates used in this study, an isolate of *Candida albicans*, *Candida glabrata* were obtained from the Public Health Laboratory of the Karbala Health Directorate. The isolates were activated and cultured on SDA medium, and their morphological and microscopic characteristics were studied

Preparation of Culture Medium

Saproid agar was used for *Candida* culture and prepared according to the manufacturer's instructions. It was then placed in an autoclave at 15 bar and 120°C. The culture medium was then extracted, cooled to 50°C, and poured into Petri dishes. It was left to solidify before use.

Preparation of Concentrations Used in the Experiment

Take 1 gram of the plant extract and dissolve it in 10 ml of 70% alcohol to obtain a concentration of 100 mg/ml, which is considered the stock solution

Prepare a series of concentrations: 100, 75, 50, and 25 mg/ml, according to the equation: $C_1 \cdot V_1 = C_2 \cdot V_2$.

The 100 mg/ml concentration is the same as the original stock solution.

The 75 mg/ml concentration is prepared by microdispensing 750 microliters of the stock solution with 250 microliters of distilled water, placing the mixture in a test tube, and mixing thoroughly.

The 50 mg/ml concentration is prepared by microdispensing 500 microliters of the stock solution with 500 microliters of distilled water, placing the mixture in a test tube, and mixing thoroughly.

The 25 mg/ml concentration is prepared by microdispensing 250 microliters of the stock solution with 750 microliters of distilled water, placing the mixture in a test tube, and mixing thoroughly.

Preparation Fungal Suspension

A sample of newly grown *Candida albicans* and *Candida gilberata* colonies (separately) is taken using a loop and mixed with normal saline in a 100 mL tube. The mixture is thoroughly mixed, and its turbidity is compared to a standard McFarland tube 0.5.

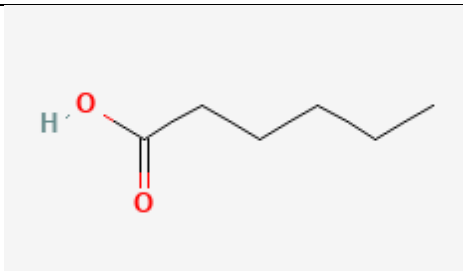
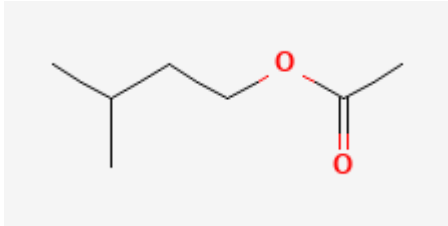
The plates containing the Streptoid agar culture medium are then inoculated by looping from all directions and left to stand for 10 minutes.

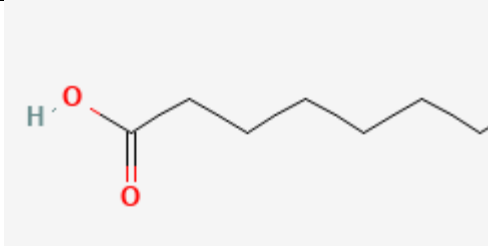
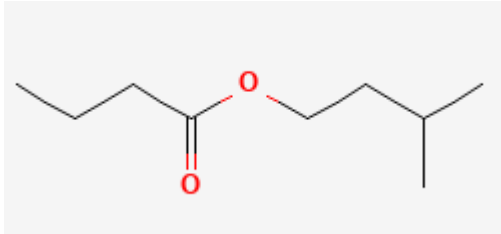
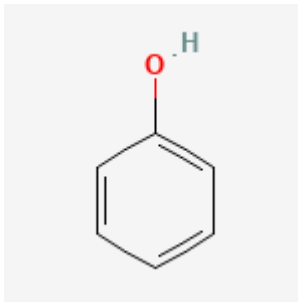
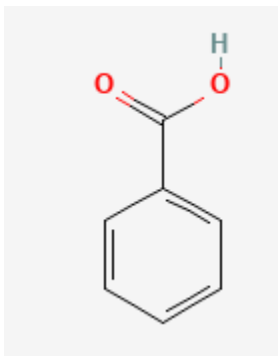
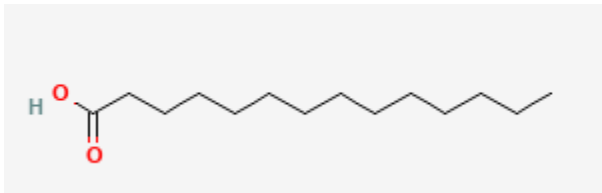
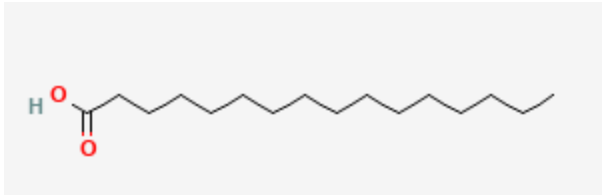
Using a cork drill, four holes are made in each plate, taking care not to puncture the edges. This process is repeated three times, and three additional plates are prepared with one hole in the center. The control solution, consisting of 70% alcohol and the antifungal fluconazole, is placed in this center. 100 μ L of each of the above concentrations is withdrawn and placed into the holes in each plate. The plates are then covered with airtight lids and incubated at 37°C for 48 hours.

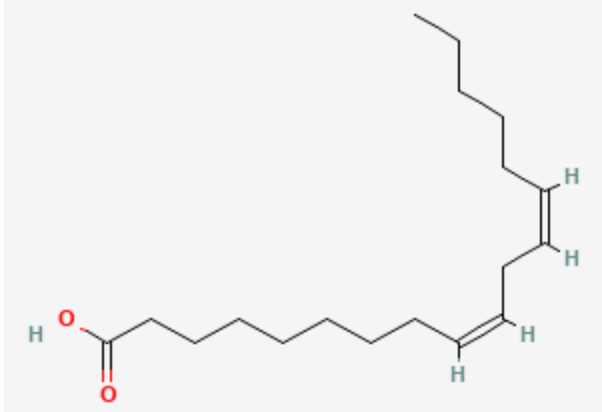
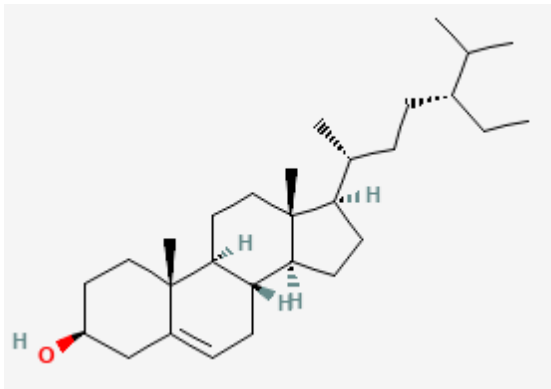
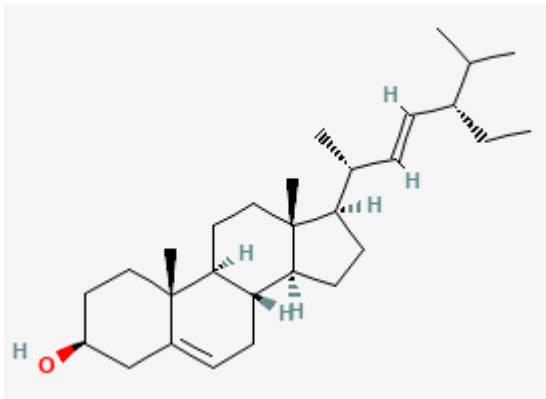
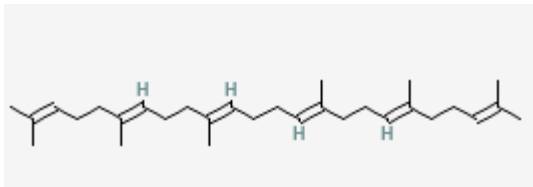
Result and discussion

Table 1 shows the qualitative results of GC-mass analysis of the alcoholic extract of aloe vera seeds using gas chromatography-mass spectrometry (GC-MS). The analysis revealed the presence of several active compounds with anti-inflammatory and antimicrobial properties, such as long-chain fatty acids like oleic acid, linoleic acid, and hexadecanoic acid, which are major components of plant seed oils. The analysis also revealed compounds known to lower cholesterol, possess antioxidant properties, and exhibit anti-inflammatory effects, such as beta-sitosterol, stigmasterol, and campesterol. Additionally, some compounds with antioxidant and antitumor properties, such as squalene, were found. This suggests that Moringa seeds contain active compounds that may explain their use in traditional medicine for treating various ailments. These results indicate that the alcoholic extract of Moringa seeds is rich in bioactive compounds that may contribute to its antifungal, antioxidant, and anti-inflammatory activity.

Table 1. Qualitative detection of the active compounds of the alcoholic extract of Moringa seeds by GC-MS analysis

Compound name	chemical formula	Chemical classification	Potential biological effect
Hexanoic acid	C ₆ H ₁₂ O ₂		Antifungal and antibacterial [11]
Heptanoic acid	C ₇ H ₁₄ O ₂		Antimicrobial [12]

Octanoic acid (Caprylic acid)	C ₈ H ₁₆ O ₂		Antifungal and antibacterial [13]
Nonanoic acid	C ₉ H ₁₈ O ₂		Antimicrobial [14]
Phenol derivatives	C ₆ H ₆ O		powerful antioxidant [15]
4-Hydroxybenzaldehyde	C ₇ H ₆ O ₂		Antioxidant and antibacterial [16]
Tetradecanoic acid (Myristic acid)	C ₁₄ H ₂₈ O ₂		anti-inflammatory [17]
Hexadecanoic acid (Palmitic acid)	C ₁₆ H ₃₂ O ₂		Antioxidant and antibacterial [18]

Linoleic acid	C ₁₈ H ₃₂ O ₂		Antioxidant and beneficial for heart health [19]
β-Sitosterol	C ₂₉ H ₅₀ O		Antioxidant and Antidiabetic [20]
Stigmasterol	C ₂₉ H ₄₈ O		anti-inflammatory [21]
Squalene	C ₃₀ H ₅₀		Anti-tumor and antioxidant [22]

Recent studies have indicated that the *Moringa oleifera* plant contains a large group of bioactive compounds that can be identified using GC-MS technology, such as fatty acids, phenols, terpenes, and plant sterols, which possess multiple biological activities such as antioxidant, antibacterial, anti-inflammatory, and hypoglycemic activity, thus enhancing the medicinal value of the plant and its use in modern medical and pharmaceutical applications [23]. *Moringa* (*Moringa oleifera*) seeds are rich in oxygen- and chlorinated volatile compounds. GC-MS is used to identify their bioactive components, such as antioxidants and insecticides. Oxygen-containing compounds (acetals, esters, alcohols) constitute the majority, reflecting the composition of the volatile oils in *Moringa* seeds, which are rich in cyclic structures and bioactive functional groups. These classifications are

derived from NIST library matches with high similarity indices (SI 78-98), and their insecticidal and fungicidal properties support their applications in natural pharmacology [24].

He concluded [25] in an experiment he conducted that the polar parts of mature moringa seeds possess antioxidant activity due to their content of volatile oils and phenolic and non-phenolic compounds.

In a study conducted by [26], the pharmacological potential of Moringa seed extracts was investigated, including fever reduction, potent anti-allergic activity, and an anti-asthmatic effect from the ethanolic extract. The aqueous extract also inhibited the growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis*. Moringa seed extracts prepared with hexane, dichloromethane, acetone, methanol, and water exhibited antibacterial activity against *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Furthermore, five of these extracts displayed antioxidant properties and offered protection against oxidative stress [27],[28]

The results in Tables 2 and 3 show that the fungal species and extract concentration significantly affect growth inhibition. Importantly, each fungal species responded differently to the extract concentrations. The alcoholic extract resulted in an inhibition zone diameter of 14.944 mm for *Candida albicans* compared to 11.378 mm for *Candida gilberata*. The aqueous extract resulted in an inhibition zone diameter of 8.672 mm for *Candida albicans* and 6.911 mm for *Candida gilberata*. This indicates that the inhibitory effect is due to the extract, not the solvent. The fungal species also affected the rate of growth inhibition, with *Candida gilberata* exhibiting greater resistance to extract concentrations compared to *Candida albicans*. Furthermore, the extract type significantly impacted growth inhibition, with the alcoholic extract showing a stronger effect than the aqueous extract.

Table 2. Effect of different concentrations of alcoholic extract of Moringa seeds on the inhibition zone (mm) of candida.

Con.	25%	50%	75%	100%	C+	C-	mean
Fungal							
Candida albicans	14.300	17.633	20.133	23.267	14.333	0.000	14.944
Candida gilberata	10.367	13.567	15.700	18.567	10.067	0.000	11.378
mean	12.333	15.600	17.917	20.917	12.200	0.000	
L.S.D .	C: 0.4687		F: 0.2706		C*F:0.6629		

Table 3. Effect of different concentrations of aqueous extract of Moringa seeds on the inhibition zone (mm) of Candida.

Con.	25%	50%	75%	100%	C+	C-	mean
Fungal							
Candida albicans	7.167	9.933	13.133	14.900	6.900	0.000	8.672
Candida gilberata	5.333	7.767	10.433	12.600	5.333	0.000	6.911
mean	6.250	8.850	11.783	13.750	6.117	0.000	
L.S.D.		C: 0.2627		F: 0.1517		C*F:0.3715	

Alcohol is an excellent organic solvent capable of extracting a wide range of phytochemicals, including:

Phenolic compounds: such as gallic acid and caffeic acid, which have strong antifungal activity.

Flavonoids: such as quercetin and kaempferol, which are known for their ability to disrupt fungal cell membranes.

Alkaloids and terpenoids: other compounds that have demonstrated antimicrobial activity.

Water is a good polar solvent, but it fails to extract many nonpolar or weakly polar compounds, which are often responsible for the potent pharmacological effect. The aqueous extract concentrates primarily on sugars, some proteins, and highly polar compounds [29]

The compounds extracted from alcohols (especially phenols and flavonoids) work in multiple ways to inhibit yeast growth. They work by Destroying the cell membrane: These compounds interfere with the integrity of the fungal cell membrane, leading to leakage of cellular contents and cell death. Or inhibiting the action of enzymes necessary for yeast growth and reproduction. Or Inducing oxidative stress this leads to the formation of free radicals within fungal cells, damaging their components. [30]

Candida albicans: This is the most common type and is generally more sensitive to antifungals.

Candida glabrata: This species is known for its increasing resistance to many conventional antifungal drugs. Therefore, it may require a higher concentration of the active extract. [31]

Enzymes in fungi are essential for vital processes such as cell wall synthesis, building blocks, and energy production. Active compounds in Moringa seeds can bind to enzyme active sites, disrupting their function and causing imbalances in fungal cell function, ultimately halting their growth.

It is believed that some of the active compounds in Moringa can inhibit the enzymes responsible for the production of ergosterol (which is an essential component of the fungal cell membrane), causing the formation of a weak and underdeveloped cell membrane and consequently the death of the fungal cell. [32],[33]

Conclusions and Recommendations

The results indicate that the extract's efficacy is related to the type and concentration of the solvent, and that increasing the concentration led to a significant increase in the areas of inhibition. Furthermore, the active compounds identified through biochemical analysis, such as fatty acids, phytosterols, and squalene, may be responsible for this antifungal effect through multiple mechanisms, including weakening the fungal cell membrane and inhibiting growth. More diverse studies are recommended to isolate multiple active compounds and evaluate their bioavailability, toxicity, and biosafety, while also precisely defining their mechanism of action. Testing on various pathogenic fungi, particularly drug-resistant strains.

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