RESEARCH PAPER

The Effectiveness of Chitosan-Coated Iron Oxide Nanoparticles as Antifungals against Various *Candida* Species

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ABSTRACT

The advent of antifungal agents' resistance has sparked the creation of more effective medications and innovative methods for treating a variety of fungal infections. Due to its biodegradability, lack of cytotoxicity, and reactive surface that may be modified with biocompatible coatings, chitosan-coated iron oxide Fe_3O_4 nanoparticles (CS-coated Fe_3O_4 NPs) have received a lot of attention. The authors were prompted to research whether CS-coated Fe_3O_4 NPs would be useful against fungal infections brought on by *Candida* species after learning about the different medical applications of CS-coated Fe_3O_4 NPs. Fourier transform infrared (FTIR) spectroscopy, transmission electron microscopy (TEM) and X-ray diffraction were used in this study to characterize CS-coated Fe_3O_4 NPs. The objective of this investigation was to compare the antifungal efficacy of CS-coated Fe_3O_4 NPs to several *Candida* spp. against fluconazole (FLC). The MIC (minimum inhibitory concentration) and MFC (minimum fungicidal concentration) values of CS-coated Fe_3O_4 NPs varied from 59 to 475 g/ml and 475 to 950 g/ml, respectively. Both the MFC and MIC of FLC were found to be in the range of 61–486 g/ml and 15–119 g/ml, respectively. According to the growth inhibition value, CS-coated Fe_3O_4 NPs were most effective against *Candida glabrata*, *Candida albicans*, and *Candida tropicalis* species. The results demonstrated that all of the tested *Candida* spp. could not develop when exposed to the CS-coated Fe_3O_4 NPs.

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INTRODUCTION

Hospital-acquired infections, often known as nosocomial infections, are increasingly being linked to *Candida* species (spp.). About 30% of patients in intensive care units are affected by nosocomial infections, which have significant morbidity and fatality rates [1–3]. In patients with impaired immune systems, invasive fungal infections are a serious health concern. Clinical symptoms can vary and range from current infection caused by local aetiologic factors to colonization in allergic bronchopulmonary

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illness. When fungal tissue involvement is seen during a histopathologic examination using specific stains, or when the aetiologic agent is culture-isolated from sterile clinical specimens, an infection has been established. One of the most significant medical issues is opportunistic *Candida* yeast infections [4]. More than 90% of invasive candidiasis infections are caused by Non-*albicans Candida* (NAC) species comprising *C. parapsilosis, C. glabrata, C. tropicalis, C. krusei* and *C. lusitaniae* and *Candida albicans* [5–7]. Over the past 20 years, the prevalence of this infection in immunocompromised hosts has substantially grown. Several antifungal medications such as amphotericin B formulations, echinocandins and fluconazole (FLC) are available as a means of treating invasive candidiasis [8–10]. However, using antifungal medications for a long time or in high doses can lead to significant issues such as treatment failure, creation of resistant organisms, and increasing antifungal medication resistance in *Candida* species [8]. For prevention, treatment, or management of human fungal diseases caused by resistant organisms, it is critically necessary to discover alternative antifungal medicines with fewer adverse effects. Decreased drug toxicity, increased therapeutic efficacy, drug rapid spread, higher drug accumulation at the spot, and drug absorption in patients are enhanced by drug delivery systems based on nanoparticles (NPs) [11–13]. One of the most adaptable, secure, and non-toxic nanomaterials are chitosan-coated iron oxide nanoparticles (CS-coated Fe_3O_4 NPs) [14,15]. A biopolymer created by deacetylating chitin, chitosan is a linear polysaccharide that is biodegradable, biocompatible, and contains amino, and primary and secondary hydroxyl groups [16,17]. These act as a foundation for targeting ligands, imaging agents and combining therapeutics [18]. Chitosan has minimal toxicity to human cells yet antibacterial action against a variety of microorganisms [19,20]. Particles of magnetite (Fe_3O_4) and/or maghemite (γ-Fe_2O_3) are found in iron oxide nanoparticles [21]. These NPs have sizes between 8 to 20 nm in diameter [22]. Due to its biochemical and magnetic characteristics, as well as their affordability for usage in a variety of medicinal applications, iron oxide nanoparticles are frequently used. The following are the essential uses for CS-coated Fe_3O_4 NPs: blood detoxification, cell tracking, magnetic hyperthermia, protein separation, biosensing, as gene carriers for gene therapy, as target-specific medication delivery, in magnetic data storage, and in magnetic resonance imaging [23]. In this research, six distinct *Candida* spp. were used to assess the antifungal activity and characteristics of CS-coated Fe_3O_4 NPs.

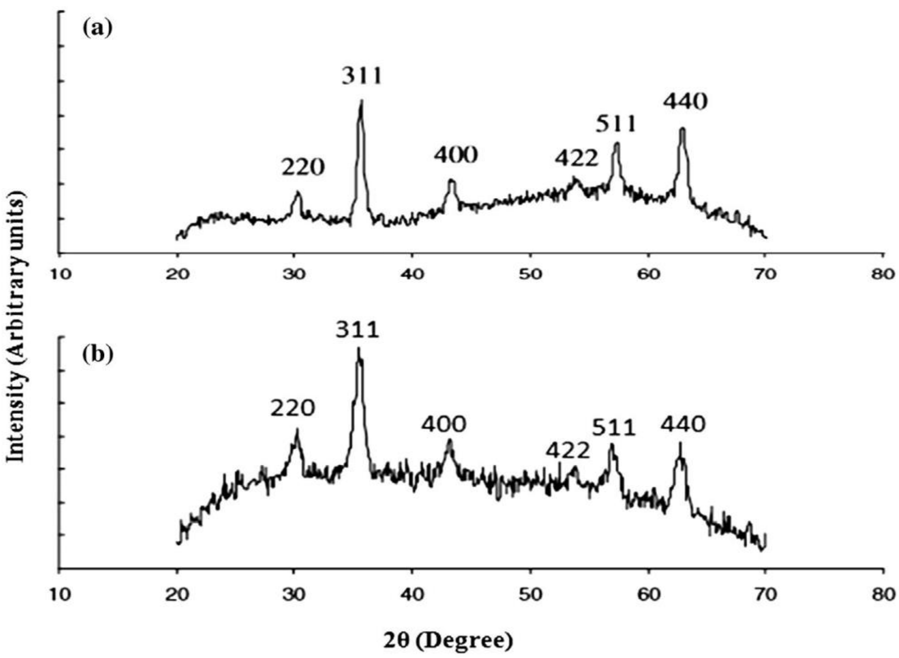

Fig. 1. XRD pattern: (a) Fe_3O_4 NPs and (b) CS-coated Fe_3O_4 NPs.

MATERIALS AND METHODS

Approximately, 35–65% of all candidaemias in the general patient population are caused by NAC species. Bone marrow transplant (BMT) recipients and cancer patients with haematological malignancies are the groups most likely to experience them (40–70%). Intensive care unit (ITU) and surgical patients (35–55%), children (1-35%), and patients with HIV infection (0–33%) are less likely to experience them. Therefore, *C. parapsilosis, C. glabrata, C. lusitaniae, C. tropicalis, C. krusei*, and *C. albicans* were the six distinct *Candida* spp. used in the current investigation. These species were found in people who had different clinical manifestations of candidiasis. The polymerase chain reaction-based approach and conventional mycological techniques utilizing particular *Msp I* enzyme and primers were previously used to identify the species [24,25]. Sabouraud dextrose agar (SDA) was used to cultivate the six *Candida* spp. The appropriate ethical committee has given its permission for this research. By using the XRD technique, the crystal structure of the NPs acquired from Sigma-Aldrich, USA, was examined. Fig. 1 displays the XRD patterns of both and CS-coated and bare Fe_3O_4 NPs.

Fig. 2 displays typical Transmission electron microscopy (TEM) images of the bare and CS-coated Fe_3O_4 NPs. Magnetic NPs are seen to be well-formed, spherical, and of uniform size.

While CS-coated NPs had a mean diameter of around 7 nm, bare Fe_3O_4 NPs had a mean diameter of about 10 nm. This demonstrated that the deagglomeration of secondary particles had been caused by the chitosan grafting. At room temperature (25°C), the FTIR analysis verified that chitosan was present on the surface of Fe_3O_4 NPs. The distinctive peaks of CS-coated and bare Fe_3O_4 NPs are depicted in Fig. 3.

By using the broth microdilution (BMD) and disc diffusion procedures based on the recommendations of the Clinical & Laboratory Standards Institute (CLSI) Guidelines [26], the growth inhibitory effects of FLC and CS-coated Fe_3O_4 NPs against the six *Candida* spp. were compared. In 96-well flat bottom tissue culture plates, the BMD technique was carried out using RPMI 1640 buffered with MOPS purchased from ATCC (Rockville, MD, USA). Deionized water and DMSO which was purchased from Sigma Aldrich were used to dissolve CS-coated Fe_3O_4 NPs and FLC powders, respectively [27]. Then, in RPMI 1640 culture media, serial two-fold dilutions were prepared with concentrations of 950-0.18 g/ml for the CS-coated Fe3O4 NPs and 950-0.3 g/ml for FLC. The inoculum was then applied to each microtiter plate wells, comprising 1.8×10^3 CFU/ml of every *Candida* species. The 96-well microtitration plates were thoroughly mixed before being placed in an incubator (Jeio-Tech, Seoul, Korea) shaken at 135 rpm for 48 hours at 30°C. At 480 nm, a microplate reader (Thermo Fisher Scientific) was applied to read the minimum inhibitory concentrations (MICs) following a 24-hour incubation period [28]. According to turbidity brought on by the development of the tested *Candida* species, the MICs were determined by microplate reader optical density determination and absorbance measurement. The above-mentioned wells and RPMI-1640 that utilized DMSO and deionized water for dissolving the FLC and CS-coated Fe_3O_4

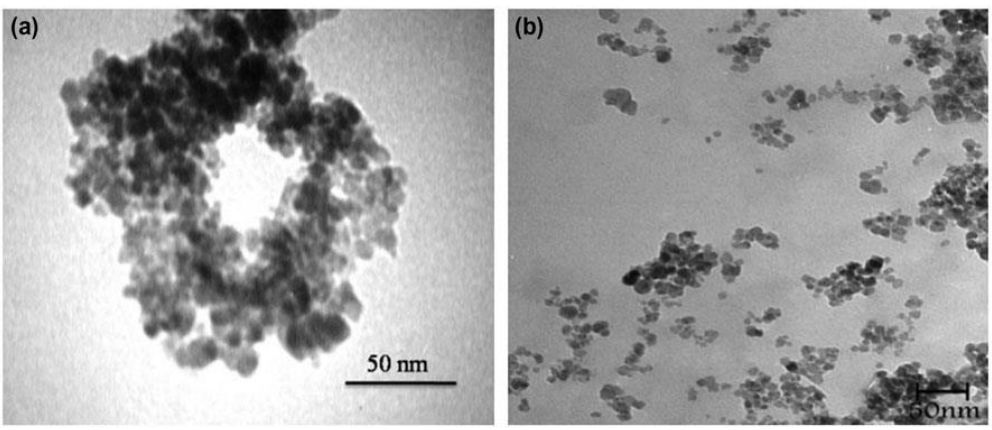

Fig. 2. TEM images: (a) the bare and (b) CS-coated Fe_3O_4 NPs.

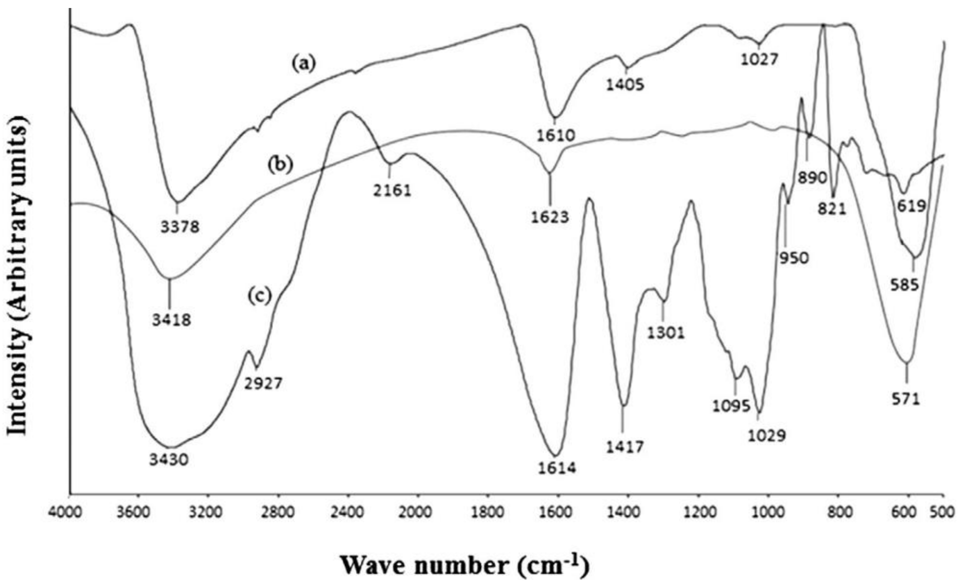

Fig. 3. FTIR analysis: (a) CS-coated Fe_3O_4 NPs, (b) Fe_3O_4 NPs and (c) CS polymer.

NPs, have been used as negative controls since it resulted in a reduction of *Candida* spp. growth inhibition by approximately 90% [29]. The MICs for the CS-coated Fe_3O_4 NPs and FLC were determined by comparing the levels of growth in the test wells with and without the agents to the levels of growth in the control wells without the CS-coated Fe_3O_4 NPs and/or FLC. Additional quality control was performed using isolates of *C. albicans* (ATCC 10231) and *C. parapsilosis* (ATCC 22019). The lowest CS-coated Fe_3O_4 NPs and/or FLC concentration that can kill 99.9% of yeast cells is called the minimum lethal concentration or minimum fungicidal concentration (MFC). 10 µl from each growth well, visible or not, were dispensed onto SDA plates, where the MFC technique was carried out. After 28 hours at 30°C of incubation, the SDA plates were examined [30]. The lowest CS-coated Fe_3O_4 NPs and FLC concentration with fewer than three colonies were used to calculate the MFC values. The mean values of MFCs and MICs for the FLC and CS-coated Fe_3O_4 NPs were recorded after each experiment was run at least three times. A Mueller-Hinton agar plate (15 cm diameter) with a 0.5 mg/ml dose of methylene blue and 2% glucose was used for the disc diffusion method [31]. The starting inoculum, equivalent to a McFarland standard of 0.5, contained $1\text{-}5\times10^6$ CFU/ml of every *Candida* species. The CS-coated Fe_3O_4 NPs and FLC were soaked in 950 and 0.3 g/ml concentrations in around 12 commercially manufactured blank paper discs. A blank paper discs containing DMSO and deionized water, an CS-coated Fe_3O_4 NPs + FLC disc, and a 25 µg FLC disc (Becton-Dickinson) have been employed as control. The inoculated agar surface was covered with all of the discs. The plates were incubated at 30±5°C for 24-28 hours [32]. By quantifying the zones of growth inhibition surrounding each disc with a meter ruler, the antifungal activity of the CS-coated Fe_3O_4 NPs and FLC were evaluated [17]. The growth zones' average was recorded after three repetitions of each experiment. The MIC for each of the six types of *Candida* spp. determined through in vitro antifungal assays was communicated to the CS-coated Fe_3O_4 NPs and FLC. A t-test and one-way ANOVA were used to examine the data. There was statistical significance at a p-value of less than 0.05.

RESULTS AND DISCUSSION

The BMD and disc diffusion methods were used to test the antifungal efficacy of the CS-coated Fe_3O_4 NPs and FLC against *Candida* spp. All of the tested strains of *Candida* spp. had their growth inhibited by the CS-coated Fe_3O_4 NPs, proving their antifungal activity against harmful strains of the fungus. Against *Candida* spp. the MIC of FLC is 15-122 µg/ml, while for CS-coated Fe_3O_4 NPs it is 59-475 µg/ml. Antifungal activity against *C. krusei, C. parapsilosis, C. lusitaniae*, and *C. albicans* spp. was considerably lower for CS-coated Fe_3O_4 NPs

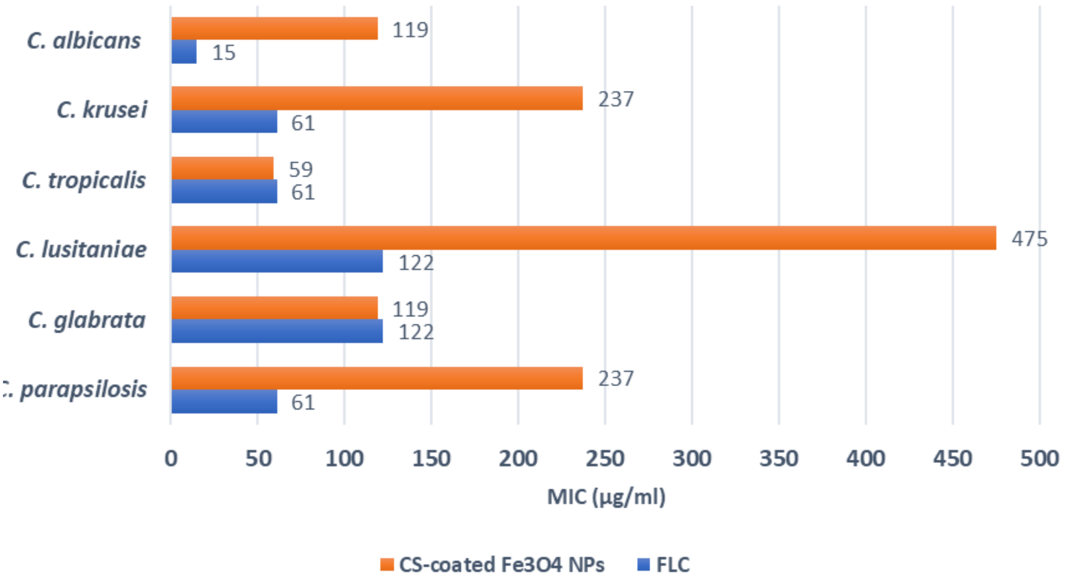

Fig. 4. The MIC of CS-coated Fe_3O_4 NPs and FLC against various *Candida* spp.

compared to that of FLC ($p \leq 0.05$). Regarding the antifungal impact on *C. glabrata* and *C. tropicalis*, there were no discernible differences between the FLC and CS-coated Fe_3O_4 NPs. For the two species, the corresponding MIC values were around 122 and 61 µg/ml. In contrast to other species like *C. krusei, C. parapsilosis,* and *C. lusitaniae* which required larger amounts of the CS-coated Fe_3O_4 NPs to stop their growth, *C. glabrata, C. albicans*, and *C. tropicalis* spp. were more sensitive to lower concentrations of the CS-coated Fe_3O_4 NPs, as shown by findings of antifungal activity for the CS-coated Fe_3O_4 NPs (Fig. 4).

The MICs of the FLC and CS-coated Fe_3O_4 NPs against several *Candida* spp. are shown in Fig. 4. In light of the data, the amounts necessary to

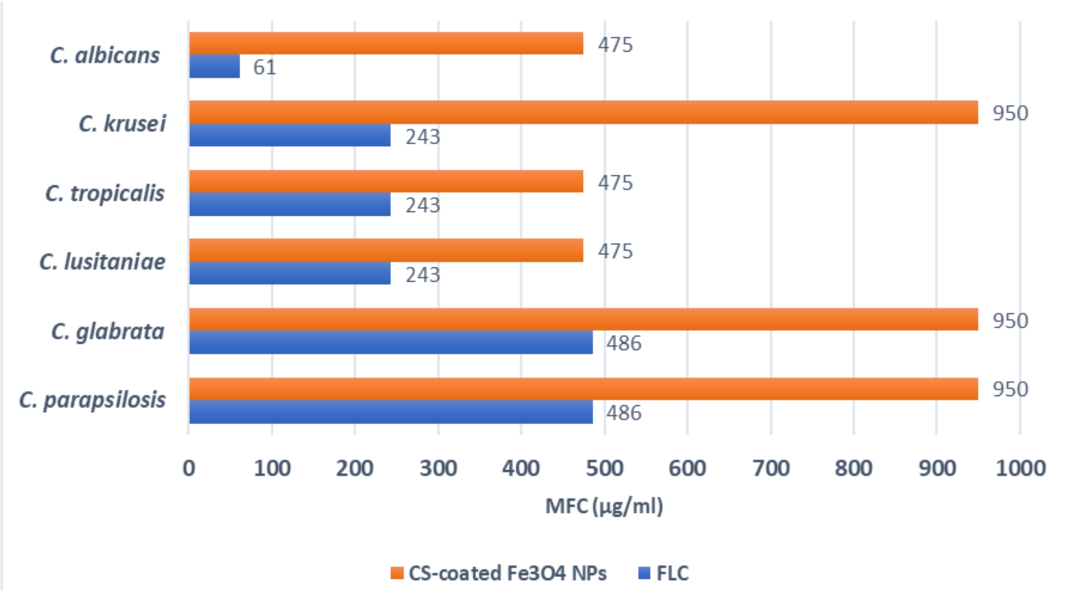

Fig. 5. The MFC of FLC and CS-coated Fe_3O_4 NPs against various *Candida* spp.

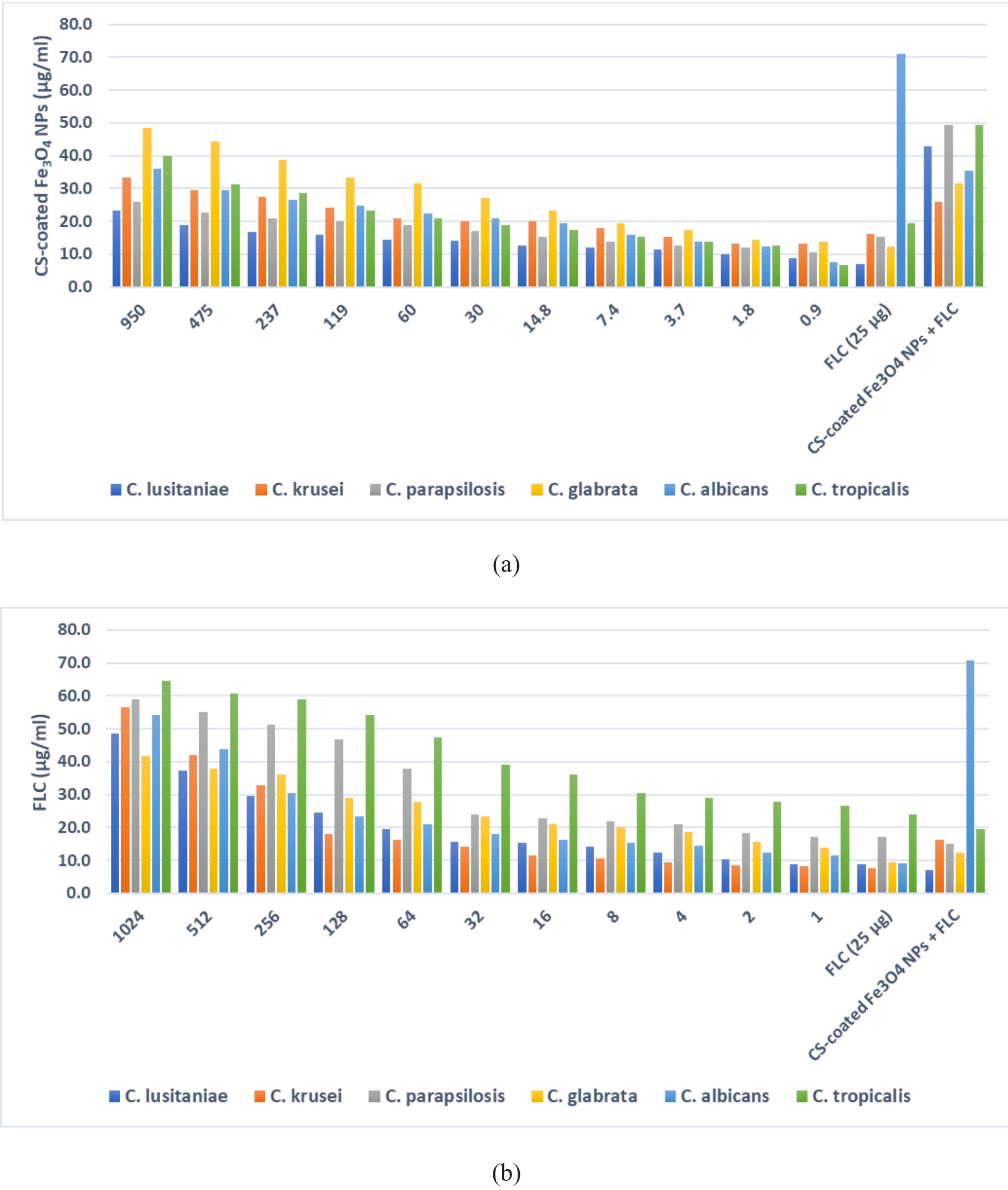

Fig. 6. Zone of inhibition: (a) CS-coated Fe_3O_4 NPs and (b) FLC at different concentrations against various *Candida* spp. (mm).

prevent growth varied depending on the Candida spp. that were put to the test. As a result, *C. lusitaniae* required the largest amount of the CS-coated Fe_3O_4 NPs to be inhibited, preceded by *C. parapsilosis* and *C. krusei*. The CS-coated Fe_3O_4 NPs had a susceptibility ranking of *C. parapsilosis>C. lusitaniae, C. glabrata>C. krusei, and C. tropicalis>C. albicans*. Out of all the examined isolates *C. parapsilosis, C. krusei,* and *C. lusitaniae* isolates exhibited the highest levels of CS-coated Fe_3O_4 NPs resistance. The FLC concentration that produced the lowest MIC was 15 g/ml when used on *C. albicans*. A MIC of 61 g/ml effectively prevented the growth of *C. parapsilosis, C. krusei,* and *C. tropicalis*. For the FLC, the average MIC value reached to 122 g/ml, making *C. glabrata* and *C.*

lusitania less sensitive than any of these examined *Candida* spp. The FLC's susceptibility ranking was *C. parapsilosis* and *C. krusei* > *C. lusitaniae* and *C. glabrata* and *C. albicans>C. tropicalis*. The MFC of the FLC and CS-coated Fe_3O_4 NPs against several Candida spp. is depicted in Fig. 5.

Fig. 5 shows the microdilution assay findings for the fungicidal activity (MFC values) of CS-coated Fe_3O_4 NPs and FLC against *Candida* spp. The MFC ranges for the FLC and CS-coated Fe_3O_4 NPs were 61 to 486 µg/ml and 475 to 950 µg/ml, respectively. For the species of *C. lusitaniae, C. tropicalis,* and *C. albicans* the MFC value of the CS-coated Fe_3O_4 NPs was 475 µg/ml, whereas it was 950 µg/ml for the species of *C. glabrata, C. parapsilosis,* and *C. krusei*. When used against the *C. albicans* species and *C. glabrata* and *C. parapsilosis* species, respectively, the FLC concentrations that produced the smallest and largest MFC were 61 µg/ml and 486 µg/ml.

In the current research, at a level of 950 µg/ml the diameters of the CS-coated Fe_3O_4 NPs' zones of inhibition against the *C. albicans, C. tropicalis,* and *C. glabrata* species were acquired as 36.1, 39.9, and 48.6 mm, respectively. Relatively smaller values of 23.4 25.8, and 33.3 mm were obtained against *C. lusitaniae, C. parapsilosis,* and *C. krusei*, respectively. *C. tropicalis* and *C. glabrata* on the other hand, were more vulnerable to the CS-coated Fe_3O_4 NPs at a concentration of 950 µg/ml. Fig. 6 displays the zones of inhibition for the FLC and CS-coated Fe_3O_4 NPs at varying doses against various *Candida* species.

CONCLUSION

Due of their particular qualities and low negative side effects, NPs are gaining more and more attention. According to the findings of this research, CS-coated Fe_3O_4 NPs possess antifungal properties and have the ability to inhibit the growth of all *Candida* species. It is important to conduct additional susceptibility, toxicity, pharmacokinetic (PK), and efficacy research, both in vitro and in vivo, in order to determine whether or not CS-coated Fe_3O_4 NPs are suitable for use in the medical field. Limitations of our study include using only six *Candida* species to investigate the effects of CS-coated Fe_3O_4 NPs, and each antifungal agent was only used in one concentration.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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