

Research Article	Pak-Euro Journal of Medical and Life Sciences
DOI: 10.31580/pjmls.v8iSp3.3515	Copyright © All rights are reserved by Corresponding Author
Vol. 8 No. Special 3, 2025-26: pp. S287-S300	
www.readersinsight.net/pjmls	Revised: March 17, 2026 Accepted: March 28, 2026
Submission: December 13, 2025	Published Online: March 31, 2026

DEVELOPMENT AND EVALUATION OF CURCUMIN- LOADED- SELF-EMULSIFYING DRUG DELIVERY SYSTEMS AGAINST *CANDIDA ALBICANS* IN VULVOVAGINAL CANDIDIASIS

Ashifa Tehseen¹, Haseena Nazneen², Riaz Ahmad³, Ghazal Ali², Sumbal Mehreen⁴, Mahnoor Farman¹, Momin Khan^{1*}

¹**Department of Microbiology**, Institute of Pathology and Diagnostic Medicine, Khyber Medical University, Peshawar, Khyber Pakhtunkhwa, Pakistan

²**Department of Microbiology**, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan

³**Department of Microbiology**, Hazara University, Mansehra 21300, Khyber Pakhtunkhwa, Pakistan

⁴**Institute of Biological Sciences**, Gomal University, Dera Ismail Khan, Pakistan

***Corresponding Author:** Momin Khan. E. mail: mominkhan.ibms@kmu.edu.pk



Abstract

Candidiasis, primarily caused by Candida albicans, poses a significant public health concern. The rising resistance to first-line antifungal azoles necessitates alternative therapeutic strategies. Curcumin, known for its potent antifungal and antibacterial activities, suffers from limited bioavailability and solubility, prompting the exploration of stable formulations. This study aimed to develop and assess self-emulsifying drug delivery system (SEDDS) formulations of curcumin for their antifungal properties against C. albicans isolated from vulvovaginal candidiasis (VVC). Eighty samples from suspected VVC cases were processed, identifying 17 (21.25%) positive for C. albicans, including two fluconazole-resistant strains. Twenty SEDDS were prepared, with the top six selected based on visual assessment. Physicochemical attributes, including zeta potential, mean droplet size, and polydispersity index (PDI), were evaluated. Antifungal activities were assessed via well diffusion and broth microdilution methods. Among the 80 samples processed, 17 (21.25%) tested positive for C. albicans, including two strains identified as fluconazole-resistant and 15 as susceptible. The physicochemical evaluation of Cu-SEDDSs revealed differences in mean droplet size, polydispersity index, and zeta potential. The zone of inhibition for fluconazole-susceptible strains ranged from 21-26 mm, while resistant strains exhibited 14-21 mm. Minimum inhibitory concentrations (MICs) ranged from 0.98 µg/mL to 7.8 µg/mL for susceptible strains and 3.9 µg/mL to 7.8 µg/mL for resistant strains. Cu-SEDDS formulations demonstrated enhanced antifungal activity, with comparable MIC values for both strains. These formulations exhibited strong fungicidal effects against C. albicans strains, suggesting their potential as effective antifungal agents.

Keywords: Bioavailability, Candida albicans, Curcumin, Self-emulsifying drug delivery system, Vulvovaginal candidiasis

INTRODUCTION

Vulvovaginal candidiasis (VVC) stands as the most prevalent gynecological condition affecting women of reproductive age globally (1). It is recognized as the second most common type of infectious vaginitis, with symptomatic VVC impacting approximately 75% of women due to hormonal fluctuations (2). Notably, the prevalence of Candida infections is significantly higher in pregnant women than in non-pregnant women (3). In 20-50 % of healthy women, Candida species are found asymptotically in the normal reproductive tract flora, with C. albicans being the most frequently encountered species among the Candida genus (4, 5).

In Pakistan, estimates suggest that around 2,821,440 women of childbearing age experience recurrent vulvovaginal candidiasis (RVVC) annually. Vaginal candidiasis is reported as the second most common reproductive tract infection among urban women in Pakistan, with a prevalence rate of 7-12% (6, 7). Approximately 70-75% of women will experience VVC at least once in their lifetime, while 6-9 % endure recurrent episodes (8, 9).

The treatment of VVC typically involves antifungal medications, including azoles, echinocandins, polyenes, and nucleoside analogues. Azoles, particularly fluconazole, are considered first-line treatment,



targeting lanosterol 14 α -demethylase, a crucial enzyme in ergosterol biosynthesis (10, 11). However, the increasing incidence of antifungal resistance presents significant challenges, driven by mechanisms such as overexpression and mutation of target enzymes, inactivation of specific genes, and overexpression of multidrug efflux pumps (12). Multidrug resistance is prevalent, underscoring the urgent need for continued development of new antifungal agents and optimized treatment strategies (13, 14).

Antimicrobial resistance, including antifungal and antibacterial resistance, has become a major global concern because resistant pathogens reduce treatment efficacy and increase the need for alternative therapeutic strategies. Recent epidemiological studies have reported important resistance and susceptibility patterns in *Candida* and *Aspergillus* species, while multidrug-resistant bloodstream infections and ESBL-, carbapenemase-, and colistin-resistant bacterial infections continue to complicate clinical management (4, 5, 10, 12, 14, 15). Surveillance of enterotoxigenic *Escherichia coli* in food sources also highlights the broader public-health relevance of microbial identification and infection control (16). Therefore, alternative delivery systems and locally effective formulations are increasingly important for difficult-to-treat fungal and bacterial infections.

Curcumin, a bioactive compound derived from turmeric, exhibits potent antifungal activity against *Candida* species, employing various mechanisms such as downregulation of ergosterol production and inhibition of hyphal growth (17). Beyond its antifungal properties, curcumin demonstrates a range of biological activities, including antioxidant, antimicrobial, antiviral, and anti-inflammatory effects (18, 19). Interestingly, the synergistic action of curcumin with existing antifungal drugs offers a promising approach to enhance therapeutic strategies. However, its clinical application is hindered by bioavailability issues, necessitating the formulation of delivery systems that improve its solubility and absorption (21-20).

The therapeutic potential of curcumin-based and nanocarrier-based formulations has also been explored beyond fungal infections, particularly in cutaneous and mucocutaneous leishmaniasis. Curcumin-loaded SEDDS have shown potential for cutaneous leishmaniasis and secondary bacterial infections, while polymer-coated miltefosine, amphotericin B-loaded chitosan nanoparticles, pentamidine-loaded chitosan nanoparticles, and vanillin-derived Schiff bases have demonstrated promising activity against *Leishmania tropica* (22-26). Furthermore, studies on intralesional Glucantime and zinc oxide nanoparticles emphasize the clinical importance of managing *Leishmania* lesions complicated by secondary bacterial infections (27, 28).

Innovative drug delivery systems, such as hydrogel nanoparticles, PLGA-encapsulated curcumin, liposomal formulations, and nanoemulsions based on nanoglobules, have been developed to enhance curcumin's water solubility and bioavailability (22, 29). Among these, SEDDS show considerable promise in improving the solubility and bioavailability of hydrophobic compounds like curcumin. SEDDS can be classified into self-microemulsifying drug delivery systems (SMEDDS) and self-nanoemulsifying drug delivery systems (SNEDDS). These formulations use stable mixtures of surfactants, co-surfactants, and oil with lipids to achieve desired therapeutic objectives (23, 30, 31).

Considering the antifungal properties of curcumin and the solubilizing capability of SEDDS for hydrophobic molecules, this study aimed to develop and evaluate SEDDS formulations of curcumin against *C. albicans* isolates.

MATERIALS AND METHODS

The chemicals, including various excipients such as Capmul PG-8, Captex 300, Cremophor RH 40, DMSO, PEG 400, Cremophor EL, Tween 80, Captex 355, PEG 300, and Caprylic acid, were obtained from the Department of Pharmacy, Khyber Medical University, Peshawar, Pakistan. Pure curcumin in powder form was procured from Sigma Aldrich (ST. Louis, USA). Nutrient broth, chrome agar, and Sabouraud dextrose agar (SDA) were purchased from Thermo Fisher Scientific (Massachusetts, USA). All other materials were of research grade and obtained from various commercial suppliers.

PREPARATION OF CURCUMIN-LOADED SEDDS

The Cu-SEDDS formulations were synthesized following a previously published protocol with slight modifications (32). For this method, different concentrations of excipients, including Capmul PG-8, Cremophor RH40 Cremophor EL, Captex 300, DMSO, PEG 300, PEG 400, Tween 80, Captex 355, and Caprylic acid, were vortexed and homogenized, followed by sonication for 30 minutes.

PHYSICOCHEMICAL CHARACTERIZATION OF CU-SEDDS

The physicochemical assessment of the synthesized emulsions, such as surface charge, PDI, and mean droplet size, was conducted using the dynamic light scattering technique with a PSS Nicomp 380 ZLS Zetasizer. Electric field strength of 10 V/cm and a wavelength of 550 nm were utilized.

The optimal blank SEDDS emulsions were selected based on physicochemical characterization and subsequently mixed with 10 mg (1% w/v) of curcumin. The mixture was then centrifuged at 13,000 rpm, covered with aluminium foil, and stored at room temperature. A total of 20 different formulations of Cu-SEDDS were prepared, and after visual assessment, the best six were selected for experiments, labelled as F1-F6 based on their ideal properties.

ISOLATION AND CULTURING OF CANDIDA ALBICANS

A total of 80 suspected VVC samples were collected from gynaecology wards of different public hospitals and private maternity clinics in District Peshawar, Pakistan. The study was approved by the Institutional Bioethical Committee, Khyber Medical University, Peshawar, Pakistan, with Approval No. (Dir/IBMS-EB/IV/000518). The fungi were cultured on SDA medium under aerobic conditions at 37°C for 16-18 hours. For further confirmation of the samples, the SDA plates were subcultured on chrome agar (Difco™, Franklin Lakes, NJ, USA), and a germ tube test was also performed. After confirmation, one fluconazole-susceptible and one fluconazole-resistant were selected for further screening.

ANTIFUNGAL SUSCEPTIBILITY TESTS

The antifungal susceptibility test was conducted following the guidelines laid down by the Clinical and Laboratory Standards Institute (CLSI) guidelines for determining fluconazole resistance in *C. albicans* (33). The efficacy of Cu-SEDDS against *C. albicans* was determined using the well diffusion method and the broth microdilution method.

DISK DIFFUSION ASSAY

Briefly, lawn cultures of *C. albicans* were prepared on an SDA agar plate using a sterile glass spreader. For this purpose, four wells, each 8 mm in diameter, were made in the agar, and 200 µl of curcumin nanoemulsions were added to each well. The plate was incubated at 37 °C for 48 h, and the inhibition zones were measured in millimeters.

BROTH MICRODILUTION ASSAY

The broth microdilution assay was conducted to determine the MIC of Cu-SEDDS nanoemulsion against *C. albicans* following CLSI guidelines (34). Initially, a 1000 µg/mL stock solution of Cu-SEDDS containing 10 mg (1%) curcumin was prepared. From this, 500 µg was transferred to Eppendorf tubes, and each tube was filled with 1 ml of distilled water to achieve a 500 µg/mL concentration. Various dilutions were prepared in tubes with 500 µl distilled water, yielding concentrations of 1, 2, 4, 7.9, 15.7, 31.3, 62.5, and 250 µg/mL. In a 96-well plate, 100 µl of yeast peptone dextrose broth with different Cu-SEDDS concentrations was added, followed by 100 µl of *C. albicans* suspension adjusted to 1×10⁶ cells/ml. The plate was incubated at 37°C for 24 hours, and growth was assessed by measuring the optical density at 530 nm. The MIC was determined as the highest dilution with no turbidity. The experiment was performed in triplicate, with fungizone (200 µl) used as a positive control and DMSO as a negative control.

MINIMUM FUNGICIDAL CONCENTRATION (MFC)

The MFC concentration was assessed by subculturing all concentrations of Cu-SEDDS formulations (F1-F6) from the tested wells of the 96-well plate onto SDA plates. A sterile wire loop was dipped into each well of the plate and streaked onto separate SDA plates for each concentration of all formulations. The plates were incubated at 37 °C for 24 h, and results were noted the following day. The concentrations showing no growth were considered fungicidal.

STATISTICAL ANALYSIS

All experiments in this study were performed in triplicate to ensure accuracy, and the mean results were reported. Describe which statistical software was used.

RESULTS

FORMULATION AND CHARACTERIZATION OF CU-SEDDS

Various concentrations of surfactants, co-surfactants, and oils were mixed using a vortex machine to achieve transparent and balanced blank SEDDS formulations. These blank formulations were then blended with 1% curcumin. The resulting formulations were characterized by their PDI, droplet size, and zeta potential. The ingredients of each formulation (F1-F6) are listed in Table I, while the emulsifying characteristics are summarized in Table II, each formulation exhibited unique sizes, zeta potential, and PDI properties. The maximum droplet size was observed in formulation F5, measuring 556.2±1.4 nm, whereas formulation F6 demonstrated the smallest droplet size at 57.19±0.4 nm. All synthesized formulations displayed negative zeta potentials, ranging from -1.38±0.3 mV for F4 to -5.82±0.4 mV for F3. Notably, the droplet sizes of all formulations remained below 600 nm. The zeta potential properties and size distributions for formulations F1, F2, F3, and F6 are illustrated in Fig. 1(a-h) respectively.

Table I. Percentage of excipients in Cu-SEDDS formulations

Cu-SEDDS	Captex 355	Captex 300	Crem RH 40	Crem EL	Campul PG-8	Tween 80	PEG 400	PEG 300	CA	DMSO	CU
F1	20	-	30	-	-	10	25	-	15	-	1
F2	20	-	-	30	-	10	-	25	15	-	1
F3	30	-	30	-	20	-	-	10	-	10	1
F4	-	25	-	30	25	-	-	10	-	10	1
F5	20	-	35	-	-	-	-	25	10	10	1
F6	20	-	-	35	-	-	25	-	10	10	1

CPTX: captex; Crem EL: Cremophor EL; crem RH 40: cremophor RH40; PEG: polyethylene glycol; CA: caprylic acid; DMSO: dimethyl sulfoxide; CU: curcumin

Table II. Characterization of curcumin-loaded SEDDS formulations

Cu-SEDDS formulations	Mean droplet size (nm)	PDI	Zeta potential (mV)
F1	128.4±0.2	0.374	-5.00 (±0.5)
F2	83.05±0.5	0.461	-4.69 (±0.1)
F3	59.98± 1.3	0.446	-5.82(±0.4)
F4	95.47±1.2	0.418	-1.38(±0.3)
F5	556.2±1.4	0.838	-5.04 (±1.1)
F6	57.19±0.4	0.208	-5.64 (±1.4)

ISOLATION AND IDENTIFICATION OF C. ALBICANS

In this study, a total of 80 vaginal swab samples were collected from women suspected of having VVC. Among these samples, 39 (48.75%) were obtained from pregnant women, while 41 (24.31%) were from non-pregnant women, which included 2 (2.5%) cases from infertile women. The samples were cultured on SDA and incubated for 1 day at 37°C. To confirm the presence of *C. albicans*, the isolates were transferred to chrome agar, where they produced characteristic greenish colonies.

ANTIFUNGAL SUSCEPTIBILITY TESTS

For the antifungal susceptibility testing, fluconazole discs (31 µg) were used, with fungizone serving as a positive control. Out of a total of 17 strains, 2 (11.7%) were identified as fluconazole-resistant, while 15 (88.3%) were found to be fluconazole-susceptible (Fig. 2).

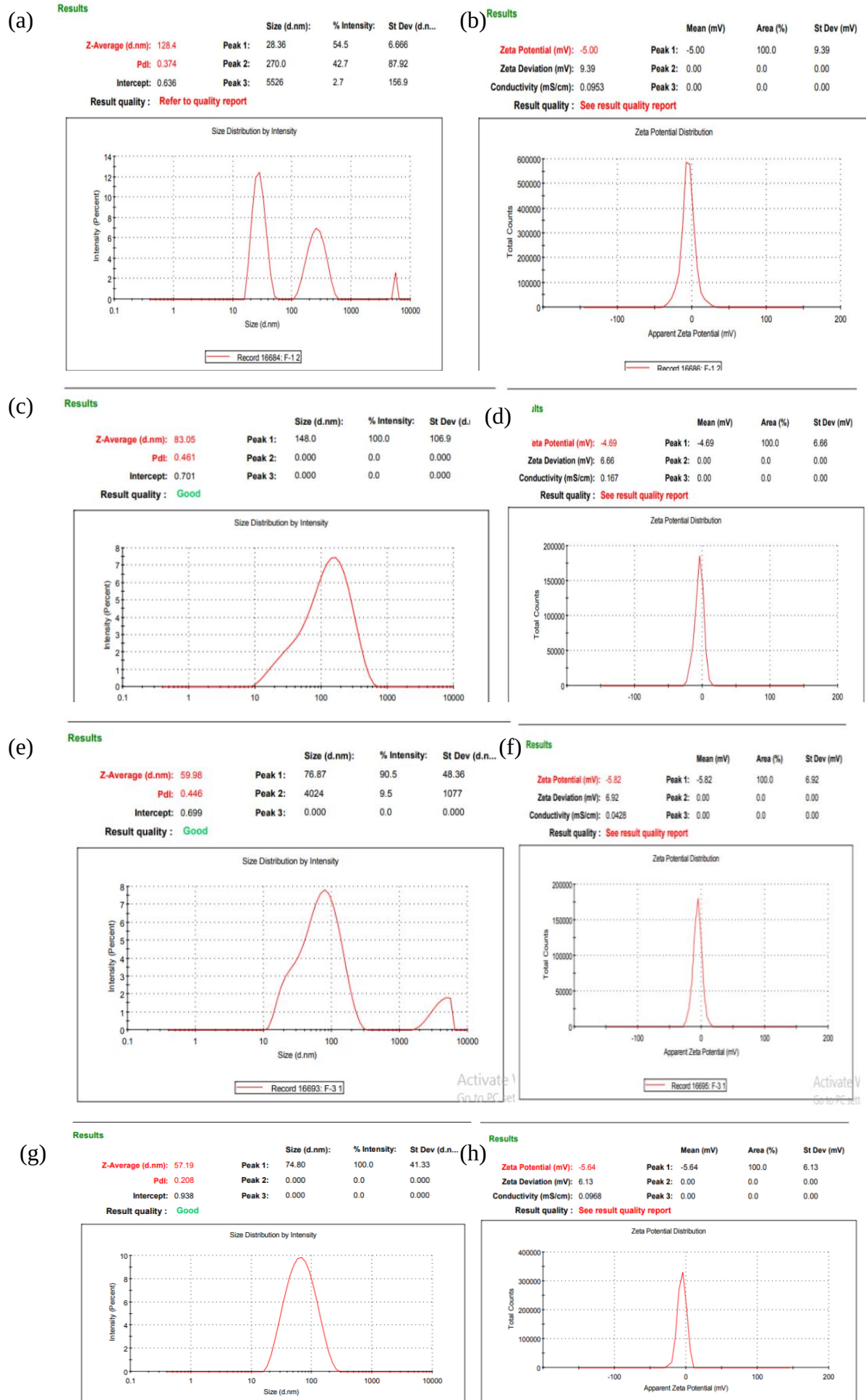


Fig. 1 (a). The size distribution of formulation F1 measured by zeta sizerr; (b). Zeta potential of formulations F1 measured by zeta sizerr; (c). The size distribution of formulation F2 measured by zeta sizerr; (d). Zeta potential of formulation F2 measured by zeta sizerr; (e). The size distribution of formulation F3 measured by zeta sizerr; (f). Zeta potential of formulation F3 measured by zeta sizerr; (g). The size distribution of formulation F6 measured by zeta sizerr; (h). Zeta potential of formulation F6 measured by zeta sizer



Fig. 2 (a). Fluconazole-resistant strain of *C. albicans*; (b). Fluconazole-susceptible strain of *C. albicans*

Antifungal activity of Cu-SEDDS was assessed by the well diffusion method. Following a 24-hour incubation period, the inhibition zones were measured for two strains of *C. albicans*: the fluconazole-susceptible strain and the fluconazole-resistant strain.

ANTIFUNGAL ACTIVITY OF CU-SEDDS AGAINST FLUCONAZOLE SUSCEPTIBLE STRAIN

The efficacy of 25 mg of each Cu-SEDDS formulation (containing 250 µg/mL curcumin) was evaluated against the fluconazole-susceptible strain of *C. albicans*. The largest zone of inhibition, measuring 26 mm, was recorded for formulations F1 and F2. Conversely, formulations F3, F4, and F6 showed the smallest zones of inhibition, measuring 21 mm (Fig. 3 and Table III).

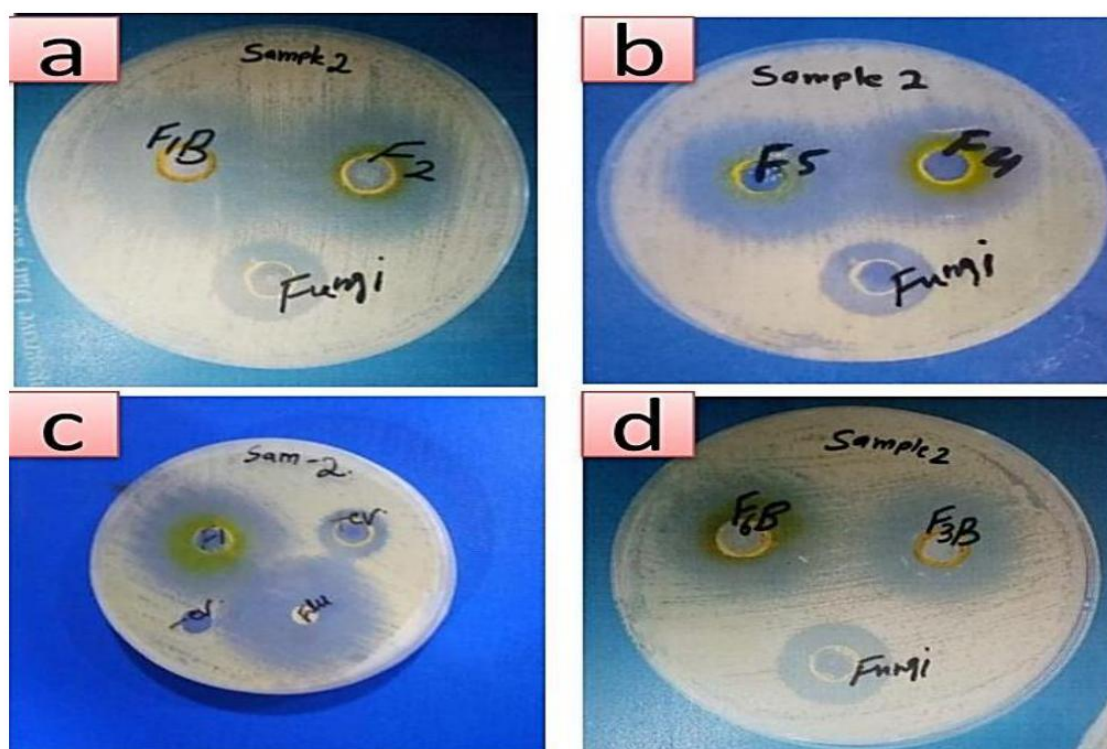


Fig. 3. Zones of inhibition of all Cu-SEDDS formulations against the fluconazole-susceptible strain of *C. albicans*; (a). Shows the zones of inhibition for formulations F2 and F3; (b). displays the zones of inhibition for formulations F4 and F5; (c). Illustrates the zones of inhibition for formulation F2, the fluconazole disc, and includes both positive and negative controls; (d). Depicts the zones of inhibition for formulations F3, F6, and the positive control

Table III. Zones of inhibition of Cu-SEDDS formulations against fluconazole-susceptible and resistant *C. albicans* strain

Cu- SEDDS Formulations	Zones of inhibition (mm) $\pm$ SD against fluconazole-susceptible <i>C. albicans</i> strain	Zones of inhibition (mm) $\pm$ SD against fluconazole-resistant <i>C. albicans</i> strain
F1	26 $\pm$ 0.5	21 $\pm$ 0.5
F2	26 $\pm$ 0.5	21 $\pm$ 0.2
F3	21 $\pm$ 0.4	16 $\pm$ 0.5
F4	21 $\pm$ 0.2	15 $\pm$ 0.8
F5	23 $\pm$ 0.5	18 $\pm$ 0.4
F6	21 $\pm$ 0.4	14 $\pm$ 0.8

ANTIFUNGAL ACTIVITY OF CU-SEDDS AGAINST FLUCONAZOLE-RESISTANT STRAIN

After 24 hours of incubation, the effects of 25 mg of all Cu-SEDDS formulations (containing 250 μ g/mL curcumin) were evaluated against the fluconazole-resistant strain of *C. albicans*. Notably, the zones of inhibition for Cu-SEDDS formulations against both fluconazole-resistant and susceptible strains showed no significant differences (Fig 4). Formulations F1 and F2 exhibited the largest zones of inhibition, measuring 21 mm, while formulation F6 had the smallest zone, measuring 14 mm (Table III).

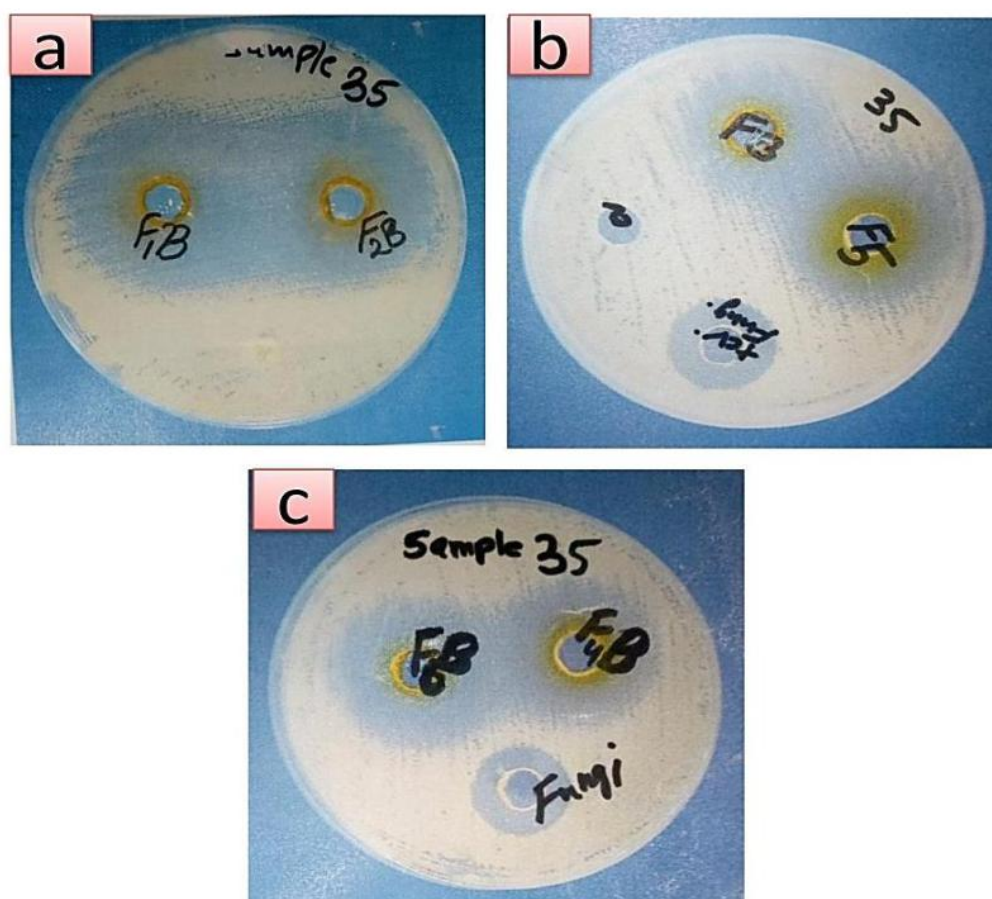


Fig. 4. Zones of inhibition of Cu-SEDDS formulations against the fluconazole-resistant strain; (a). Zones of inhibition for formulations F1 and F2 and the fluconazole disc; (b). Zones of inhibition for formulations F3 and F5, along with positive and negative controls; (c). Zones of inhibition for formulations F6 and F4

MIC OF CU-SEDDS DETERMINED BY THE BROTH MICRODILUTION METHOD

The MIC for the fluconazole-susceptible and fluconazole-resistant strains of *C. albicans* was determined using the broth microdilution method. Following a 24-hour incubation period at 37°C, the MIC results were evaluated. Formulation F2 displayed the lowest MIC value of 0.98 μ g/mL, while formulation F6 had the highest MIC value of 7.8 μ g/mL. Table IV lists the MIC values of curcumin in all Cu-SEDDS formulations against the fluconazole-susceptible strain of *C. albicans*. F2 showed the best antifungal performance, which may be related to its balanced composition of Captex 355, Cremophor EL, Tween 80, and PEG 300, producing small droplets (83.05 nm), acceptable PDI, and stable negative zeta potential. These

properties may improve curcumin dispersion and interaction with fungal cells. Table IV shows the MIC of curcumin in each Cu-SEDDS formulation against the fluconazole- susceptible and resistant strain of *C. albicans*.

Table IV. MIC values of curcumin (in all Cu- SEDDS formulations) against Fluconazole-susceptible and resistant strain of *C. albicans*

CU- SEDDS formulations	MIC Values ($\mu\text{g/ml}$) $\pm$ SD against Fluconazole susceptible strain of <i>C. albicans</i>	MIC Values ($\mu\text{g/ml}$) $\pm$ SD against Fluconazole resistant strain of <i>C. albicans</i>
F1	3.9 $\pm$ 0.02	3.9 $\pm$ 0.02
F2	0.98 $\pm$ 0.04	3.9 $\pm$ 0.02
F3	3.9 $\pm$ 0.01	7.8 $\pm$ 0.05
F4	3.9 $\pm$ 0.02	7.8 $\pm$ 0.05
F5	1.95 $\pm$ 0.01	7.8 $\pm$ 0.01
F6	7.8 $\pm$ 0.01	7.8 $\pm$ 0.01

MINIMUM FUNGICIDAL ACTIVITY

To ensure accuracy, the MFC of the Cu-SEDDS formulations was determined alongside their MIC. The MFC values were found to be identical to the MIC values for all formulations (F1-F6) against the fluconazole-susceptible strain. Specifically, the MFC values for curcumin were 1.95 $\mu\text{g/mL}$ for formulation F5, 7.8 $\mu\text{g/mL}$ for formulation F6, and 0.98 $\mu\text{g/mL}$ for formulation F2. An MFC value of 3.9 $\mu\text{g/mL}$ was recorded for formulations F1, F3, and F4 (Fig. 5).

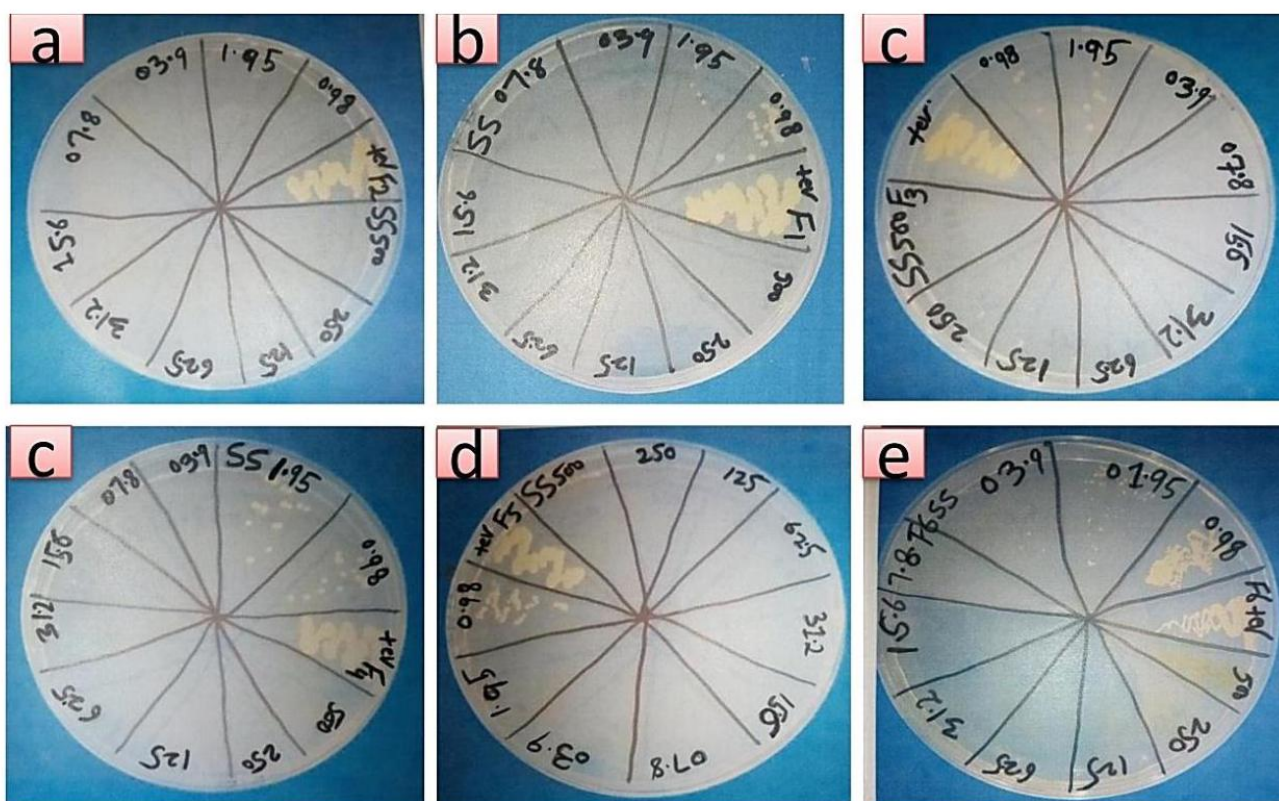


Fig. 5. MFC values of all Cu-SEDDS formulations (F1-F6) against fluconazole-susceptible strain; (a). MFC of F2; (b). MFC of F1; (c). MFC of F3; (d). MFC of F4; (e). MFC of F5; (f). MFC of F6

MFC OF CU-SEDDS FORMULATIONS AGAINST FLUCONAZOLE-RESISTANT STRAIN

MFC provided additional confirmation of the MIC for each formulation against the fluconazole-resistant strain. Fig. 6 displays the MFC values of all Cu-SEDDS formulations (F1-F5) against the fluconazole-resistant strain.

Among the 80 vaginal samples, 44 samples showed *Candida* growth. *C. albicans* accounted for 17 isolates, while 27 isolates were identified as non-*albicans* *Candida* species. The present study focused only on *C. albicans* because the objective was to evaluate Cu-SEDDS activity against the major VVC-associated species and against fluconazole-resistant *C. albicans* strains.

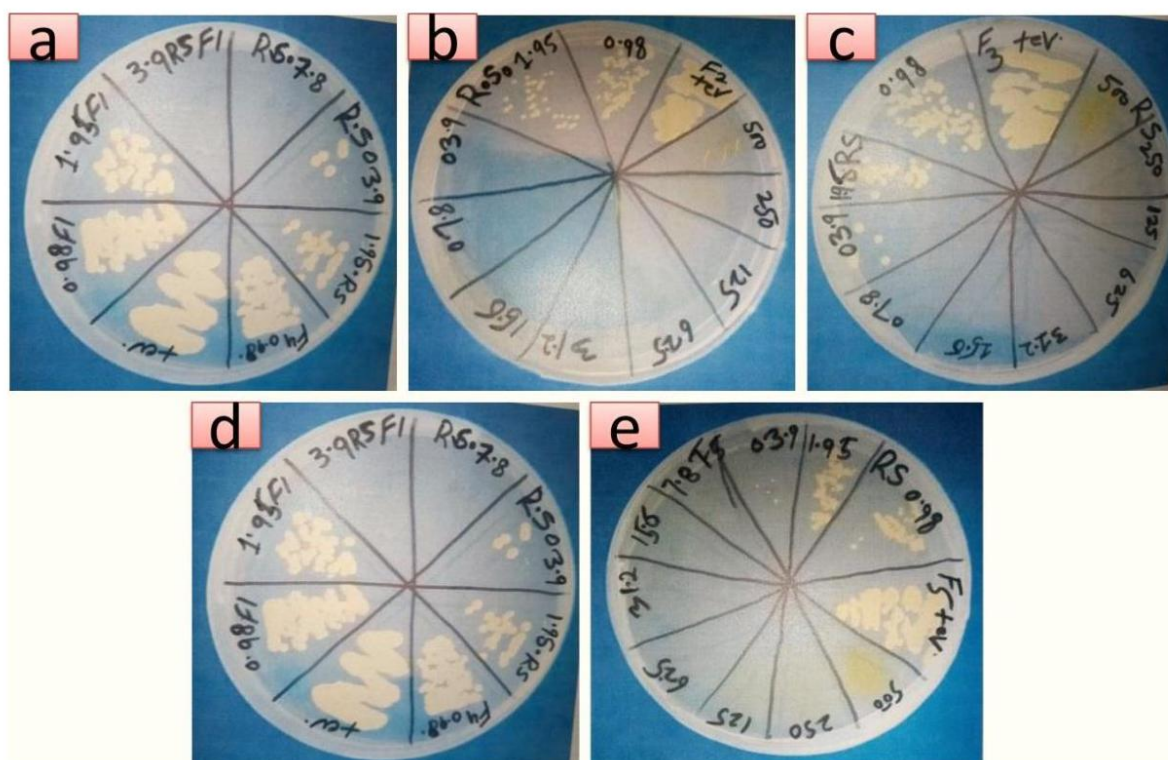


Fig. 6. MFC values of Cu-SEDDS formulations (F1-F5) against fluconazole-resistant strain. (a) shows MFC of F1. (b) shows MFC of F2. (c) reveals MFC of F3. (d) indicates the MFC of F4. (e) depicts the MFC of F5

DISCUSSION

From 1995 to 2002, only 9% of newly developed drugs achieved BCS Class I status, characterized by high permeability and solubility, while over 40% suffered from low oral bioavailability. Self-emulsifying drug delivery systems (SEDDS) have been increasingly employed to enhance the permeability, solubility, and absorption of drugs in BCS Classes II, III, and IV. This study evaluated a Cu-SEDDS formulation containing curcumin against *Candida albicans* strains, producing fine oil-in-water emulsions in aqueous media through gentle agitation. All excipients selected for the formulation were from the FDA's GRAS list, ensuring safety for human use.

The formulation incorporated medium-chain triglycerides, such as Captex 300 and Captex 355, which comprised 20–30% of the lipid phase. These excipients are essential for SEDDS, enhancing drug solubility and facilitating self-emulsification. Surfactants in the formulation, maintained at 30-50% w/w, effectively reduced surface tension and bridged the hydrophilic and hydrophobic phases. Non-ionic surfactants with an HLB value greater than 12 are recommended for SEDDS formulations due to their ability to create fine nano-emulsions with particle sizes under 200 nm. Tween 80, with an HLB of 15, was included at 10% concentration in formulations F1 and F2, while Cremophor EL (HLB 12-14) was added at 30-35% in F2, F4, and F6. Additionally, Cremophor RH40 (HLB 13) was incorporated at 30-35% in F1, F3, and F6. Capmul PG-8, categorized as a hydrophobic surfactant with an HLB below 12, was included in formulations F3 and F4 at 20-25%.

Co-solvents like PEG 300 and PEG 400 were utilized in formulations F1-F6 (10–31%) to enhance the dispersion of hydrophilic surfactants and improve stability. These co-solvents also mitigate skin irritation from surfactants and minimize permanent gastrointestinal changes. PEG 400 aids in emulsification by forming hydrogen bonds, thus enhancing emulsion stability.

DMSO was included in formulations F3 to F6 due to its effectiveness in dissolving curcumin and its ability to permeate biological membranes, making Cu-SEDDS formulations suitable for topical applications like treating vaginal candidiasis, thanks to their analgesic, antioxidant, and anti-inflammatory properties.

The formulations exhibited negative zeta potentials, crucial for stability, with values of -5.00, -4.69, and -5.82 for F1, F2, and F3, respectively, while F4, F5, and F6 had zeta potentials of -1.38, -5.04, and -5.64. F1

was classified as an SMEDDS due to its average particle size of 128.4 nm, whereas F2 (83.05 nm), F3 (59.98 nm), F4 (95.47 nm), and F6 (26.35 nm) were classified as SNEDDS.

Cu-SEDDS formulations exhibited significant anticandidal activity against both fluconazole-susceptible and fluconazole-resistant strains of *Candida albicans*. Among the 80 samples collected, 55% tested positive for *Candida* species, consistent with studies in Baghdad (26.3%) and varying reports from Islamabad (39.76% to 65.8%) due to differences in study design and demographics.

The observed activity of Cu-SEDDS against both fluconazole-susceptible and fluconazole-resistant *C. albicans* is clinically relevant in the context of increasing antimicrobial and antifungal resistance. Similar concerns have been reported in studies describing *Candida* species distribution, *Aspergillus* epidemiology, multidrug-resistant bloodstream infections, and resistant uropathogens, highlighting the need for alternative formulations and adjunct therapeutic approaches (4, 5, 9, 10, 12, 14, 56).

Of the positive samples, 27 (33.75%) were non-*albicans*, and 17 (22.31%) were *C. albicans*, with one instance of co-infection. The prevalence of *C. albicans* in this study was 13.4%, similar to findings by Jamil *et al.* Another study reported a higher prevalence in pregnant women (47%) compared to non-pregnant women.

In terms of fluconazole susceptibility, 11.7% of *C. albicans* strains were resistant, comparable to the 18.75% reported by Tasneem *et al.* Conversely, 88.3% were sensitive, aligning with reports of 89.5% sensitivity from Badiie and Alborzi. Cu-SEDDS formulations, each containing 25 mg of curcumin (250 µg/mL), demonstrated significant inhibitory efficacy against both types of strains, with mean inhibition zones of 23 mm for susceptible strains and 17.4 mm for resistant strains.

The MIC of curcumin across all formulations ranged from 0.98 µg/mL to 7.8 µg/mL for fluconazole-susceptible strains and from 3.9 µg/mL to 7.8 µg/mL for resistant strains. These values are substantially lower than those reported by NK *et al.*, which ranged from 250 to 650 µg/mL for susceptible strains and 250 to 500 µg/mL for resistant strains. Additionally, Katiraei *et al.* showed that dendrosomal nanocurcumin (MIC 2 mg/mL) was more effective than curcumin (MIC 0.5 mg/mL) against both *C. albicans* and non-*albicans* species. Variability in reported MIC values for curcumin against *C. albicans* is notable; Sharma *et al.* reported 185 mg/mL, while Lee *et al.* found 4.2 mg/mL. Neelofer and colleagues observed a range of 250–2000 µg/mL, and Martins *et al.* reported 64 mg/mL. In contrast, our Cu-SEDDS formulations displayed significantly lower MIC values, highlighting their potential as effective therapeutic agents against *C. albicans*.

The improved MIC profile of Cu-SEDDS in the present study may be associated with enhanced dispersion, improved curcumin solubilization, and better interaction of nano-sized droplets with fungal cells. This is consistent with previous work on curcumin-loaded SEDDS for cutaneous and mucocutaneous leishmaniasis, where SEDDS improved the delivery potential of curcumin and showed promising activity against the primary pathogen as well as secondary bacterial infections (21, 23). Similarly, polymer-coated miltefosine, amphotericin B-loaded chitosan nanoparticles, pentamidine-loaded chitosan nanoparticles, and vanillin-derived Schiff bases have shown promising activity against *Leishmania tropica*, further supporting the value of formulation-based approaches for infectious disease treatment (22, 51, 52, 57).

Taken together, these findings suggest that Cu-SEDDS may contribute to the development of alternative antifungal strategies in settings where conventional drugs are limited by resistance, poor solubility, or reduced bioavailability. The relevance of this approach is strengthened by previous evidence showing the potential of nanocarrier-based systems in fungal infections, leishmaniasis, secondary bacterial infections, and broader antimicrobial resistance-related challenges (14, 21-23, 51-57).

CONCLUSION

This study investigated the anticandidal activity of SEDDS formulations against both fluconazole-susceptible and fluconazole-resistant strains of *Candida albicans*. Formulation F1, with an average particle size of 128.4 nm, was classified as SMEDDS, while formulations F2 (83.05 nm), F3 (59.98 nm), F4 (95.47 nm),

and F6 (26.35 nm) were classified as SNEDDS. The negative zeta potential across all formulations indicated good colloidal stability, a critical factor for their effectiveness.

Although direct comparison with unmodified curcumin was not performed in this study, Cu-SEDDS formulations showed strong inhibitory activity against *C. albicans*, suggesting their potential to improve the fungicidal effect on *C. albicans* strains. These findings highlight that the selected formulations are effective against both fluconazole-susceptible and fluconazole-resistant strains, offering a promising strategy for enhancing the treatment of candidiasis. The ability of these formulations to improve the bioavailability and efficacy of curcumin positions them as valuable candidates for the development of novel antifungal therapies. Overall, this study underscores the potential of SEDDS formulations in advancing treatment options for fungal infections.

Limitations and future application

Non-albicans *Candida* species were identified in this study but were not included in antifungal testing. Future studies should evaluate Cu-SEDDS activity against different *Candida* species and perform stability testing over extended storage periods. For clinical translation, these formulations may be further developed as vaginal creams, gels, or other local delivery systems after appropriate safety and formulation studies.

Acknowledgment:

We extend our appreciation to the Office of Research Innovation and Commercialization (ORIC), Khyber Medical University, for providing partial support for this study.

Conflict of interest:

The authors report no conflicts of interest

Ethical Approval:

The study was approved by the Institutional Bioethical Committee, Khyber Medical University Peshawar, Pakistan with Approval No. (Dir/IBMS-EB/IV/000518).

Author's contributions:

AT & HN Conceived, designed the study, performed the experiments, collected the samples, and analyzed the data; RA & GA Microbiological investigation and interpretation of results; SM & MF Contributed to data analysis and manuscript revision; MK Supervised the study, provided scientific guidance, and critically revised the manuscript. All authors read and approved the final version of the manuscript.

Declaration of generative AI-Assisted Tools:

No AI-assisted tools were used.

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