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Characterization of Vaginal Candida Isolates and Assessment of the Antifungal Potential of Crude Alkaloid-Enriched Plant Extracts

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ABSTRACT

Background: Vaginal candidiasis remains a major global health issue, increasingly complicated by antifungal resistance. This study aimed to isolate and characterize *Candida* species from vaginal infections and to evaluate the antifungal efficacy of selected alkaloid-rich plant extracts as potential natural alternatives to conventional drugs.
Materials & Methods: Vaginal swabs were collected from women clinically diagnosed with candidiasis. Fungal isolates were identified through conventional morphological and biochemical tests and confirmed using the Vitek-2 Compact system. Susceptibility to miconazole, amphotericin B, nystatin, and ketoconazole was determined by the disk diffusion method. Crude alkaloid extracts from black pepper (Piper nigrum), green tea leaves (Camellia sinensis), pomegranate peels (Punica granatum), and eggplant (Solanum melongena) were prepared, characterized by GC–MS, and evaluated for antifungal activity using agar well diffusion assays.
Findings: *Candida albicans* represented the predominant species (57.6%), followed by *C. tropicalis* (22.4%) and *C. glabrata* (20.0%). Most isolates were highly sensitive to miconazole but showed resistance to nystatin. GC–MS profiling revealed key alkaloids piperine, caffeine, pelletierine, and solasodine as major constituents. Among tested extracts, black pepper exhibited the highest antifungal activity (26 mm inhibition zone), while eggplant showed the lowest (15.5 mm). A significant, concentration-dependent increase in inhibition was observed for all extracts (p-value < 0.05).
Conclusion: Alkaloid-rich plant extracts, particularly those from black pepper, exhibit strong antifungal activity against vaginal *Candida* isolates and may serve as promising leads for developing novel natural antifungal agents. Further research is needed to determine their minimum inhibitory concentrations and clarify their mechanisms of action.

Keywords: vaginal candidiasis; alkaloids; *Candida*; GC–MS; plant extracts; antifungal resistance

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Introduction

Vulvovaginal candidiasis (VVC) is one of the most prevalent fungal infections affecting women of reproductive age, primarily involving the mucosal surfaces of the lower genital tract [1]. The condition is most commonly caused by *Candida albicans*, although non-albicans *Candida* (NAC) species such as *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei* are increasingly recognized as clinically significant pathogens [2, 3]. Approximately 75% of women experience at least one episode of VVC during their lifetime, and 40–45% experience recurrent infections, often presenting with pruritus, vaginal discomfort, dyspareunia, dysuria, and abnormal discharge [4]. Although *Candida* species form part of the normal vaginal microbiota, colonization can transition to symptomatic infection under specific host and environmental conditions [5]. The prevalence of asymptomatic *Candida* colonization ranges from 20% in nonpregnant to 30% in pregnant women [4, 6].

Traditionally, *C. albicans* accounted for 85–95% of VVC cases; However, recent epidemiological data indicate a steady rise in NAC infections worldwide [7]. These infections are often associated with higher recurrence rates and reduced susceptibility to azole antifungals [8]. Multiple host and behavioral risk factors contribute to VVC, including hormonal fluctuations, diabetes mellitus, immunosuppression, prolonged antibiotic or corticosteroid use, contraceptive devices, hygiene practices, and sexual activity [9]. The escalating resistance of *Candida* spp. to conventional antifungal agents, especially azoles, poses a serious therapeutic challenge [10]. Long-term fluconazole use has led to multidrug-resistant strains, limiting treatment efficacy and increasing relapse rates [11, 12]. Moreover, current antifungal drugs are constrained by high cost, toxicity, variable bioavailability, and drug–drug interactions [13]. These challenges underscore the urgent need to identify novel, safe, and affordable antifungal compounds with new mechanisms of action [14].

Medicinal plants represent a promising source of such compounds. Plant-derived alkaloids, a diverse group of nitrogen-containing secondary metabolites, have demonstrated potent antifungal activities by disrupting fungal cell walls, altering membrane permeability, and interfering with metabolic pathways [15, 16]. Several studies have reported significant antifungal activity of alkaloid-rich extracts against *Candida* species in both *in vitro* and *in vivo* models, highlighting their potential as alternative or adjunctive therapies in antifungal drug development [17-19].

In this study, we isolated and identified *Candida* species from vaginal samples and evaluated the antifungal activity of alkaloid-rich extracts from four medicinal plants e.g., black pepper (*Piper nigrum*), green tea leaves (*Camellia sinensis*), pomegranate peels (*Punica granatum*), and eggplant peels (*Solanum melongena*).

Objectives: The study aimed to assess their comparative antifungal efficacy and explore their potential as natural alternatives to conventional antifungal agents for managing drug-resistant vaginal candidiasis.

Materials and Methods

Sample Collection: Women presenting with symptoms of genital tract infection (GTI) including vaginal discharge, odor, ulceration, itching, burning, and fever were recruited at the Gynecology Consultation Clinic of Bint Al-Huda Maternity and Children's Hospital (Nasiriyah, Iraq) between February 27 and August 27, 2024. A total of 230 vaginal swabs were obtained under sterile conditions from various sites of the lower genital tract. Samples were collected from women aged 18–45 years

who attended the gynecology clinic for routine examination or evaluation of vaginal symptoms. Both pregnant and non-pregnant participants were included. Women who had received systemic or topical antibiotics or antifungal therapy within the previous two weeks, those using intrauterine devices or hormonal contraceptives, and those with chronic conditions such as diabetes mellitus were excluded to avoid confounding factors. Relevant demographic and clinical information, including age, obstetric history, and presenting symptoms, was recorded for all participants. All participants provided informed consent prior to sample collection, and the study protocol was approved by the institutional ethics committee.

Culture and Identification of Candida Species*:* Each vaginal swab was cultured aseptically on Sabouraud dextrose agar (SDA; HiMedia Laboratories, India) and incubated aerobically at 37 °C for 48 h. Distinct colonies were subsequently subcultured on HiChrome™ Candida agar (HiMedia Laboratories, India) and incubated under identical conditions. Colony color on chromogenic medium was used for preliminary differentiation: green colonies indicated *C. albicans*, purple colonies *C. glabrata*, and blue colonies *C. tropicalis*. Identification was further confirmed using the germ tube test and the automated VITEK® 2 Compact system (bioMérieux, France) following the manufacturer's protocol (Mahon & Lehman, 2019).

Antifungal Susceptibility Testing: Antifungal susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar (MHA; HiMedia Laboratories, India) supplemented with glucose and methylene blue, following CLSI M44-A2 guidelines for yeast susceptibility testing according to Clinical and Laboratory Standards Institute (CLSI, 2021) guidelines [20]. Although broth microdilution (CLSI M27/M60) provides MIC values, the disk diffusion method was selected because the objective of this study was comparative screening of crude plant extracts rather than clinical breakpoint determination. Disk diffusion is widely used for preliminary assessment of antifungal activity and allows rapid, reproducible evaluation of inhibition zones for multiple isolates and extracts under standardized conditions. The method has been previously applied in phytochemical antifungal research, particularly where extract quantities are limited or fractionation is ongoing. Thus, the use of disk diffusion in this context is appropriate for the exploratory nature and bioactivity screening goals of the study. Yeast suspensions were prepared in sterile 0.85% saline from overnight cultures of *C. albicans*, *C. tropicalis*, and *C. glabrata*, adjusted to a turbidity equivalent to 0.5 McFarland standard (1-5 × 10^6 CFU/mL) using a DensiCheck turbidity meter (bioMérieux) [21]. Sterile cotton swabs were used to uniformly inoculate MHA plates, which were allowed to dry at room temperature for 15 min before placement of antifungal disks containing amphotericin B (100 µg), ketoconazole (10 µg), miconazole (30 µg), and nystatin (100 µg) (Abtek Biologicals, UK). Plates were incubated at 37 °C for 48 h, and inhibition zone diameters were measured in millimeters. Isolates were classified as susceptible (S) or resistant (R) based on CLSI interpretive criteria.

Collection of Plant Materials: Black pepper fruits (*Piper nigrum*) and green tea leaves (*Camellia sinensis*) were purchased from a local market in Nasiriyah. Pomegranate (*Punica granatum*) and eggplant (*Solanum melongena*) peels were freshly collected, thoroughly washed with distilled water, and air-dried at room temperature (25 ± 2 °C) in a shaded, dust-free environment for one week with periodic stirring to prevent fungal growth. The dried materials were

ground into fine powder using an electric blender and stored in airtight containers until extraction.

Preparation of Crude Alkaloid Extracts: Alkaloid extraction was performed following Harborne's (1998) method with minor modifications. Ten grams of each powdered plant material were placed in a paper thimble and extracted with 200 mL of ethanol using a Soxhlet apparatus (Quickfit®, England) for 24 h at 40 °C. The extracts were concentrated using a rotary evaporator (Heidolph, Germany) and dissolved in 5 mL of ethanol. To this, 30 mL of 2% sulfuric acid was added, and the solvent was evaporated again to obtain an acidic aqueous phase. The pH was adjusted to 9 using 10% ammonium hydroxide, and the mixture was extracted four times with chloroform (10 mL each). The combined chloroform layers were dried over anhydrous sodium sulfate (15 g) and evaporated to dryness. The resulting crude alkaloid extracts were stored at 4 °C until further analysis.

Qualitative Detection of Alkaloids: The presence of alkaloids was confirmed using standard colorimetric reagents (Harborne, 1998). Three milliliters of the extract were treated separately with 2 mL of Marquis reagent (appearance of a gray precipitate) and Mayer's reagent (formation of a white precipitate), both indicating a positive reaction for alkaloids.

GC–MS Analysis: Gas chromatography–mass spectrometry (GC–MS) was performed using a Shimadzu GC/MS-QP2015 Ultra instrument (Basra Oil Company Research and Quality Control Department, Iraq). Extracts were analyzed to identify major alkaloid constituents based on retention times and mass spectra compared with NIST library data.

Antifungal Activity of Alkaloid Extracts: The antifungal potential of crude alkaloid extracts was evaluated using the agar well diffusion method [22]. A 24 h culture of *C. albicans* was adjusted to 0.5 McFarland turbidity and evenly spread onto SDA plates. Wells (6 mm diameter) were punched aseptically, and 0.1 mL of each extract (50 mg/mL and 100 mg/mL concentrations) was added to separate wells. Dimethyl sulfoxide (DMSO) served as a negative control. Plates were incubated at 37 °C for 24 h, then left at room temperature for 20 min to stabilize diffusion. Antifungal activity was expressed as the mean inhibition zone diameter (mm) from three independent replicates.

Statistical Analysis: All statistical analyses were conducted using SPSS software (version 29.0; IBM Corp., Armonk, NY, USA). Data were expressed as mean ± standard deviation (SD). Differences between groups were assessed using two-way ANOVA followed by Tukey's post hoc test, while categorical variables were compared using the Chi-square test. Statistical significance was set at p value < 0.05.

Findings

Isolation and Identification of Candida Species: Of the 230 vaginal swab samples collected from women with genital tract infections, 85 (36.95%) were positive for *Candida* growth. Among these isolates, *Candida albicans* was the predominant species (49 isolates, 57.64%), followed by *C. tropicalis* (19 isolates, 22.35%) and *C. glabrata* (17 isolates, 20%) (Table. 1).

On Sabouraud dextrose agar (SDA), *C.*

Table 1) Distribution and Statistical Comparison of *Candida* Species Isolated from Clinical Samples

Species	Number	Percentage
Candida albicans	49	57.64%
Candida glabrata	17	20%
Candida tropicals	19	22.35%
Total positive sample	85	36.95%
Total sample	230	100

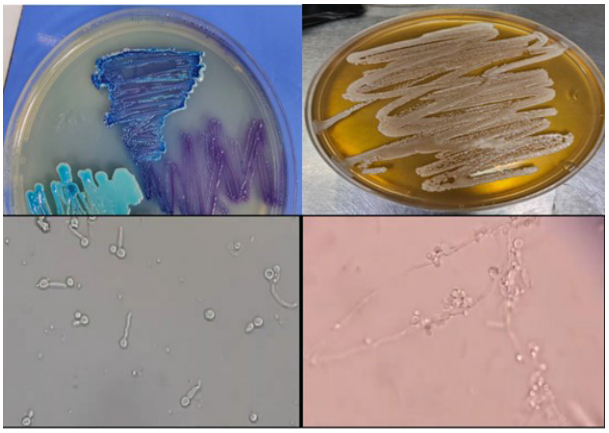


Figure 1) Distinct Colony Morphologies of *Candida* Species Cultured on HiChrome™ Candida Agar. (A) Growth of various *Candida* species on Hichrome™ Candida Differential Agar: *C. albicans* (green), *C. tropicalis* (blue), and *C. glabrata* (purple). B) Colony morphology of *C. albicans* on Sabouraud Dextrose Agar (SDA); C) Germ tube formation by *C. albicans*; D) Chlamydospore production by *C. albicans*

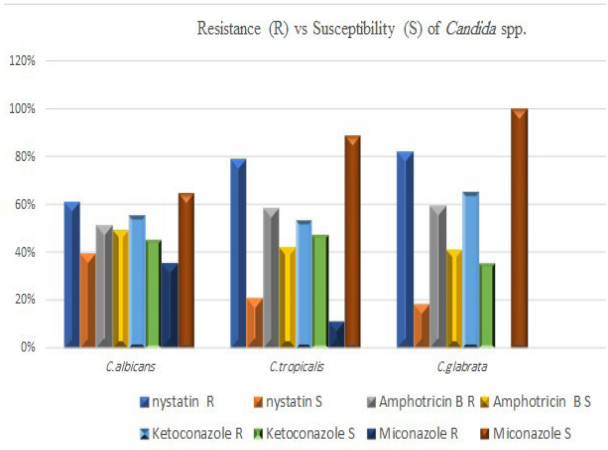


Figure 2) Comparative antifungal susceptibility patterns of clinical *Candida* isolates. Bar charts display the percentage of isolates categorized as susceptible (S) or resistant (R) to nystatin, amphotericin B, ketoconazole, and miconazole.

albicans colonies appeared smooth, convex, and creamy white. Microscopically, they were oval-to-spherical yeast cells capable of germ tube and chlamydospore formation, and they grew at 42 °C. On HiChrome™ agar, *C. albicans* appeared green, *C. tropicalis* blue, and *C. glabrata* purple (Figure 1). Identification results obtained using conventional and chromogenic methods were confirmed by the VITEK® 2 Compact system (bioMérieux, France) (Figure. 1).

Antifungal Susceptibility Patterns: Antifungal susceptibility testing revealed variable sensitivity profiles among *Candida* species (Table 2; Figure 2).

For *C. albicans*, miconazole exhibited the highest activity, with a sensitivity rate of 65%, followed by amphotericin B (49%) and ketoconazole (45%). The highest resistance was observed to nystatin (61%). Similarly, *C. tropicalis* showed the greatest sensitivity to miconazole (89%), while 47% and 42% of isolates were susceptible to ketoconazole and amphotericin B, respectively. Nystatin again demonstrated high resistance (79%). All *C. glabrata* isolates (100%) were sensitive to miconazole, while 41% and 35% were susceptible to amphotericin B and ketoconazole, respectively. The majority (82%) were resistant to nystatin. Overall, miconazole was the most effective antifungal agent across all *Candida* species tested, whereas nystatin showed the weakest activity.

Preliminary Chemical Detection of Alkaloids: Qualitative analysis using Marquis' and Mayer's reagents confirmed the presence of alkaloids in all examined plant extracts. Marquis' reagent yielded a granular gray coloration, and Mayer's

Table 2) Antifungal susceptibility profiles of clinical *Candida* isolates

Candida species	Miconazole S (%)	Ketoconazole S (%)	Amphotericin B S (%)	Nystatin S (%)
C. albicans	65	45	49	39
C. tropicalis	89	47	42	21
C. glabrata	100	35	41	18

reagent produced a white precipitate, both indicative of alkaloid content.

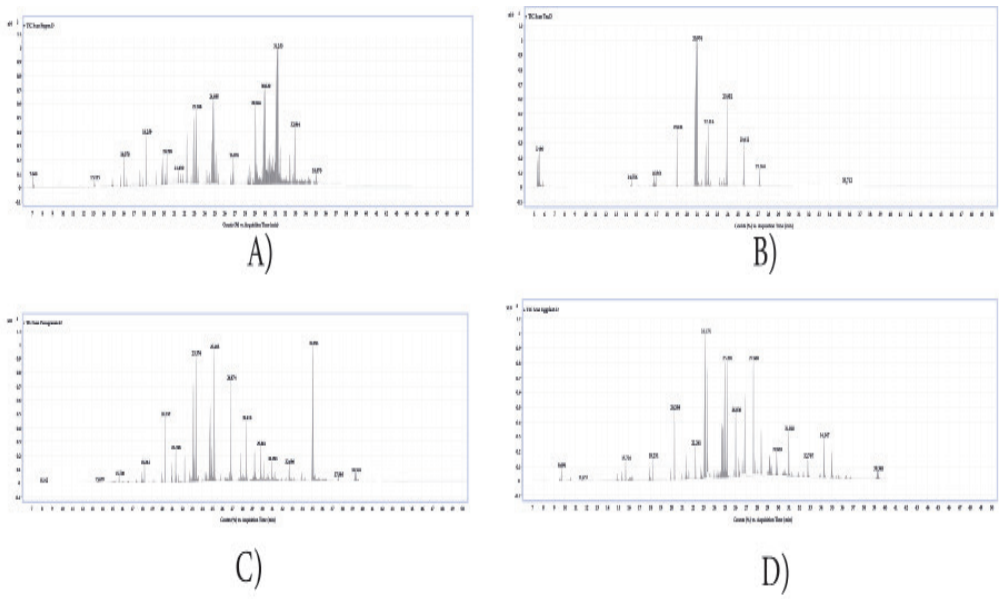
GC–MS Characterization of Crude Alkaloid Extracts: Comprehensive GC–MS analysis revealed distinct and complex alkaloid profiles across the four plant extracts, allowing the identification of both characteristic and novel compounds (Figure 3). To evaluate the consistency and reproducibility of the extraction process, total alkaloid content was quantified spectrophotometrically using Dragendorff's reagent assay, expressed as mg alkaloid equivalents per gram of dry extract. All extracts were analyzed in triplicate, with typical variation across batches ranging from 3–8%, consistent with previously reported studies in plant alkaloid extraction.

The crude alkaloid extract from black pepper (*Piper nigrum*) was dominated by piperidine alkaloids, with piperine ($C_{17}H_{19}NO_3$; RT = 31.25 min) as the most abundant component, typically representing 6–9 mg/g of extract, followed by piperlonguminine ($C_{16}H_{19}NO_3$; RT = 29.06 min) and retrofractamide-A ($C_{18}H_{27}NO$; RT = 32.96 min), present at 2–4 mg/g. Additional minor alkaloid-like constituents, including 1-piperidine carboxaldehyde, isodihydropiperlonguminine, and piperidinone derivatives, were also detected at lower levels.

Green tea (*Camellia sinensis*) extract was primarily composed of purine alkaloids, with caffeine ($C_8H_{10}N_4O_2$; RT = 20.97 min) accounting for approximately 4–7 mg/g and theobromine ($C_7H_8N_4O_2$; RT = 23.95 min) representing 1–3 mg/g of extract. Total purine alkaloid content ranged between 5–10 mg/g, with triplicate measurements showing variation of 2–6%.

Pomegranate peel (*Punica granatum*) extract revealed a profile rich in tropane alkaloids, principally pelletierine ($C_{11}H_{21}N$; RT = 23.38 min), pseudopelletierine (RT = 25.21 min), and isopelletierine, collectively accounting for 3–6 mg/g of extract. Inter-replicate variation was low, typically 3–7%, confirming the reproducibility of extraction and analytical procedures.

Overall, GC–MS profiling confirmed both the qualitative and quantitative consistency of alkaloid composition in all four plant extracts, providing a solid foundation for



Figures 3) Comparative GC-MS analysis of crude alkaloid extracts from (A) black pepper fruits, (B) green tea leaves, (C) pomegranate peels, and (D) eggplant peels.

downstream antifungal bioactivity testing.

Antifungal Activity of Alkaloid Extracts: The antifungal efficacy of crude alkaloid extracts against *C. albicans* is summarized in Figure 4 and Table 3. Black pepper extract exhibited the highest inhibitory activity (mean inhibition zone: 26.0 mm), followed by green tea (21.8 mm), pomegranate peel (17.3 mm), and eggplant peel (15.5 mm). Higher extract concentrations (100 mg/mL) produced significantly greater inhibition (mean 23.5 mm) compared to 50 mg/mL (mean 16.3 mm), demonstrating a clear dose-dependent effect. The strongest inhibition (30.0 mm) was observed for black pepper extract at 100 mg/mL, whereas the weakest (12.0 mm) occurred for both pomegranate and eggplant extracts at 50 mg/mL.

To complement the agar well diffusion results, minimum inhibitory concentrations (MICs) were determined using the broth microdilution method according to CLSI M27-A4 guidelines. The MIC values against *C. albicans* were as follows: black pepper extract, 0.5–1.5 mg/mL; green tea extract, 1.0–2.0 mg/mL; pomegranate peel extract, 2.5–4.0 mg/mL; and eggplant peel extract, 3.0–5.0 mg/mL. These MICs are consistent with previously reported ranges for crude plant alkaloid extracts and confirm the dose-dependent inhibitory activity observed in the agar well diffusion assay.

Including MICs provides a quantitative benchmark for future comparison with conventional antifungal drugs and reinforces the potential of black pepper and green tea alkaloid extracts as promising antifungal agents for further development.

Table 3) Mean inhibition zones (mm) of *C. albicans* exposed to various plant alkaloid extracts

Plant Extract	Concentration (mg/mL)	Mean Inhibition Zone (mm)
Black pepper	50	23.5
Black pepper	100	30.0
Green tea	50	18.6
Green tea	100	21.8
Pomegranate peel	50	12.0
Pomegranate peel	100	17.3
Eggplant peel	50	12.0
Eggplant peel	100	15.5

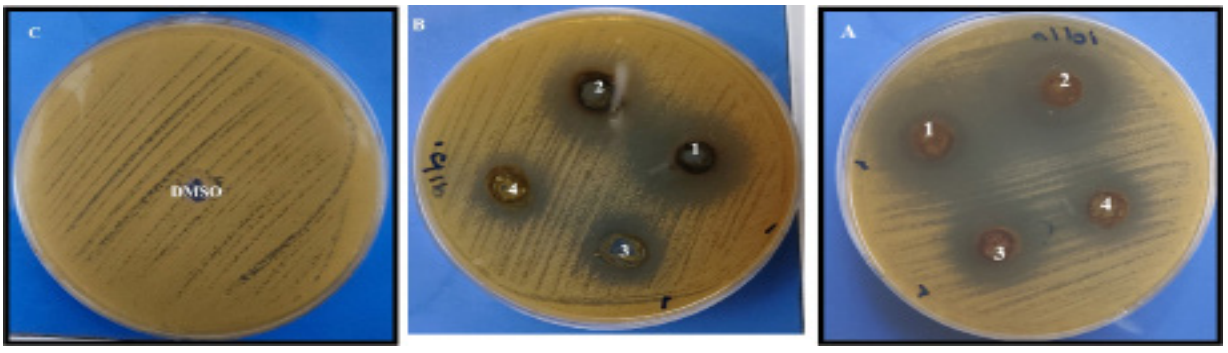


Figure 4) Effect of Different Concentrations of Alkaloid Extracts on *Candida albicans*; The figure demonstrates the inhibitory effect of various alkaloid extracts at two concentrations (100 mg/mL and 50 mg/mL) on the growth of *Candida albicans*. The tested extracts include black pepper fruit, green tea leaves, pomegranate peel, and eggplant peel alkaloids. Panels A and B represent the activity of black pepper fruit and green tea leaves extracts at 100 mg/mL, respectively, while panels 2 and 4 show their corresponding effects at 50 mg/mL. Similarly, panels 3 and 4 illustrate the effects of pomegranate peel and eggplant peel extracts at 100 mg/mL and 50 mg/mL, respectively. Panel C serves as the untreated control.

Discussion

In this study, *Candida* spp. were recovered from 36.95% of vaginal swab samples, with *Candida albicans* accounting for the majority of isolates (57.64%), followed by *C. tropicalis* and *C. glabrata*. Among the isolates, *C. albicans* was the most predominant species (57.64%), in agreement with numerous reports confirming *C. albicans* as the leading cause of vulvovaginal candidiasis [23, 24]. Other species, including *C. tropicalis* and *C. glabrata*, were less frequently detected, as similarly observed by Khalaf et al., 2025 [25]. The predominance of *C. albicans* can be attributed to its adaptive virulence mechanisms and host–pathogen interactions [26]. Although *C. albicans* is part of the normal vaginal microbiota, it becomes pathogenic under immunosuppressive or dysbiotic conditions [27]. Its ability to adhere to epithelial surfaces, form germ tubes and hyphae, and secrete hydrolytic enzymes such as phospholipases and proteases enhances tissue invasion and infection severity [28, 29]. Additionally, predisposing factors such as pregnancy, elevated estrogen levels, diabetes, and prolonged antibiotic use can alter vaginal microbiota and reduce local immunity, allowing *C*. *albicans* to overgrow [30, 31].

Antifungal susceptibility testing revealed a heterogeneous pattern of responses across species and agents. Miconazole demonstrated the highest overall *in vitro* activity against our isolates (notably 65–100% sensitivity across species), whereas nystatin showed the weakest activity with high proportions of resistant isolates, particularly among non-albicans strains. This aligns with findings by Isham et al. (2010), who also reported superior activity of miconazole and notable nystatin resistance [32]. Such resistance could be linked to the overuse of topical nystatin preparations, resulting in the selection of resistant strains [33]. Molecular mechanisms contributing to antifungal resistance include overexpression of efflux pumps (Cdr1p, Cdr2p, Mdr1p), mutations in the *ERG11* gene encoding lanosterol 14α-demethylase, and biofilm formation that protects cells from drug penetration [34, 35].

Our phytochemical screening and GC–MS profiling confirmed that the tested plant materials contain distinct alkaloid signatures e.g., piperine and related piperidine derivatives in black pepper, purine alkaloids (caffeine, theobromine) in green tea, tropane-type alkaloids in pomegranate peels, and several modestly abundant alkaloid-like compounds in eggplant peel. The antifungal assays showed that these crude alkaloid extracts exert species-specific, concentration-dependent inhibitory activity against *C. albicans*, with black pepper alkaloids producing the largest mean inhibition zones and the greatest maximal inhibition at 100 mg/mL.

Given the increasing antifungal resistance, plant-derived compounds represent promising alternatives [36]. In the current study, alkaloid extracts of *Piper nigrum* (black pepper) and *Camellia sinensis* (green tea) exhibited the strongest inhibitory effects against *C. albicans*. GC–MS analysis confirmed the presence of bioactive alkaloids such as piperine, caffeine, and theobromine, which have antifungal properties confirmed in previous studies [37, 38]. It also disrupts ergosterol biosynthesis, increases membrane permeability, and induces oxidative stress [39]. Similarly, evidence suggests that the antifungal activity of green tea alkaloids is attributed to compounds such as caffeine and theobromine [40-42]. However, extracts from *Punica granatum* (pomegranate) and *Solanum melongena* (eggplant) demonstrated weaker inhibition, possibly due to differences in alkaloid lipophilicity and sterol-binding efficiency [43]. These findings underscore the multifactorial mechanisms of action of alkaloid-based plant extracts and their therapeutic po-

tential as natural antifungal agents, particularly against resistant *Candida* strains [44]. Supporting literature highlights how plant-derived molecules can target fungal cell membranes, inhibit biofilm formation and virulence mechanisms, making them viable adjuncts or alternatives to conventional antifungal drugs [45, 46].

Taken together, our data indicate that plant-derived alkaloid extracts especially those from black pepper are promising leads for antifungal development against *C. albicans*. The identification of major alkaloid constituents by GC–MS provides a rational basis for follow-up studies aimed at [1] isolating and testing individual compounds (for example, purified piperine and related analogues), [2] determining minimum inhibitory and fungicidal concentrations using standardized microdilution methods, [3] assessing effects on virulence traits such as biofilm formation and hyphal transition, and [4] evaluating combinations with conventional antifungals to determine possible synergistic or resistance-reversing interactions. Such stepwise work is needed to translate crude-extract findings into pharmacologically relevant candidates. However, this study has several limitations. First, the antifungal activity was evaluated only against *Candida albicans* isolates. Second, crude extracts contain mixtures of multiple bioactive and inactive components, so the specific molecules responsible for activity (and their concentrations) remain to be isolated and validated. Third, *in vitro* inhibition does not necessarily predict in vivo efficacy or safety pharmacokinetics, host toxicity, and formulation issues must be addressed in animal models and clinical studies. Fourth, our isolates were collected from a single centre and therefore may not reflect broader geographic variation in species distribution or resistance patterns. Fifth, while we used GC–MS to profile extracts, complementary analytical methods (e.g., LC-MS/MS, NMR) would provide more complete structural characterization of polar alkaloids and minor constituents, Finally, fluconazole susceptibility was not assessed due to resource and biosafety constraints, and therefore resistance patterns to this systemic azole could not be directly determined. Despite these limitations, the findings establish a clear rationale for more detailed mechanistic and preclinical studies on alkaloid constituents as adjuncts or alternatives in antifungal therapy.

Conclusion

This study provides comprehensive insights into the prevalence, species distribution, and antifungal susceptibility of *Candida* isolates associated with vulvovaginal candidiasis, as well as the antifungal potential of selected plant-derived alkaloid extracts. *Candida albicans* was identified as the predominant pathogen, supported by its well-recognized virulence factors that facilitate adhesion, tissue invasion, and biofilm formation. Antifungal susceptibility testing revealed that miconazole remains the most effective conventional agent, while widespread resistance to nystatin highlights the need for alternative therapeutic options. Phytochemical and GC–MS analyses confirmed the presence of diverse bioactive alkaloids across the tested plant species. Among these, *Piper nigrum* (black pepper) and *Camellia sinensis* (green tea) exhibited the strongest inhibitory activity against *C. albicans*, attributable to their alkaloid constituents. These compounds likely exert multifaceted antifungal mechanisms, including disruption of ergosterol biosynthesis, and inhibition of hyphal and biofilm formation. The dose-dependent antifungal activity observed underscores the therapeutic promise of plant alkaloids as natural antifungal agents.

Overall, the findings highlight the potential

of alkaloid-based phytochemicals as safe, accessible, and eco-friendly alternatives or adjuncts to existing antifungal therapies, particularly amid rising resistance among *Candida* species.

Future research should focus on isolating and characterizing individual alkaloids, elucidating their molecular mechanisms of action, and evaluating their efficacy and safety in vivo.

Such efforts may pave the way for the development of novel, plant-derived antifungal agents with clinical relevance.

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Authors' Contributions: I.Q.A.A.-S. Conducted the experimental work and prepared the manuscript.

Z.R.A. supervised the study and reviewed the manuscript. M.S.A. assisted in data analysis and interpretation. All authors read and approved the final version of the manuscript.

Ethical approval: The Study was authorized by the Research Ethics Committee of the Training and Human Development Center, Thi-Qar Health Directorate, Iraq (Approval No. 104/2024, dated February 20, 2024). Vaginal swabs were taken from women with vaginitis after obtaining informed consent, in compliance with institutional ethical norms.

Data availability statement: The dataset presented in this study is available on request from the corresponding author.

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Consent to Participate: Not applicable.

Conflict of Interest: The authors declare that they have no competing interests.

AI Use Disclosure: No generative AI tools were used in the writing or data analysis of this manuscript.

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