Comparative *In Vitro* Antifungal Activities of *Zingiber officinale*, *Justicia carnea*, *Hibiscus sabdariffa* Nanosynthesized Extracts against Uropathogenic *Candida albicans*

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Abstract: Urogenital infections caused by *Candida* species (candiduria) are a growing public health concern, exacerbated by the rise of antifungal-resistant strains. This necessitates the search for novel, effective antifungal agents, with medicinal plants being a promising source. Ethanol, chloroform, and hot water (aqueous) extracts of *Zingiber officinale* (rhizome), *Justicia carnea* (leaf), and *Hibiscus sabdariffa* (calyx) sourced from Akure, Ondo State, were prepared and tested at 200 mg/ml against uropathogenic *Candida albicans* isolated from midstream urine samples of patients attending government hospitals in Ondo State. The Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were determined for the active extracts using a broth microdilution method. Green synthesis of silver nanoparticles (AgNPs) using *H. sabdariffa* was also characterized and assessed. The hot water extract of *J. carnea* and the ethanol extract of *H. sabdariffa* demonstrated the largest zones of inhibition (29.00±0.58 mm) against the isolate. The hot water extract of *Z. officinale* also showed significant activity, with a peak inhibition of 18.67±0.33 mm. Nanoparticles synthesized with *H. sabdariffa* showed potent activity, peaking at 24.00±0.58 mm. *Z. officinale* aqueous extract exhibited the most potent fungicidal activity, with an MFC of 100 mg/ml. The broad-spectrum fungicidal activity of *Z. officinale* and *H. sabdariffa* aqueous extracts highlights their potential as therapeutic agents. These findings indicate that nanosynthesized and polar extracts of these indigenous plants offer a viable alternative for developing novel antifungal therapeutics against multidrug-resistant candiduria.

Key word: Antifungal**,** *Candida*, Candiduria, Medicinal plants**,** Uropathogens

INTRODUCTION

Fungal infections represent a significant and increasing threat to global public health, with *Candida* species standing as the most common causative agent of opportunistic mycoses in humans (Piancson *et al.*, 2023). Candidiasis, particularly urogenital infections (candiduria) caused by *Candida* strains, is frequently associated with high morbidity, especially in immunocompromised patients, diabetics, and those with indwelling catheters (Alshami and Alharbi, 2024; Gavanji *et al.*, 2025). The therapeutic landscape is further complicated by the emergence of strains resistant to conventional azole antifungals, such as fluconazole, creating an urgent need for novel antifungal drugs (Ake *et al.*, 2023). *Candida* species stand as the most common causative agent of opportunistic mycoses in humans (Alshami and Alharbi, 2024). While *Candida albicans* remains the primary etiological agent responsible for the majority of cases, the prevalence of non-*albicans* species such as *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis* is increasing, particularly in complicated urinary tract infections (Alshami and Alharbi, 2024; Gavanji *et al.*, 2025).

Medicinal plants have historically been a rich repository of bioactive compounds and serve as a primary source for drug discovery. Their complex phytochemical mixtures often provide a multi-target approach to antimicrobial activity, potentially bypassing common resistance mechanisms (Hussein and El-Anssary 2018). The rhizome of *Zingiber officinale* is used globally as a spice and a remedy for various ailments. Its antimicrobial properties are well-documented, with modern research attributing its potent antifungal activity against *Candida* to principal phytochemicals like gingerol and shogaol (Gavanji *et al.*, 2025). *Hibiscus sabdariffa* (Roselle) calyces are popularly consumed as a beverage (*zobo*)

and are traditionally used, particularly in Nigeria, for treating urinary tract infections (UTIs) and hypertension. Studies have validated its efficacy against uropathogenic *C. albicans*, linking its activity to a high content of polyphenols (e.g., gossypetin, hibiscetin) and organic acids, which are known to inhibit fungal growth and biofilm formation (Dgheim *et al*., 2023; Alshami and Alharbi, 2024). *Justicia carnea* known as the "flamingo flower," is used in traditional medicine, particularly for its hematinic (blood-boosting) properties. While less studied for its antimicrobial effects, phytochemical analyses confirm it is a rich source of flavonoids, alkaloids, and saponins, which are compound classes known to possess antifungal properties (Oloruntola *et al*., 2024; Ajuru *et al*., 2025). Numerous plant-based phytochemicals, including tannins, alkaloids, flavonoids, saponins, and other metabolites, are important for the synthesis of nanomaterials with important applications (Kaushik *et al*., 2023). Plant nanosynthesis has diverse applications, including in medicine for drug delivery and imaging, in agriculture for enhanced fertilizers and pesticides, and in environmental management for pollutant removal. While individual studies on these plants exist, comparative data on their antifungal efficacy using different extraction solvents against a panel of uropathogenic *Candida* isolates is limited. This study aims to provide a comprehensive *in vitro* comparison of the inhibitory and fungicidal potential of *Z. officinale*, *J. carnea*, and *H. sabdariffa* as well as nanosynthesized extracts, thereby validating their ethnobotanical use and assessing their potential as sources for novel antifungal agents.

MATERIALS AND METHODS

Ethical Consideration and Sample Collection: The ethical approval was issued by the Ondo State Health research Ethics Committee (OSHREC) with the protocol number OSHREC 23/9/2021/385. Permission was also sought from the Permanent Secretary of the Hospital Management Board, for the usage of the facilities in selected General hospitals with Ondo State. Informed consent was also used, to assure the confidentiality of their results and also the opportunity to withdraw at any time from the study. Participants were provided with sterile, wide-mouthed universal containers and instructed on collecting a 10–20 mL clean-catch midstream urine (MSU) sample. All urine samples were transported to the laboratory immediately. Samples that could not be processed within two hours of collection were refrigerated at 4 °C and processed within 24 hours to prevent bacterial overgrowth (Cheesbrough, 2006).

A total of 535 participants were recruited for the larger study, comprising 182 (34%) males and 353 (66%) females. The age range of the participants was 10–50 years. Socio-demographic data, including educational status, occupation, and access to sanitary facilities, were captured via structured questionnaires (Table 1).

Fungal Isolation, Culture and Identification: Following collection, urine samples were vortexed for gentle mixing. An inoculating loop was dipped vertically into the urine and inoculated onto Sabouraud Dextrose Agar (SDA) Supplemented with chloramphenicol (50 mg/L) to inhibit bacterial contamination. Chromogenic *Candida* Agar (CHROMagar™ Candida) was used for the differential, presumptive identification of common *Candida* species. The plates were incubated aerobically at 37 °C for 48 to 72 hours. Colony counts were performed after incubation. A colony count of ≥ 10^4 CFU/mL was considered indicative of significant candiduria (Kauffman, 2005). Colonies exhibiting yeast-like morphology (creamy, white-to-beige, opaque, smooth) on SDA were selected for further analysis. Gram Staining technique was performed on a smear prepared from a pure colony, heat-fixed, and stained (Cheesbrough, 2006). Colony pigmentation on the chromogenic medium was recorded as a primary differential characteristics. Germ tube test

was performed for the rapid presumptive identification of *C. albicans*. A small inoculum from a pure colony was suspended in 0.5 mL of human or sheep serum and incubated at 37 °C for 2–3 hours. A drop was placed on a slide, covered with a coverslip, and observed under × 40 magnification. A positive test was indicated by the formation of long, non-septate filamentous extensions (germ tubes) originating directly from the yeast cell without constriction at the base. To confirm the identity of all isolates (including germ-tube-negative and non-*albicans* species from CHROMagar) (Table 2), a standardized commercial biochemical assimilation panel, such as the API 20C AUX (bioMérieux), was used. Isolates were suspended in the provided medium to a 2.0 McFarland standard and used to inoculate the 20 microtubes containing dehydrated carbohydrate substrates.

Fresh samples of *Hibiscus sabdariffa* (calyx), *Justicia carnea* (leaves), and *Zingiber officinale* (rhizomes) were obtained from Akure metropolis, Ondo State. The plants were identified and authenticated at the Herbarium of the University of Ibadan with voucher numbers UIH-23344 (*H. sabdariffa*), UIH-23356 (*J. carnea*), and UIH-23346 (*Z. officinale*). Honey was obtained from the School of Agriculture, Federal University of Technology, Akure (FUTA). The plant materials were air-dried and soaked for 72 h with intermittent agitation. The plates were incubated aerobically at 37 °C for 48 to 72 h.

Plant Extract Preparation: Ethanol, chloroform and hot water extracts were derived from *H. sabdariffa* calyx, *J. carnea* leaves, and *Z. officinale* rhizomes. The plant materials were air-dried at room temperature for 7–10 days to constant weight, then ground into fine powder using a mechanical blender. The dried, powdered material (100 g) was mixed with the respective solvents at a 1:10 ratio (100 g in 1000 mL of solvent) for each solvent and soaked for 72 hours with intermittent agitation to facilitate extraction. The mixture was then filtrated/sieved using muslin cloth followed by Whatman No. 1 filter paper to remove solid residues (Kaur *et al*., 2021; Peiris *et al*., 2023). The filtrates were concentrated under vacuum using a rotary evaporator (RE-52A, Union Laboratories, England) at 37°C to obtain the crude extracts. The 100% stock concentrates of the extracts were obtained and stored in a refrigerator at 4°C in sterile universal bottles until further use. Silver nanoparticle (AgNP) synthesis of *H. sabdariffa* involved preparing an aqueous solution (1 mM) of silver nitrate ($AgNO_3$) in 250 mL Erlenmeyer flasks, to which plant extract was added for reduction into Ag+ ions for each type of plant extracts. The composite mixture was kept on turntable of the magnetic hot plate or microwave oven for complete bioreduction at a power of 300 W for 4 min discontinuously to prevent an increase of pressure. In the meantime, the colour change of the mixture from faint light to yellowish brown to reddish brown to colloidal brown was monitored periodically for maximum 30 min. The reactions were carried out in dark (to avoid photoactivation of $AgNO_3$) at room temperature. Complete reduction of $AgNO_3$ to Ag^+ ions was confirmed by the change in colour from colourless to colloidal brown. After irradiation, the dilute colloidal solution was cooled to room temperature and kept aside for 24 hrs for complete bioreduction and saturation. Then, the colloidal mixture was sealed and stored properly for further use.

Preparation of Fungal Isolates and Inoculum: The fungal isolates were first grown on Sabouraud Dextrose Agar (SDA) plates for 24-48 hours to ensure viable, active cultures. A standardized inoculum was then prepared. Fungal colonies were collected and suspended in sterile saline. The turbidity of this suspension was adjusted to match the 0.5 McFarland standard to ensure a consistent concentration of fungal cells for the assay.

Agar Well Diffusion Assay (Antifungal Susceptibility): The standardized fungal inoculum was uniformly swabbed onto the entire surface of fresh, sterile SDA plates.

Using a sterile cork borer, wells were punched into the agar. A fixed volume of each plant extract (Ethanol, Chloroform, and Hot Water) at a concentration of 200 mg/ml was dispensed into a separate well. Controls include Ketoconazole (positive control), and DMSO (negative control). The plates were incubated at 30°C for 24-48 hours. Antifungal activity was assessed by measuring the diameter of the complete zone of inhibition (in mm) around each well.

Minimum Inhibitory Concentration (MIC) Determination: A broth microdilution method was used to determine the MIC. Serial dilutions of the active plant extracts were prepared in Sabouraud Dextrose Broth (SDB) within a 96-well microtiter plate. The final concentrations tested in the wells were 100 mg/ml, 50 mg/ml, and 25 mg/ml. Each well was then inoculated with the standardized fungal inoculum. After incubation, the MIC was recorded as the lowest extract concentration that showed no visible fungal growth (no turbidity).

Minimum Fungicidal Concentration (MFC) Determination: The MFC was determined using the results from the MIC test. Aliquots were taken from all wells in the MIC plate that showed no visible growth (wells at the MIC and above). These aliquots were sub-cultured onto new, extract-free SDA plates. The plates were incubated for another 24-48 hours. The MFC was defined as the lowest original extract concentration from which no fungal colonies grew on the new SDA plate, indicating that the fungi had been killed rather than just inhibited.

Statistical Analysis: All experiments were conducted in triplicate (n=3). The results are expressed as Mean ± Standard Error (S.E.). The statistical significance of the differences between the inhibition zones of different extracts was determined using Tukey's Honestly Significant Difference (HSD) test. A P-value greater than 0.05 ($P > 0.05$) was considered not statistically significant.

RESULTS

Antifungal Activity of *Zingiber officinale* on Uropathogenic *Candida albicans*

Table 3 shows that the antifungal activity of *Z. officinale* at 200 mg/ml is highly dependent on the extraction solvent. The hot water (aqueous) extract (ZOH) was the most effective, demonstrating broad-spectrum activity against all tested *Candida* strains, with its largest zone of inhibition (18.67 ± 0.33 mm) observed against *Candida I*. The ethanol extract (ZOE) showed only moderate and selective activity, with a peak inhibition of 11.33 ± 0.67 mm against *Candida IX*. The chloroform extract (ZOC) was completely ineffective, recording 0.00 ± 0.00 mm inhibition for all strains. Notably, the standard Ketoconazole control also showed no inhibition (0.00 ± 0.00 mm).

Antifungal Activity of *Justica carnea* on Uropathogenic *Candida albicans*

The hot water extract (JCH) of *J. carnea* demonstrated exceptional antifungal potency at 200 mg/ml. It produced the largest zones of inhibition (29.00 ± 0.58 mm) against both *Candida VI* and *Candida VII*. The ethanol extract (JCE) also showed very strong but highly selective activity, producing a 23.00 ± 0.58 mm zone against *Candida IV* but showing no activity (0.00 ± 0.00 mm) against three other strains. This suggests the active compounds are most effectively extracted by hot water. The chloroform extract (JCC) was almost completely inactive, showing only a minimal 5.00 ± 0.00 mm zone against *Candida VI* (Table 4)

Antifungal Activity of *Hibiscus sabdariffa* on Uropathogenic *Candida albicans*

The ethanol extract (HSE) of *H. sabdariffa* was the most potent extract at 200 mg/ml, exhibiting broad and strong activity against all *Candida* strains. It achieved a 29.00 ± 0.58 mm zone of inhibition against *Candida IX* and maintained significant inhibition across all isolates, with its lowest being 15.00 ± 0.58 mm. The hot water extract (HSH) was also consistently effective, with a peak inhibition of 18.00 ± 0.00 mm against *Candida I*. As with the other plants, the chloroform extract (HSC) was completely inactive (0.00 ± 0.00 mm) as shown in Table 5.

Comparative Fungal Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) for the fungal isolates, revealed concentrations ranges from 100 mg/mL to 200 mg/mL. The most potent MIC was 100 mg/mL for *Z. officinale* aqueous extract against *Candida IV*, and an MIC of 100 mg/mL for the *H. sabdariffa* aqueous extract against *Candida IX*. Several extracts, such as the *J. carnea* ethanol extract, showed no detectable inhibition (ND) against certain strains even at the highest concentrations tested (200 mg/ml) as shown in Table 6.

Comparative Minimum Fungicidal Concentration

The aqueous (hot water) extract of *Zingiber officinale* (B) was the most effective, demonstrating a broad-spectrum fungicidal effect at a concentration of 100 mg/mL. The aqueous extract of *H. sabdariffa* was also highly potent at MFC of 100 mg/mL against the isolates. *J. carnea* ethanol extract was not fungicidal (ND) against any strain at the tested concentrations, indicating its action is primarily inhibitory (fungistatic) rather than fungicidal as shown in Table 7.

Antifungal Activity of *H. sabdariffa* Nanoparticles on Uropathogenic *Candida albicans*

The antifungal efficacy of *H. sabdariffa* was enhanced on synthesized nanoparticles extracts. The activity was strongly concentration-dependent, i.e. 1 M concentration was the most effective (24.00 ± 0.58 mm), followed by 50 mM, and 25 mM. Most importantly, the 1M nanoparticle solution was far more effective than Ketoconazole (10.33 ± 0.33 mm for *Candida IV*) and *H. sabdariffa* hot water extract (HSH) without nanoparticle synthesis as shown in Table 8.

Table 1: Demographic and data collection

Parameter	Statistics / Description
Total Participants	535
Gender Distribution	Male: 182 (34%); Female: 353 (66%)
Age Range	10–50 years
Data Captured	Socio-demographics (Educational status, Occupation, Access to sanitary facilities)
Instrument	Structured Questionnaires

Table 2: Morphological and biochemical characterization of uropathogenic *Candida albicans*

Test Parameter	Observation / Result	Inference
Macroscopy (SDA)	Creamy, white-to-beige, opaque, smooth, and convex colonies.	Yeast isolate
Microscopy (Gram Stain)	Large, Gram-positive, ovoid budding yeast cells (blastoconidia).	*Candida* sp.
Chromogenic Agar (CHROMagar™)	Distinctive emerald-green colored colonies.	*Candida albicans*
Germ Tube Test	Formation of germ tubes without constriction at the mother cell junction.	Positive (*C. albicans*)
Biochemical Assay (API 20C AUX)	Sugar assimilation profile yielding a specific 7-digit code.	*C. albicans* confirmed

Table 3: Antifungal activity of *Zingiber officinale* (ZO) at 200 mg/ml on uropathogenic *Candida albicans*

Fungal Isolates	ZOE (Ethanol)	ZOC (Chloroform)	ZOH (Hot Water)	Honey	Ketoconazole (Control)	DMSO (Control)
Candida I	11.00 ± 0.58^{b}	0.00 ± 0.00^{a}	18.67 ± 0.33^{c}	10.33 ± 0.33^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Candida IV	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	17.00 ± 0.58^{c}	10.67 ± 0.33^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Candida VI	5.00 ± 0.00^{b}	0.00 ± 0.00^{a}	15.00 ± 0.58^{c}	13.33 ± 0.88^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Candida VII	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	11.33 ± 0.33^{b}	14.33 ± 0.33^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Candida IX	11.33 ± 0.67^{bc}	0.00 ± 0.00^{a}	13.00 ± 1.00^{c}	8.67 ± 0.88^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same rows are not significantly different ($P>0.05$) according to Tukey's Honestly Significant Difference
Keys: ZOE- *Z. officinale* ethanol, ZOC- *Z. officinale* chloroform, ZOH- *Z. officinale* hot water, DMSO - Dimethyl sulfoxide

Table 4: Antifungal activity of *Justica carnea* (JC) at 200 mg/ml on uropathogenic *Candida albicans*

Fungal Isolates	JCE (Ethanol)	JCC (Chloroform)	JCH (Hot Water)	Honey	Ketoconazole (Control)	DMSO (Control)
Candida I	10.33 ± 0.33^{b}	0.00 ± 0.00^{a}	18.67 ± 0.67^{c}	11.00 ± 0.58^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Candida IV	23.00 ± 0.58^{d}	0.00 ± 0.00^{a}	14.67 ± 0.33^{c}	12.00 ± 0.58^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Candida VI	0.00 ± 0.00^{a}	5.00 ± 0.00^{b}	29.00 ± 0.58^{d}	13.67 ± 0.88^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Candida VII	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	29.00 ± 0.58^{c}	13.67 ± 0.88^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Candida IX	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	23.00 ± 0.58^{d}	13.67 ± 0.33^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same rows are not significantly different (*P*>0.05) according to Tukey's Honestly Significant Difference
Keys: JCE- *J. carnea* ethanol, JCC- *J. carnea* chloroform, JCH- *J. carnea* hot water, DMSO - Dimethyl sulfoxide

Table 5: Antifungal activity of *Hibiscus sabdariffa* (HS) calyx at 200 mg/ml on uropathogenic *Candida albicans*

Fungal Strain	HSE (Ethanol)	HSC (Chloroform)	HSH (Hot Water)	Honey	Ketoconazole (Control)	DMSO (Control)
Candida I	21.67 ± 0.33^{d}	0.00 ± 0.00^{a}	18.00 ± 0.00^{b}	20.00 ± 0.00^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Candida IV	15.00 ± 0.58^{b}	0.00 ± 0.00^{a}	15.67 ± 0.33^{c}	13.67 ± 0.67^{bc}	11.67 ± 0.88^{b}	0.00 ± 0.00^{a}
Candida VI	23.67 ± 0.33^{c}	0.00 ± 0.00^{a}	14.00 ± 0.58^{b}	14.00 ± 0.58^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Candida VII	20.67 ± 0.33^{c}	0.00 ± 0.00^{a}	15.67 ± 0.33^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Candida IX	29.00 ± 0.58^{c}	0.00 ± 0.00^{a}	10.33 ± 0.33^{b}	11.00 ± 0.58^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same rows are not significantly different (*P*>0.05) according to Tukey's Honestly Significant Difference
Keys: HSE- *H. sabdariffa* ethanol, HSC- *H. sabdariffa* chloroform, HSH- *H. sabdariffa* hot water, DMSO - Dimethyl sulfoxide

Table 6: Comparative minimum inhibitory concentration (MIC) of plant extracts on uropathogenic *Candida albicans*

Isolates	ZOE (mg/ml)	ZOH (mg/ml)	HSE (mg/ml)	HSH (mg/ml)	JCE (mg/ml)	JCH (mg/ml)
Candida I	200	200	200	200	200	200
Candida IV	ND	100	200	200	200	200
Candida VI	ND	200	200	200	200	200
Candida VII	ND	200	200	200	ND	200
Candida IX	200	200	200	100	ND	200

Keys: ZOE - *Z. officinale* Ethanol, ZOH - *Z. officinale* Aqueous, HSE - *H. sabdariffa* Ethanol, HSH - *H. sabdariffa* Aqueous, JCE - *J. carnea* Ethanol, JCH - *J. carnea* Aqueous, ND - Not Detectable

Table 7: Comparative minimum fungicidal concentration (MFC) of plant extracts on uropathogenic *Candida albicans*

Isolates	ZOE (mg/ml)	ZOH (mg/ml)	HSE (mg/ml)	HSH (mg/ml)	JCE (mg/ml)	JCH (mg/ml)
Candida I	ND	100	ND	100	ND	ND
Candida IV	ND	100	100	ND	ND	ND
Candida VI	100	100	ND	100	ND	100
Candida VII	ND	100	200	100	ND	100
Candida IX	200	100	100	100	ND	100

Keys: ZOE - *Z. officinale* Ethanol, ZOH - *Z. officinale* Aqueous, HSE - *H. sabdariffa* Ethanol, HSH - *H. sabdariffa* Aqueous, JCE - *J. carnea* Ethanol, JCH - *J. carnea* Aqueous, ND - Not Detectable

Table 8: Antifungal activity of *H. sabdariffa* synthesized nanoparticles on uropathogenic *Candida albicans*

Isolates	1 M (HSN)	50 mM (HSN)	25 mM (HSN)	AgNO3 (Control)	Ketoconazole (Control)	HSH (Control)
Candida I	19.33 ± 0.33^{d}	15.00 ± 1.15^{c}	15.33 ± 0.33^{c}	0.00 ± 0.00^{a}	9.67 ± 0.33^{b}	10.00 ± 0.57^{b}
Candida IV	24.00 ± 0.58^{d}	21.00 ± 0.58^{d}	17.00 ± 0.58^{c}	0.00 ± 0.00^{a}	10.33 ± 0.33^{b}	14.00 ± 1.15^{c}
Candida VI	19.00 ± 0.58^{d}	16.00 ± 1.15^{cd}	12.67 ± 0.33^{bc}	0.00 ± 0.00^{a}	10.33 ± 0.33^{b}	14.00 ± 1.15^{c}

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same rows are not significantly different (*P*>0.05) according to Tukey's Honestly Significant Difference
Keys: HSN - *H. sabdariffa* Nanoparticles, HSH - *H. sabdariffa* hot water extract

DISCUSSION

This comparative study successfully demonstrated that *Zingiber officinale*, *Justicia carnea*, and *Hibiscus sabdariffa* possess significant *in vitro* antifungal properties against uropathogenic *Candida* isolates. The universal ineffectiveness of the chloroform extracts strongly suggests that the active antifungal compounds in these three plants are polar in nature. This aligns with the known phytochemistry of these plants, that their activity is typically derived from water- and ethanol-soluble compounds such as flavonoids, saponins, tannins, and organic acids, rather than non-polar lipids or terpenes (Zheng *et al.,* 2016; Xu *et al.,* 2017). The agar diffusion assay revealed exceptional potency from the *J. carnea* hot water extract (JCH) and the *H. sabdariffa* ethanol extract (HSE), which both produced 29.00 mm inhibition zones. This demonstrates the presence of highly active, diffusible compounds. The potent activity of HSE (ethanol extract) aligns with literature reporting *H. sabdariffa* as a source of polyphenols and flavonoids that effectively inhibit uropathogenic *C. albicans* (Dgheim *et al*., 2023; Alshami and Alharbi, 2024). The strong performance of the *J. carnea* hot water extract is a significant finding. While *J. carnea* is less studied for its antifungal properties, its richness in flavonoids and alkaloids supports this observed bioactivity (Anthonia *et al*., 2019; Ajuru *et al*., 2025). The MIC and, MFC data provide a deeper insight into the extracts' true potential. While *J. carnea* produced the largest inhibition zone, it was the aqueous (hot water) extracts of *Z. officinale* and *H. sabdariffa* that showed the most powerful and broad-spectrum *fungicidal* action, killing all or nearly all *Candida* strains at 100 mg/ml. This is a crucial distinction as a large inhibition zone (fungistatic) is not as therapeutically valuable as a low fungicidal concentration. The finding that *Z. officinale* aqueous extract is fungicidal against *Candida* albicans isolates is consistent with research identifying gingerol and shogaol as potent antifungal molecules (Gavanji *et al*., 2025).The study's observation that the bio-reductive synthesis of silver nanoparticles (AgNPs) using *Hibiscus sabdariffa* produced markedly greater antifungal zones (24.00 ± 0.58 mm) than the crude extract is consistent with recent reports that plant-mediated AgNPs show larger inhibition zones and lower MIC/MFCs against *Candida* and other fungal species than their parent extracts (AlJindan and AlEraky, 2022; Kerimkulova *et al.,* 2025). Targeted reviews and experimental studies of green synthesis that correlate particular phytochemical classes (phenolics, flavonoids, anthocyanins, terpenoids) in *Hibiscus sabdariffa* with improved nanoparticle stability and antimicrobial potency against urinary candiduria (Villagrán *et al.,* 2024; Shahzadi *et al.,* 2025).

The broad resistance of the isolate to chloroform fractions across all tested plants aligns with standard extraction-chemistry expectations: chloroform is a low-polarity solvent that preferentially recovers nonpolar constituents, whereas key antimicrobial phytochemicals such as anthocyanins and many phenolic acids (dominant in *H. sabdariffa*) and the principal ginger phenolics (gingerols/shogaols) are polar to moderately polar and are better extracted by aqueous/alcoholic media; therefore the chloroform inactivity supports the inference that the active antifungal principles are largely polar. Empirical solvent-fractionation studies and extraction reviews corroborate that polar solvents (water, methanol, ethanol, aqueous mixtures) yield higher phenolic/anthocyanin content and greater antifungal/antioxidant activity than nonpolar solvents (Álvarez *et al.,* 2021; Shahzadi *et al.,* 2025). Chemical and pharmacological profiles for *H. sabdariffa* and *Zingiber officinale* confirm that anthocyanins/phenolic acids and gingerols/shogaols are abundant and are typically concentrated in polar fractions (Mao *et al.,* 2019).

The potency reported for *J.* (Justicia) *carnea* hot-water extract is consistent with ethnopharmacological records and modern

phytochemical analyses: Justicia spp. are repeatedly reported to contain flavonoids and other polar metabolites that often explain aqueous-extract activity in traditional “blood-boosting” and anti-infective uses; several recent genus-level reviews highlight flavonoids (naringenin and related compounds) as recurring bioactive constituents with antimicrobial effects (Carneiro *et al.,* 2023). Finally, the finding that *Z. officinale* aqueous extract displayed fungicidal activity (MFC ≈ 100 mg/mL in your report) accords with multiple studies demonstrating that ginger extracts (and specific constituents such as zingerone/zingerols) inhibit *Candida* growth and importantly can interfere with ergosterol biosynthesis and membrane integrity in *Candida* species in vitro, providing a mechanistic basis for fungicidal activity (Mao *et al.,* 2019; Chougule *et al.,* 2024).

Furthermore, the results from the nanoparticle assay are noteworthy. The *H. sabdariffa* hot water extract-synthesized nanoparticles (1 M) showed a zone of 24.00 mm compared to the raw extract and Ketoconazole. This suggests that green synthesis not only produces nanoparticles but also creates a synergistic composite where the plant's phytochemicals (acting as capping and reducing agents) enhance the antifungal activity of the silver. The positive control, Ketoconazole, also showed poor activity in the diffusion assay (0.00 mm in most cases), which may be due to its poor solubility and diffusion in agar, making direct comparison difficult.

CONCLUSION

This study confirms the ethnobotanical use of *Z. officinale*, *J. carnea*, and *H. sabdariffa* as effective antifungal agents. The polar extracts (hot water and ethanol) demonstrated significant *in vitro* activity against uropathogenic *Candida albicans*, while the non-polar (chloroform) extracts were inactive. The hot water extract of *J. carnea* and the ethanol extract of *H. sabdariffa* showed the largest zones of inhibition, indicating potent static activity. However, the aqueous extracts of *Z. officinale* and *H. sabdariffa* demonstrated the most promising therapeutic potential, exhibiting broad-spectrum *fungicidal* activity at 100 mg/ml. These findings strongly support the use of these plants as a source for novel antifungal compounds. Further research should focus on the isolation and characterization of the specific active compounds from these extracts and their evaluation in *in vivo* models of candiduria.

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