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Evaluation Of Antifungal Activity of Selected Plant Extracts Against *Candida krusei* Isolated from Urinary Tract Infections

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Abstract

This study evaluated the antifungal activity of Allium sativum (garlic), Allium cepa (onion), and Citrus limon (lemon) extracts against Candida krusei isolated from urinary tract infections (UTIs). Plant materials were extracted using ethanol, chloroform, and hexane via Soxhlet extraction. Phytochemical screening was conducted to identify bioactive constituents. Urine samples from patients were cultured on Sabouraud Dextrose Agar, and isolates were identified using morphological and molecular methods (18S rRNA sequencing). Ethanolic extracts showed higher concentrations of phytochemicals, particularly alkaloids and flavonoids. The ethanolic garlic extract demonstrated the highest antifungal activity, with zones of inhibition comparable to ketoconazole. The findings suggest that solvent type significantly influences phytochemical yield and antifungal efficacy. These plant extracts, especially garlic, may serve as potential alternative antifungal agents.

Keywords: Antifungal activity, Candida krusei, phytochemicals, plant extracts, urinary tract infection

INTRODUCTION

Urinary tract infections (UTIs) remain among the most common infectious diseases worldwide, affecting over 150 million people annually and contributing significantly to morbidity, healthcare costs, and antimicrobial use (Flores-Mireles et al., 2019; Medina & Castillo-Pino, 2019). Although bacterial pathogens such as Escherichia coli are the primary causes of UTIs, fungal infections—particularly those caused by Candida species—are increasingly reported, especially among immunocompromised individuals, catheterized patients, diabetics, and hospitalized populations (Kauffman, 2020; Pappas et al., 2020).

Among Candida species, Candida albicans remains the most frequently isolated pathogen in cases of candiduria, accounting for a significant proportion of fungal UTIs (Kauffman, 2020; Bongomin et al., 2020). However, non-albicans Candida species are also emerging, some of which demonstrate reduced susceptibility to commonly used antifungal agents (Pappas et al., 2020).

The increasing emergence of antifungal resistance and the limited number of effective antifungal drug classes pose serious global health concerns (Fisher et al., 2022; WHO, 2022). The overuse and misuse of antifungal medications have accelerated the development of resistant strains, reducing treatment effectiveness and increasing mortality rates (Arastehfar et al., 2020). These challenges highlight the urgent need for alternative, affordable, and effective antifungal agents.

Natural products, including medicinal plants, have gained renewed attention as potential sources of novel

antimicrobial compounds. Plants such as garlic (Allium sativum), onion (Allium cepa), and lemon (Citrus limon) have been widely reported to possess antimicrobial, antioxidant, and anti-inflammatory properties due to their rich phytochemical composition (Batiha et al., 2020; Sharifi-Rad et al., 2021).

Garlic contains bioactive compounds such as allicin, diallyl sulfide, and diallyl disulfide, which exhibit strong antifungal and antibacterial activities (Batiha et al., 2020). Onion contains sulfur-containing compounds including thiosulfinates and cepaenes known for antimicrobial properties (Fredotović et al., 2021). Lemon is rich in citric acid, flavonoids, and essential oils that demonstrate inhibitory effects against several microbial pathogens (García-Cayuela et al., 2019).

Despite extensive studies on their antibacterial effects, fewer investigations have focused specifically on their antifungal activity against Candida albicans in urinary tract infections. Given the rising prevalence of fungal UTIs and antifungal resistance, investigating this plant extracts may contribute to alternative therapeutic strategies (Fisher et al., 2022; WHO, 2022).

Epidemiological data indicate that serious fungal infections affect over one billion people globally and cause more than 1.5 million deaths annually (Bongomin et al., 2020). The increasing burden of antifungal resistance further emphasizes the necessity to explore medicinal plants as potential sources of new antifungal agents (Arastehfar et al., 2020).

Justification of the Study

In Nigeria, fungal infections contribute significantly to disease burden, yet antifungal treatment options remain limited. Additionally, fungal UTIs are often underdiagnosed. Therefore, there is a need to explore plant-based antifungal agents that are affordable, accessible, and effective. The aim of the study was to evaluate the antifungal activity of selected plant extracts against Candida krusei isolated from UTIs.

MATERIALS AND METHODS

Study Area

The study was conducted at Abubakar Imam Urology Centre, Kano, Nigeria. Ethical approval was obtained from the Kano State Ministry of Health.

Sample Collection and Isolation

Urine samples were collected aseptically and cultured on CHROMagar and Sabouraud Dextrose Agar. Plates were incubated at 35–37°C for 24–48 hours. Colonies were identified based on morphology and microscopy.

Molecular Identification

Confirmed isolates were subjected to 18S rRNA sequencing for accurate identification.

Plant Extraction

Garlic, onion, and lemon were washed, dried, and pulverized. Extraction was carried out using ethanol, chloroform, and hexane via Soxhlet apparatus. Extracts were concentrated and stored at 4°C.

Phytochemical Screening

Extracts were screened for:

Alkaloids

Flavonoids

Saponins

Tannins

Phenols

Glycosides

Antifungal Activity

The agar well diffusion method was used. Extract concentrations ranged from 400 to 50 mg/mL. Ketoconazole served as positive control.

Minimum Inhibitory Concentration (MIC)

MIC was determined using broth dilution. The lowest concentration inhibiting visible growth was recorded.

Statistical Analysis

Data were analyzed using SPSS version 20. ANOVA was used with significance set at $p < 0.05$.

RESULTS AND DISCUSSION

Identification of Isolates, Candida krusei was identified based on Creamy colonies, with the budding yeast cells and Presence of pseudohyphae, 94.5% sequence similarity with reference strain.

Ethanolic extracts showed the highest phytochemical content. Alkaloids and flavonoids were more abundant in garlic and onion extracts, while lemon extracts contained higher phenolic compounds. The ethanolic garlic extract showed the highest antifungal activity (up to 24.91 mm inhibition zone). Hexane extracts showed minimal activity. Onion chloroform extract and lemon hexane extract demonstrated moderate activity.

Ketoconazole showed comparable activity but was slightly lower than garlic extract at higher concentrations. MIC values ranged from 12.5 mg/mL to 100 mg/mL, with garlic extract showing the lowest MIC (highest potency).

Table 1: Isolates confirmation based on the Cultural, Morphological Characteristics and 18S rRNA Sequencing

Isolate	Cultural Characteristics	Microscopic Characteristics	Sequence (bp)	% Identity	Accession No.
Candida krusei (*Pichia kudriavzevii*) strain NG-7	Whitish, creamy-colored, smooth, shiny colonies	Spherical yeast cells with multiple buds and pseudohyphae	290	94.5%	KM016456.1

Keys: bp – base pairs; (%) – percentage.

Table 2: Qualitative Phytochemicals and Physical Screening of Garlic, Onion and Lemon Extracts Using Ethanol, Hexane and Chloroform

	Garlic Extracts			Lemon Extracts			Onion Extracts		
Phytochemicals	**EGE**	**HGE**	**CGE**	**ELE**	**HLE**	**CLE**	**EOE**	**HOE**	**COE**
Alkaloids	+	-	+	+	+	-	-	-	-
Flavonoids	+	+	-	-	-	-	+	+	+
Glycosides	+	-	+	-	-	-	-	+	-
Phenols	-	-	+	+	+	+	-	-	-
Reducing sugar	-	+	+	+	+	+	+	+	+
Saponins	+	-	+	+	+	+	+	+	+
Steriods	-	+	-	+	+	-	-	-	-
Tannins	+	+	-	+	+	+	+	+	-
Terpenoids	-	+	-	-	-	-	+	+	-
Texture	VL	VL	VL	Gm	Ol	Gm	VL	VL	VL
Colour	PY	Cl	YB	Br	Yl	Br	PY	PY	PY
Odors	GS	GS	GS	LS	LS	LS	OS	OS	OS

Key Words; EGE, HGE, CGE – ethanolic, hexanolic, and chloroform garlic extracts, EOE, HOE, and COE – ethanolic, hexanolic, and chloroform onion extracts, ELE, HLE and CLE – ethanolic, hexanolic and chloroform lemon extracts, Cl – Colorless, VL – Viscous Liquid, Gm – Gummy, Ol – Oily, Yl – Yellow, Br – Brown, YB-Yellowish Brown, PY – Pale Yellow, GS – Garlic Scent, LS – Lemon Scent and OS – Onion Scent

Table 3: Antifungal Activities of Ethanol, Hexane, and Chloroform Extracts of Garlic, Lemon, and Onion against *Candida krusei*

Conc. (mg/ml)	GEE	GEH	GEC	LEE	LEH	LEC	OEE	OEH	OEC	KE
400	24.91 ± 0.04[a]	5.00 ± 0.00[a]	13.22 ± 0.08[a]	5.00 ± 0.00[a]	18.39 ± 0.37[a]	15.35 ± 0.20[a]	14.88 ± 0.05[b]	5.00 ± 0.00[a]	18.14 ± 0.03[b]	20.25 ± 0.04[a]
200	20.64 ± 0.04[a]	5.00 ± 0.00[a]	10.66 ± 0.03[a]	5.00 ± 0.00[a]	16.74 ± 0.04[a]	14.30 ± 0.04[a]	11.76 ± 0.04[a]	5.00 ± 0.00[a]	16.76 ± 0.04[b]	—
100	16.25 ± 0.04[a]	5.00 ± 0.00[a]	8.35 ± 0.04[a]	5.00 ± 0.00[a]	15.32 ± 0.28[a]	11.43 ± 0.06[a]	9.58 ± 0.02[a]	5.00 ± 0.00[a]	11.53 ± 0.03[a]	—
50	11.33 ± 0.01[a]	5.00 ± 0.00[a]	5.00 ± 0.00[a]	5.00 ± 0.00[a]	12.47 ± 0.16[a]	5.00 ± 0.00[a]	9.56 ± 0.04[a]	5.00 ± 0.00[a]	5.00 ± 0.00[a]	—

Keys; GE, LE, OE, KE – Garlic extracts, lemon extracts, Onion extract, Ketoconazole (Control – 200mg**)** ([a,b]) – means ± standard deviation with different superscripts differ significantly at p(.05).

DISCUSSION

The study demonstrates that plant extracts possess significant antifungal activity against Candida krusei. The superior performance of ethanolic garlic extract confirms the importance of solvent polarity in extracting active compounds.

Garlic's antifungal activity is attributed to allicin, which disrupts fungal cell membranes. Onion and lemon also showed activity due to flavonoids and terpenoids.

The findings align with previous studies indicating that medicinal plants can serve as alternative antifungal agents, particularly in regions with limited access to conventional drugs.

CONCLUSION

This study confirms that Candida krusei is a significant fungal pathogen in UTIs. It also demonstrates that plant extracts—especially ethanolic garlic extract—have strong antifungal activity.

The results support the potential use of these plants as alternative or complementary antifungal therapies. Further studies, including clinical trials, are recommended.

RECOMMNDATIONS

1. Further in vivo studies should be conducted.
2. Standardization of plant extracts is necessary.
3. Clinical trials should be performed.
4. Combination therapy should be explored.

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