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Research Article

Evaluation of the Antifungal Activity of *Moringa oleifera* and *Ziziphus abyssinica* Root Extracts against *Candida albicans*

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ABSTRACT
The increasing prevalence of antifungal resistance in *Candida albicans* the primary agent of candidiasis, necessitates the discovery of new therapeutic agents. Medicinal plants like *Moringa oleifera* and *Ziziphus abyssinica* are promising sources of bioactive compounds. This study aimed to evaluate the antifungal activity of *M. oleifera* and *Z. abyssinica* root extracts against *C. albicans,* determine their minimum inhibitory concentration (MIC), and analyze their phytochemical constituents. Root powders were extracted using aqueous, ethanol, and n-hexane solvents. Antifungal activity was assessed via the agar well diffusion method at concentrations of 25, 50, 75, and 100 mg/ml. The MIC was determined using the tube dilution method. Phytochemical analysis was performed using Gas Chromatography-Mass Spectrometry (GC-MS). All extracts exhibited dose-dependent antifungal activity. The ethanol extracts showed the highest efficacy, with zones of inhibition up to 14 mm for *M. oleifera* and 16 mm for Z. abyssinica at 100 mg/ml, a result comparable to the Fluconazole control (16 mm). The MIC for the most effective extracts was 75 mg/ml. GC-MS analysis identified key bioactive compounds, including beta-Sitosterol (42.72%) and Valerenol (15.16%) in *Z. abyssinica* and a predominant pentacyclic compound (42.87%) and 16-Pregnenolone (13.97%) in *M. oleifera.* The root extracts of *M. oleifera and Z. abyssinica,* particularly their ethanol fractions, possess significant antifungal properties against *C. albicans,* with efficacy rivaling a standard drug. These findings validate their traditional use and suggest their potential as sources for developing new antifungal drugs to combat resistant candidiasis.

Keywords: Antifungal Activity; Concentration; *Candida albicans;* GC-MS, Minimum Inhibitory; *Moringa oleifera;* Phytochemicals; *Ziziphus abyssinica*

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INTRODUCTION

Fungal infections, particularly opportunistic mycoses like candidiasis, are a significant cause of morbidity and mortality, especially in immunocompromised individuals (Bouopda, 2020). *Candida albicans* is the most prevalent species, capable of causing infections ranging from superficial mucocutaneous conditions to life-threatening systemic candidiasis (Dabas, 2013). The management of these infections is challenged by the limited arsenal of antifungal drugs and the emergence of resistance, particularly to azoles (Odds *et al.,* 2003). Furthermore, conventional drugs like amphotericin B can cause severe side effects such as nephrotoxicity (Arif *et al.,* 2009). This underscores the urgent need for novel, safe, and effective antifungal agents.

Medicinal plants represent a valuable reservoir of bioactive compounds. Over 80% of the world's

population relies on traditional plant-based medicines for primary healthcare (Caceres *et al.,* 1992). *Moringa oleifera,* often called the "miracle tree," is renowned for its nutritional and broad therapeutic properties, including antimicrobial, anti-inflammatory, and antioxidant activities (Fahey, 2005; Farooq *et al.,* 2012). Similarly, *Ziziphus abyssinica* is used in traditional medicine to treat various ailments, and previous studies have indicated its antimicrobial potential (Nyaberi *et al.,* 2010).

While some studies have explored the antimicrobial properties of *M. oleifera* leaves and seeds, and *Z. abyssinica* fruits, there is a paucity of research focused on their root extracts against fungal pathogens. This study therefore aimed to investigate the antifungal efficacy of *M. oleifera* and *Z. abyssinica* root extracts against *C. albicans,* determine their minimum inhibitory concentration, and identify the phytochemical constituents responsible for their activity.

MATERIALS AND METHODS

Plant Material Collection and Extraction

Dried roots of *Moringa oleifera* and *Ziziphus abyssinica* were purchased from local markets in Lokoja, Nigeria, and authenticated by an expert at the Herbarium of the Department of Biological Sciences, Federal University Lokoja. The roots were pulverized into a fine powder. The powder (30 g) was macerated separately with 300 ml of distilled water, ethanol, and n-hexane for 24 hours. The mixtures were filtered, and the filtrates were concentrated at 50°C to obtain dry extracts.

Microbial Strain and Culture Conditions

A clinical isolate of *Candida albicans* was obtained from the Federal Teaching Hospital, Lokoja. The strain was maintained on Potato Dextrose Agar (PDA) slants at 4°C and sub-cultured for fresh growth before use.

Antifungal Susceptibility Testing

The antifungal activity was evaluated using the agar well diffusion method (Sarka *et al.,* 2020). Briefly, the surface of sterile Potato Dextrose Agar (PDA) plates was uniformly swabbed with a standardized microbial suspension of C. albicans, adjusted to a 0.5 McFarland standard. Using a sterile cork borer, wells of 6 mm diameter were aseptically punched into the inoculated agar. Subsequently, 100 µL of each plant extract, at concentrations of 25, 50, 75, and 100 mg/mL, were introduced into the respective wells. Control wells contained Fluconazole (standard antifungal drug) as a positive control and the corresponding extraction solvents (aqueous, ethanol, n-hexane) as negative controls. The plates were then incubated at 37°C for 24–48 hours. After incubation, the antifungal activity was quantified by measuring the diameter of the zones of inhibition (ZOI) in millimeters (mm) from the well's edge to the zone's periphery.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined using the broth dilution method. Two-fold serial dilutions of the extracts were prepared in liquid PDA medium. Each tube was inoculated with a standardized *C. albicans* suspension and incubated at 37°C for 48 hours. The MIC was defined as the lowest concentration that showed no visible turbidity, indicating complete inhibition of fungal growth.

Phytochemical Analysis by GC-MS

The chemical constituents of the most active extracts were analyzed using an Agilent Intuvo 9000 GC system coupled with a 5977B MSD detector. A DB-5 MS capillary column was used with helium as the carrier gas. The oven temperature was programmed from 50°C (hold 5 min) to 300°C at 10°C/min. The compounds were identified by comparing their mass spectra with the NIST library.

Data Analysis

Statistical software for social sciences (SPSS) was the package used to analysed the data. The data obtained from antifungal assays are presented as mean ± standard error. Statistical significance was determined using ANOVA followed by Tukey-Kramer Multiple Comparisons Test, with a p-value of < 0.05 considered significant.

RESULTS

Antifungal Activity

The root extracts of both plants demonstrated concentration-dependent antifungal activity against *C. albicans* (Table 1). Ethanol extracts consistently showed the highest activity. For *M. oleifera*, the ethanol extract produced a ZOI of 14.0 mm at 100 mg/ml. For *Z. abyssinica*, the ethanol extract at 100 mg/ml produced a ZOI of 16.0 mm, which was comparable to the standard control drug (Fluconazole, 16.0 mm). Aqueous extracts generally showed the lowest activity.

Minimum Inhibitory Concentration (MIC)

The MIC results confirmed the findings from the diffusion assay. The ethanol extract of *M. oleifera* showed clear inhibition (no turbidity) at 75 mg/ml and 100 mg/ml. Similarly, the ethanol extract of *Z. abyssinica* showed complete inhibition at 100 mg/ml (Table 2).

Table 1. Antifungal activity of root extracts against *Candida albicans* (Mean Zone of Inhibition in mm ± Standard Error)

Plants	Concentrations (mg/ml)	Control	Aqueous	Ethnol	n-Hexane
M. oleifera	100	16±0.02a	14±0.1a	14±0.05b	14±0.06a
	75	16±0.07a	12±0.02b	14±0.07b	13±0.40b
	50	16±0.01a	10±0.08	12±0.06c	10±0.10d
	25	16±0.08a	08±0.07d	10±0.50d	08±0.06
Z. abyssinica	100	16±0.08a	12±0.05b	16±0.04a	14±0.03a
	75	16±0.08a	10±0.04c	14±0.06b	12±0.01c
	50	16±0.03a	08±0.40d	12±0.20c	10±0.07d
	25	16±0.01a	06±0.06e	10±0.07d	09±0.03e

Values with different superscripts in a column are significantly different ($p < 0.05$). The control was Fluconazole at a standard concentration.

Table 2. Minimum Inhibitory Concentration (MIC) of active extracts

Plant Samples	Extract	25mg/ml	50mg/ml	75mg/ml	100mg/ml
Moringa oleifera	Aqueous	++	+	+	-
	Ethanol	+	+	-	-
Ziziphus abyssinica	Aqueous	+++	++	+	-
	Ethanol	++	+	+	-

Key: +++ = Highly Turbid; ++ = Moderately Turbid; += Slightly Turbid; -= Clear (No Growth)

GC-MS Analysis

GC-MS analysis revealed a diverse profile of bioactive compounds in the root extracts of both plants. The analysis identified thirty-three (33) chemical constituents in *Ziziphus abyssinica* and twenty-eight (28) in *Moringa oleifera.*

For *Ziziphus abyssinica*, the major bioactive components were beta-Sitosterol (42.72%), a sesquiterpenoid derivative (3a,7-Methano-3aH-cyclopentacyclooctene..., 18.13%), Valerenol (15.16%), Erucic acid (11.85%), and a phenolic compound (4,4'-((2R,3S)-2,3-Dimethylbutane-1,4-diyl)bis (2-methoxyphenol), 10.23%). The ten most abundant compounds are summarized in Table 3.

For *Moringa oleifera*, the extract was dominated by a complex pentacyclic compound (42.87%), 16-Pregnenolone (13.97%), a phthalate ester (11.45%), and a nitrogenous compound (9-Azabicyclo [4.2.1] nonane, 7.52%). The key compounds are listed in Table 4.

Table 3. Major bioactive compounds identified in *Ziziphus abyssinica* root extract by GC-MS analysis.

Peak	Retention Time (min)	Area (%)	Compound Name	Molecular Formular	Weight (g/mol)
1	35.203	42.72	beta-Sitosterol	$C_{29}H_{50}O$	414.71
2	20.2797	18.13	3a,7-Methano-3aH-cyclopentacyclooctene,1,4,5,6,7,8,9,9a-octahydro-1,1,7-trimethyl	$C_{15}H_{24}$	220.35
3	20.2319	15.16	Valerenol	$C_{15}H_{24}O$	220.35
4	38.5109	11.85	Erucic Acid	$C_{22}H_{42}O_2$	338.57
5	33.6817	10.23	4,4'-((2R,3S)-2,3-Dimethylbutane-1,4-diyl)bis(2-methoxyphenol)	$C_{20}H_{26}O_4$	330.42

6	33.7375	6.23	4,4'-((2R,3S)-2,3-Dimethylbutane-1,4-diyl)bis (2-methoxyphenol) (Isomer)	$C_{20}H_{26}O_4$	330.42
7	330.42	2.88	1-Cyclododecanol, aminomethyl-	$C_{13}H_{27}NO$	213.36
8	35.4828	1.95	Ergosta-4,22-dien-3-one	$C_{28}H_{44}O$	396.65
9	36.4458	0.81	9-Octadecen-1-ol, (Z)	$C_{18}H_{36}O$	268.48
10	36.5424	0.72	Phenanthrene, tetradecahydro-	$C_{14}H_{24}$	192.34

Table 4. Major bioactive compounds identified in *Moringa oleifera* root extract by GC-MS analysis

Peak	Retention Time (min)	Area (%)	Compound Name	Molecular Formular	Weight (g/mol)
1	37.999	42.87	Pentacyclo [19.3.1.1(3,7).1(9,13).1(15,19)] octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecaene-25,26,27,28-tetrol, 5,11,17,23-tetrakis(1,1-dimethylethyl) -	- *	-
2	37.9278	13.97	16-Pregnenolone	$C_{21}H_{32}O_2$	316.50
3	20.7007	11.45	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	$C_{20}H_{30}O_4$	334.46
4	5.7347	7.52	9-Azabicyclo[4.2.1] nonane, 9-methyl-	$C_9H_{17}N$	139.24
5	30.5422	4.73	Cycloeicosane	$C_{20}H_{40}$	280.53
6	30.6767	3.45	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}$	280.50
7	30.9314	2.49	Oleic Acid	$C_{18}H_{34}O_2$	282.46
8	33.6717	2.02	Methyl tetratriacontyl ether	$C_{35}H_{72}O$	508.96
9	25.3195	1.51	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.45
10	30.5913	30.5913	3-Eicosene, (E)-	$C_{20}H_{40}$	280.53

DISCUSSION

The superior efficacy of ethanol extracts aligns with the findings of Tiwari *et al.* (2011) and Bashir *et al.* (2017), who noted that ethanol is a potent solvent for extracting a wide range of phytochemicals. The observed dose-dependent inhibition is a classic indicator of bioactive compound presence. Notably, the ethanol extract of Z. abyssinica at 100 mg/ml exhibited an inhibition zone equal to that of the standard antifungal drug, Fluconazole, highlighting its potent efficacy. The results corroborate previous studies of Chepuri *et al.* (2018) on *M. oleifera* and that of Nyaberi *et al.* (2010) on *Z. abyssinica*, which reported antimicrobial properties in other plant parts. This study, however, provides specific evidence for the efficacy of their root extracts, validating their use in traditional medicine for treating infections.

The potent activity can be attributed to the rich phytochemical profile identified by GC-MS. Beta-Sitosterol, the most abundant compound in *Z. abyssinica*, is known for its antimicrobial and anti-inflammatory properties (Ahmad *et al.*, 2014). Valerenol has documented bioactive potential, and Erucic acid derivatives have been associated with antimicrobial effects. In *M. oleifera*, 16-Pregnenolone, a steroid, may contribute to the antifungal activity, as various steroids are known to disrupt fungal membrane integrity. The presence of these compounds, along with others like flavonoids and terpenoids (inferred from the complex structures identified), likely acts synergistically to inhibit fungal growth, potentially by disrupting cell membranes or inhibiting essential enzymes (Gavamukulya *et al.,* 2014).

CONCLUSION

This study conclusively demonstrates that the ethanol root extracts of *Moringa oleifera* and *Ziziphus abyssinica* are promising sources of novel antifungal agents against *Candida albicans*, warranting further investigation for development into therapies for drug-resistant candidiasis. Base on the finding of this study, it is recommended that further work focus on in-vivo validation, compound purification synergy testing with conventional drugs, mechanic studies

and public health education translate this finding into practical antifungal solutions

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