



Within and Beyond the MIC: Unveiling the Antifungal Potency of Green-Synthesized Silver Nanoparticles Against Clinical Isolates of *Candida albicans*

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Abstract

Biogenic silver nanoparticles (AgNPs) synthesized using aqueous extracts of *Pyracantha koidzumii* (AgNPs-P) and *Schinus molle* (AgNPs-S) were evaluated for antifungal activity against two clinical isolates (HCI-I and HCI-II) and an ATCC strain of *Candida albicans*. Synthesis was confirmed by UV–Vis, Fourier transform infrared spectrophotometry (FTIR), dynamic light scattering (DLS), electrophoretic light scattering (ELS), nanoparticle tracking analysis (NTA), and transmission electron microscopy (TEM) revealing stable, quasi-spherical particles averaging 15.13 nm (AgNPs-P) and 10.81 nm (AgNPs-S). Both types of AgNPs exhibited a minimum inhibitory concentration (MIC) of 1.56 µg/mL across all strains. However, AgNPs-P demonstrated superior efficacy against HCI-I, with the lowest minimum fungistatic (6.25 µg/mL) and fungicidal concentrations (12.5 µg/mL). Notably, AgNPs-P eliminated fungal cells at a lower particle concentration (1.76×10^{10} NPs/mL) yielding a lower particle efficiency index (2.8×10^4 NPs/CFU), underscoring their enhanced antifungal efficacy. Mechanistically, the AgNPs compromised cell wall integrity, inhibited hyphal growth, and reduced proteolytic activity. These results highlight the need to evaluate antifungal performance beyond MIC values and suggest AgNPs-P as a promising therapeutic candidate.

Keywords *C. albicans* · Silver nanoparticles · Green synthesis · Antifungal susceptibility test · Minimum fungicidal concentration · Minimum fungistatic concentration

1 Introduction

Fungal infections caused by *Candida albicans* represent a critical global health challenge, specifically for immuno-compromised individuals. In Mexico, candidiasis continues to be a significant concern, with *C. albicans* identified as one of the primary causes of nosocomial infections [1]. Recent epidemiological studies indicate that invasive candidiasis is among the top ten bloodstream infections in Mexican health-care facilities, with mortality rates exceeding 40% [2]. The ever-growing resistance of *C. albicans* strains to conventional antifungal treatments, particularly azoles and echinocandins [3–6], alongside the increasing global incidence of infections caused by these types of resistant strains, highlights the urgent need for other alternative antifungal agents [7].

In recent years, silver nanoparticles (AgNPs) have garnered significant attention due to their potent antifungal properties, particularly against multidrug-resistant fungal pathogens such as *C. albicans* [8]. One of the most commonly used approaches

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for synthesizing this type of nanoparticle is green chemistry, which relies on eco-friendly methods such as plant-derived extracts or microorganism-mediated processes involving bacteria, fungi, yeasts, microalgae, and bacteriophages [9]. Thus, it is possible not only to reduce the need for toxic chemicals but also to leverage the natural reducing and stabilizing properties of phytochemicals, protein, or peptides. This approach also minimizes the environmental impact while enhancing the biological activity of the nanoparticles, making them highly effective against microbial pathogens [10]. In this context, plants such as *Schinus molle* L. (pink pepper) and other related *Pyracantha* species [distinct from *Pyracantha koidzumii* (Hayata) Rehder] have previously been utilized for the synthesis of AgNPs [11–14]; however, their antimicrobial activity against human fungal pathogens remains largely overlooked. *P. koidzumii* and *S. molle* are widely distributed in Mexico and other regions of the world and are commonly used for ornamental purposes and providing shade in arid areas. Their abundance makes them a sustainable and locally accessible resource for nanoparticle synthesis. Despite the growing interest in plant-mediated synthesis of AgNPs, there remains a limited understanding of the antifungal potential of AgNPs synthesized using underexplored plant species. *Pyracantha koidzumii* and *S. molle* are two such species with documented phytochemical profiles but little to no prior application in nanomaterial synthesis. This study addresses this gap by demonstrating, for the first time, the successful green synthesis of AgNPs and evaluating their antifungal activity.

The minimum inhibitory concentration (MIC) is a crucial parameter in antimicrobial assays for nanoparticles, as it defines the lowest concentration required to inhibit visible microbial growth. In nanoparticle research, MIC determination is essential for evaluating antimicrobial potency, optimizing dosage for therapeutic use, understanding mechanisms of action, assessing resistance potential, and standardizing antimicrobial testing [15]. While the MIC is widely used as a standard endpoint in antifungal evaluations, it does not always reflect the full biological activity or functional performance of nanomaterials. Consequently, this research explores additional parameters including the minimum fungistatic concentration (MFuC), the minimum fungicidal concentration (MFC), the nanoparticle concentration, the particle efficiency index (PEI), and the mode of action of nanoparticles on fungal cells to comprehensively elucidate the antifungal activity of biogenically synthesized AgNPs against human clinical isolates of *C. albicans*.

2 Materials and Methods

2.1 Chemical Reagents

Silver nitrate (AgNO_3), bovine serum albumin, Roswell Park Memorial Institute Medium 1640 (RPMI-1640), dipotassium

phosphate (K_2HPO_4), ammonium hydroxide (NH_4OH , 29%), magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), sodium chloride (NaCl), calcofluor white, and propidium iodide were purchased from Merck KGaA, Darmstadt, Germany. Yeast extract, glucose, bacteriological agar, and Sabouraud Dextrose Agar (SDA) were sourced from BD Bioxon, Mexico. Sterile 96-well plates (3599) were obtained from Corning Inc. Life Sciences. The *C. albicans* strain (ATCC-10231) was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA).

2.2 Plant Specimens and Aqueous Extract Preparation

The plants used in this study were authenticated by the Biologist Alejandro Torres-Montufar, responsible for the management of the Herbarium of the Biological Sciences Department (NYBG Steere Herbarium code: FESC). The accession numbers for the specimens were 9888 [*P. koidzumii* (Hayata) Rehder] and 5726 (*Schinus molle* L.) (Fig. S1). Leaves (without any visual physical damage) were washed with distilled water, oven-dried (40°C for 48 h), and ground with an electric plate-style mill type C-11-1 (Glen Mills Inc. Clifton, NJ, USA). Finally, the finely ground material was screened through a 60-mesh US sieve (particle size of $<250\ \mu\text{m}$). To prepare the aqueous extracts, 2.5 g of powdered leaves (*P. koidzumii* and *S. molle*) were added to amber flasks containing 50 mL of deionized water. The mixtures were boiled for 10 min. After boiling, the solutions were left to cool to room temperature and then centrifuged ($274 \times g$, 7 min). The resulting supernatants were filtered using a PTFE membrane syringe filter with a $0.22\text{-}\mu\text{m}$ pore size.

2.3 Synthesis of AgNPs

For the synthesis, 500 μL of AgNO_3 solution (0.1 M) was added to two separate amber flasks. Afterward, 640 μL and 1000 μL of *P. koidzumii* and *S. molle* aqueous extracts were introduced into each respective flask (these volumes were selected based on preliminary assessments aimed at maximizing the surface plasmon resonance (SPR) intensity near 429 nm in the UV–Vis spectrum). This approach ensured that any observed differences in antifungal efficacy could be attributed to the specific phytochemical composition of each plant extract, rather than disparities in nanoparticle yield. Subsequently, to each mixture, 4.2 μL of NH_4OH was added. The total reaction volume in each flask was then adjusted to 5 mL using deionized water. The reaction mixture was kept under constant stirring for another 10 min at room temperature ($25 \pm 2^\circ\text{C}$).

2.4 Characterization

2.4.1 UV–Vis Spectroscopy

UV–Vis absorption spectroscopy was employed to investigate the optical characteristics of the AgNPs. Absorption spectra were recorded in the range of 300–900 nm utilizing a Cary 8454 UV–Vis diode array spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). All measurements were performed in triplicate at room temperature in quartz cuvettes featuring a path length of 1 cm.

2.4.2 Fourier Transform Infrared Spectrophotometry (FTIR)

A Frontier SP8000 FTIR spectrometer (Perkin Elmer, Waltham, MA, USA) equipped with an attenuated total reflection (ATR) accessory was utilized to identify the functional groups on the surface of the as-prepared NPs. The FTIR spectra were recorded from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} . The FTIR spectrometer was calibrated using a mid-infrared (MIR) polystyrene traceable reference material (L1365334, Perkin Elmer, Waltham, MA, USA), and a background spectrum was recorded to eliminate atmospheric interferences. The characteristic absorption bands were identified and analyzed using the Spectrum 10.4.2 software. All measurements were performed in triplicate.

2.4.3 Dynamic Light Scattering (DLS) and Electrophoretic Light Scattering (ELS) Measurements

Particle size, polydispersity index (PDI), and ζ -potential measurements were carried out using a particle size analyzer (ZetaSizer Pro, Malvern Instruments, Worcestershire, UK). All samples were analyzed in a disposable capillary cell DTS1070 with an equilibration period of 120 s. The suitable parameters chosen were for material (colloidal silver): refractive index 0.135 and absorption 3.99; for dispersant (water): refractive index 1.33 and viscosity 0.8872 mPa/s at 25 °C. Quintuplicates of each sample were measured, and each measurement comprised 20 runs. Results were analyzed using the ZS Xplorer software.

2.4.4 Nanoparticle Tracking Analysis (NTA)

A NanoSight NS300 (Malvern Instruments, Worcestershire, UK) equipped with a 532-nm green laser module was utilized. Data was recorded using a 20 $\times$ objective and a 60-s video clip. Three 60-s video measurements were recorded to provide the average mean and mode values as well as the concentration of AgNPs. Briefly, samples of AgNPs (4.8 μM) were prepared in Milli-Q water. Thereafter, an aliquot

was taken with a sterile syringe and injected into the equipment's chamber, preventing the formation of air bubbles. The capture setting parameters used were camera type: super-high-sensitivity complementary metal oxide semiconductor camera (sCMOS), camera level: 13, slider shutter: 1232, slider gain: 219, number of frames: 1498, temperature: 23 °C, and viscosity: (water) 0.9 cP. The software used to capture and analyze data was nanoparticle NTA 3.2 Dev Build 3.2.16. Each measurement was repeated three times under identical conditions to confirm reproducibility.

2.4.5 Transmission Electron Microscopy (TEM)

Particle size and morphology were determined using a JEM-1010 transmission electron microscope (JEOL, Peabody, MA, USA) operated at an accelerating voltage of 80 kV with a nominal resolution of 0.25 nm. TEM grids were prepared by placing one 10 μL drop of a 3-mM AgNPs solution on carbon-coated copper grids, which were dried at room temperature. The ImageJ 1.52 version software (US National Institutes of Health, Bethesda, MD, USA) was used to determine the average diameter of NPs in the TEM images. A minimum of 200 particles were considered for analysis.

2.5 Antifungal Assay

2.5.1 Fungal Growth and Inoculum Preparation

Two clinical isolates of *C. albicans* from recurrent vulvovaginal candidiasis (denoted as HCI-I and HCI-II) and one reference strain (ATCC-10231) were used. The HCIs were kindly provided by the Social Security Institute of the State of Mexico and Municipalities (ISSEMYM, Ecatepec, State of Mexico, Mexico). Initial characterization was performed using CHROMagar™ Candida, which allows for both selective growth of *Candida* species and easy, rapid identification through the color changes in colonies based on species-specific enzymatic activity [16]. Thereafter, the strain and isolates were cultured on SDA plates at 37 °C for 24 h. The inoculum was prepared in RPMI–1640 medium by adjusting the cell concentration to 0.5 McFarland standard.

2.5.2 Antifungal Susceptibility Test

The antifungal activity of the AgNPs was assessed following the Clinical and Laboratory Standards Institute (CLSI) guidelines M27-S3 and M27-S4 for yeast species [17, 18] with minor adequations concerning the incubation temperature and the time of reading. Sterile 96-well plates were employed using the serial dilution technique. For AgNPs-P, AgNPs-S, and nystatin, the concentration ranged from 0.097 to 200

and 0.24 to 500 µg/mL, respectively. Plates were incubated at 37 °C for 24 h; then, the minimum inhibitory concentration (MIC) was determined visually as the lowest concentration that inhibited the fungus growth, based on the contrast between wells with and without visible growth, facilitated by the color change of the RPMI medium. Furthermore, the minimum fungistatic concentration (MFuC) and the minimum fungicidal concentration (MFC) were determined by inoculating an aliquot of all treatment-wells onto SDA plates. For this purpose, plates were divided into 12 sections. In each section, a drop (10 µL) of sample (*C. albicans* treated with nystatin or AgNPs) was deposited in duplicate. Subsequently, the plates were incubated at 37 °C for 24 h, and fungal growth's absence was evaluated. The MFuC was identified as the lowest concentration of the antifungal agent at which visible fungal growth remained. In contrast, the MFC was considered as the concentration at which no fungal growth was observed upon subculture onto SDA plates [19]. Samples were tested in triplicate for each independent experiment.

2.6 The Relationship Between the Concentration of AgNPs and the Colony Forming Units (CFU)

To determine the relationship between the concentration of AgNPs and the CFU in the wells, the following methodology was used: In the SDA plates (described in the previous section), the CFU near to the MFC were counted using the ImageJ software. The CFU/mL average value was estimated using the dilution factor of 100. Finally, the nanoparticle concentration (NPs/mL) obtained via the NTA technique was estimated and correlated.

2.7 Cell Wall Integrity

The potential damage to the cell wall of the *C. albicans* was evaluated following the methodology reported by Rivera-Yañez et al. [20]. Briefly, 50 µL of sample treated with 1.56 µg of AgNPs/mL (the concentration that corresponds to the MIC) were taken and placed on a glass microscope slide (Waldemar Knittel Glass GmbH, Braunschweig, Germany). Subsequently, 20 µL of calcofluor white plus 10 µL of propidium iodide (2.4 mM) were added. The mixture was thoroughly mixed and allowed to react for 5 min under a 24 × 50 mm cover slip (Waldemar Knittel Glass GmbH). Control samples (without AgNPs treatment) were also considered. Subsequently, 10 zones were randomly selected per slide and analyzed with an Axio Imager A1 fluorescent microscope equipped with an AxioCam MRc (Carl Zeiss Microscopy GmbH, Germany), using the ZENcore 2.0 software. The total number of cells (cells in blue) and the cells with wall damage (cells in red) were counted using the ImageJ software. Three independent experiments were performed.

2.8 Protease Activity Assay

Proteolytic activity was studied using the plate method reported elsewhere [21, 22]. For this purpose, the agar was prepared by dissolving 0.5 g K₂HPO₄, 0.04 g MgSO₄•7H₂O, 1 g NaCl, 0.2 g yeast extract, 4 g glucose, and 4 g agar in 150 mL of deionized water. The pH was adjusted to 6.5. The solution was steam sterilized (121 °C, 15 psi, 15 min); subsequently, 0.5 g bovine serum albumin—as the sole nitrogen source—was dissolved in 50 mL of deionized water; the solution was filtered using a PTFE membrane syringe filter with a 0.22-µm pore size and then added to the previously described sterilized agar. Thereafter, aliquots (20 µL) of the well corresponding to the MFuC and the positive control were transferred to separate sterile Eppendorf tubes, and 180 µL of sterile deionized water was added. Tubes were vortexed thoroughly, and 10 µL of each tube was taken and dropped onto the agar plates. The plates were incubated at 37 °C for 5 days. Then, the plates were stained with 0.6% amido black and subsequently decolorized with a 20% acetic acid solution. The diameter of the CFU and the generated halo were measured in triplicate. The enzymatic activity index (*Pz*) was calculated using the following equation:

$$Pz = \frac{a}{b} \quad (1)$$

where *a* = CFU diameter (mm) and *b* = CFU diameter plus the generated halo (mm). *Pz* = 1 corresponds to non-enzymatic activity, values from 0.9 to 0.99 reflect very weak activity, 0.8–0.89 indicate weak activity, 0.70–0.79 represent moderate activity, and *Pz* < 0.69 signifies high enzymatic activity.

2.9 Experimental Design and Statistical Analysis

The experiment was conducted using a completely randomized design. Since the data did not meet the assumptions of normality and homogeneity of variances required for parametric tests, the nonparametric Kruskal–Wallis test was applied. All statistical analyses were performed using the GraphPad Prism software Version 10.2.3 (GraphPad Software, San Diego, CA, USA). A significance level of $\alpha = 0.05$ was used to determine statistical significances.

3 Results and Discussion

3.1 Characterization

The representative UV–Vis absorption spectra of the engineered AgNPs-P and AgNPs-S are shown in Fig. 1a. In general, there was a prominent absorption band due to the excitation of the SPR. As seen, the band was centered

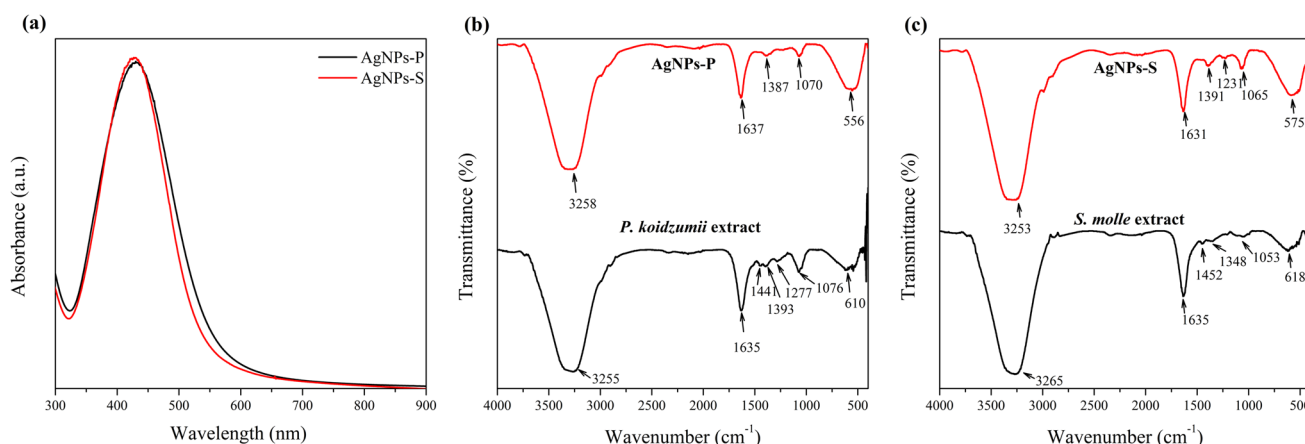


Fig. 1 Representative UV–Vis absorption spectra of the synthesized AgNPs (a), and comparative FTIR spectra of the extracts and the engineered AgNPs using the *P. koidzumii* (b) and *S. molle* (c) aqueous extracts

around 429 nm for both systems. A previous study involving the synthesis of AgNPs with *S. molle* reported a similar SPR maximum [13]. The synthesis of AgNPs involving the aqueous extract of *P. koidzumii* has not been reported, but the absorption band of AgNPs synthesized using the aqueous extract of *P. crenulata* (455 nm) corresponds to the typical value for biogenically synthesized AgNPs [14]. Furthermore, no additional absorbance maximums associated with silver oxide (Ag_2O) were observed, indicating that NPs are in a reduced metallic state without significant oxidation. This observation is critical, as AgNPs exhibit optimal antimicrobial properties in their metallic form, while oxidation may affect their stability and functionality for biological applications [23].

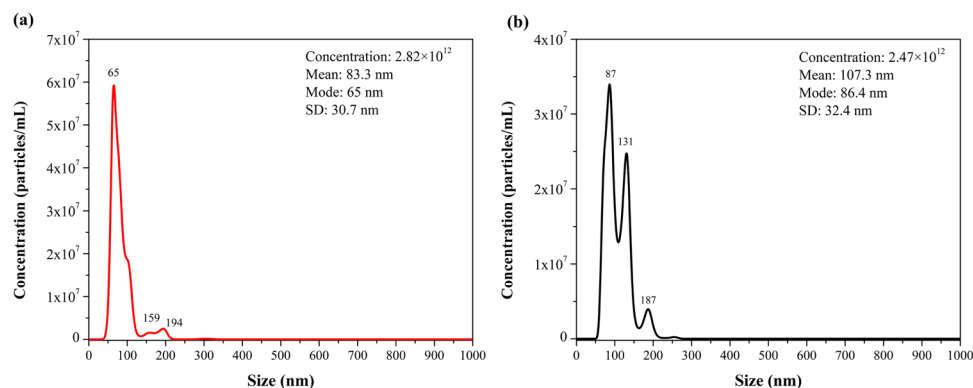
The FTIR spectra showed shifts in some characteristic vibrations, revealing interactions between the functional groups in the extract and the NPs surface (Fig. 1 b and c). For instance, the broad band observed at around $3255\text{--}3265\text{ cm}^{-1}$ attributed to O–H groups such as alcohols and phenols, shifted slightly. The band near 1635 cm^{-1} , which corresponds to C=O stretching or N–H bending from carbonyl or amide groups, also showed a minor shift, indicating its involvement in the stabilization process [23]. The bands at 1076 and 1053 cm^{-1} are found in both extracts, and the bands at 1070 and 1065 cm^{-1} for AgNPs are associated with C–O, C–O–C, and C–N vibrations caused by polyphenols, proteins, organic acids, glycosides, alcohols, terpenoids, polysaccharides, and polyols [24]. The bands observed in the $610\text{--}575\text{ cm}^{-1}$ region can be attributed to C–H out-of-plane bending vibrations in aromatic compounds, reflecting the presence of substituted benzene rings. These changes in the FTIR spectra confirmed the role of certain phytochemicals present in the aqueous extract during NP stabilization. The participation of hydroxyl, carbonyl, and aromatic groups aligns with

previous studies, emphasizing the critical role of phenolic compounds, flavonoids, and carboxylic acids in the green synthesis of AgNPs [25].

According to the DLS measurements, the average size of the NPs was 48.9 ± 2.7 and 59.5 ± 3.4 nm for AgNPs-P (PDI = 0.35) and AgNPs-S (PDI = 0.29), respectively. Thus, AgNPs-S exhibited a more uniform size distribution due to their lower PDI, which may reduce the risk of agglomeration and improve colloidal stability. These slight variations in hydrodynamic diameter could be attributed to the differences in the phytochemical composition of the aqueous extracts. Regarding the ζ -potential, AgNPs-P and AgNPs-S yielded -38.25 ± 1.9 mV and -36.8 ± 1.7 mV, respectively. Both values fall within the range commonly reported for plant-mediated synthesized AgNPs, typically between -20 and -40 mV, due to the capping and stabilization effects provided by bioactive compounds present in the aqueous extract [26, 27]. Moreover, the particle concentration obtained via the NTA technique were 2.82×10^{12} and 2.47×10^{12} particles/mL for AgNPs-P and AgNPs-S, respectively (Fig. 2). The mode values for AgNPs-P and AgNPs-S were 65 and 86.4 nm, respectively.

Other studies on AgNPs synthesis have reported different nanoparticle concentrations. For instance, Rheder et al. [28] using 0.02 M of the metallic salt reported a concentration of 7.2×10^{10} NPs/mL during the synthesis of AgNPs using the extract of the plant *Althaea officinalis*. Moreover, Pérez-de León et al. [29] synthesized AgNPs through chemical reduction of 0.01 M AgNO_3 ; the authors reported a slight value in the particle concentration (2.3×10^{12} NPs/mL). In this research, the use of *P. koidzumii* and *S. molle* aqueous extracts yielded a slightly higher nanoparticle concentration (2.82×10^{12} and 2.47×10^{12} particles/mL), possibly due to its high phytochemical content. It is well known that a high particle concentration may enhance antifungal activity due

Fig. 2 Particle size distribution and particle concentration of AgNPs-P (a) and AgNPs-S (b) measured by the NTA technique



to several key factors: higher particle concentration can provide a greater number of NPs available to interact with the fungal cells, leading to membrane disruption and intracellular penetration [30]. This also leads to an enhanced ion release, which stimulates the production of reactive oxygen species (ROS), causing oxidative stress and damage to certain cellular components like lipids, proteins, and DNA [31]. Additionally, a higher particle concentration can ensure greater surface coverage and cumulative interactions with the fungal cells, amplifying the antifungal effect of the NPs [32].

Moreover, Fig. 3 (profiles a and b) shows representative TEM micrographs for the synthesized AgNPs. Images showed predominantly quasi-spherical morphologies, with average sizes of 15.13 ± 6.6 and 10.80 ± 2.2 nm for AgNPs-P and AgNPs-S, respectively (Fig. 3c and d). In general, the synthesized AgNPs-P showed a broader size distribution. The small differences in size can be attributed to the distinctive phytochemical composition of the plant extracts used during the synthesis, which influences their nucleation and stabilization.

3.2 Antifungal Assay

MIC is defined as the lowest concentration of an antimicrobial agent that visibly inhibits the growth of the microorganism after a specified incubation period [33]. For tests that involve metallic NPs in suspensions, determining the MIC visually can be a challenging task due to the inherent coloration of the systems, which may interfere with optical clarity and make it difficult to assess the endpoint. This phenomenon is particularly noted in the AgNP-based antifungal studies, where the characteristic yellow–brown coloration complicates the determination of the MIC value using the standard visual inspection methodology (Fig. S2). Despite these challenges, visual assessment remains a commonly used method for determining the MIC of AgNPs in the antifungal studies. Although color interference poses a limitation, this approach continues to be one of the most accessible and widely adopted techniques in the field [34]. In

our study, for the *C. albicans* tested (Fig. S2), visual examination determined the MIC at 1.56 µg/mL and 1.95 µg/mL for AgNPs and nystatin, respectively.

3.3 Determination of the Fungistatic and Fungicidal Concentrations

Figure 4 shows the results of the fungistatic and fungicidal concentration for the ATCC strain and the two clinical isolates. According to Fig. 4, the MFuC for the HCI-I isolate was found in wells 6 and 4 for AgNPs-P and AgNPs-S, respectively. For the clinical isolate HCI-II, the MFuC were in wells 5 and 4 for AgNPs-P and AgNPs-S, respectively. However, the MFC for HCI-I and HCI-II isolates treated with AgNPs-P was found in wells 5 and 4; while for AgNPs-S, the MFC was in well 3 (both isolates). Moreover, the ATCC strain showed the MFuC in wells 7 and 6, and the MFC was observed in wells 6 and 5 for AgNPs-P and AgNPs-S, respectively. Based on these results, the ATCC strain was more susceptible to the treatment with AgNPs.

Usually, in studies evaluating the antifungal potential of metallic NPs, it is common to report only the MIC value. However, this research pointed out that MIC does not always correspond to the MFuC or MFC values. Therefore, reporting the MIC solely may not provide a comprehensive thorough understanding of the antifungal efficacy of metallic NPs. In this research, the antifungal evaluation demonstrated significant differences in the MIC, MFuC, and MFC values among treatments across both the reference strain and the clinical isolates. For comparison purposes, Table 1 shows the MIC, MFuC, and MFC values. The particle concentration (NPs/mL) determined via NTA is also provided.

As can be seen, for the ATCC-10231 strain, the visual determined MIC value for nystatin (1.95 µg/mL) was statistically different from those of AgNPs-P and AgNPs-S (1.56 µg/mL). However, to reach the MFuC, 3.12 and 6.25 µg/mL were required for AgNPs-P and AgNPs-S, respectively. These values are significantly lower than the MFuC value (7.81 µg/mL) for nystatin. For the clinical isolates HCI-I and

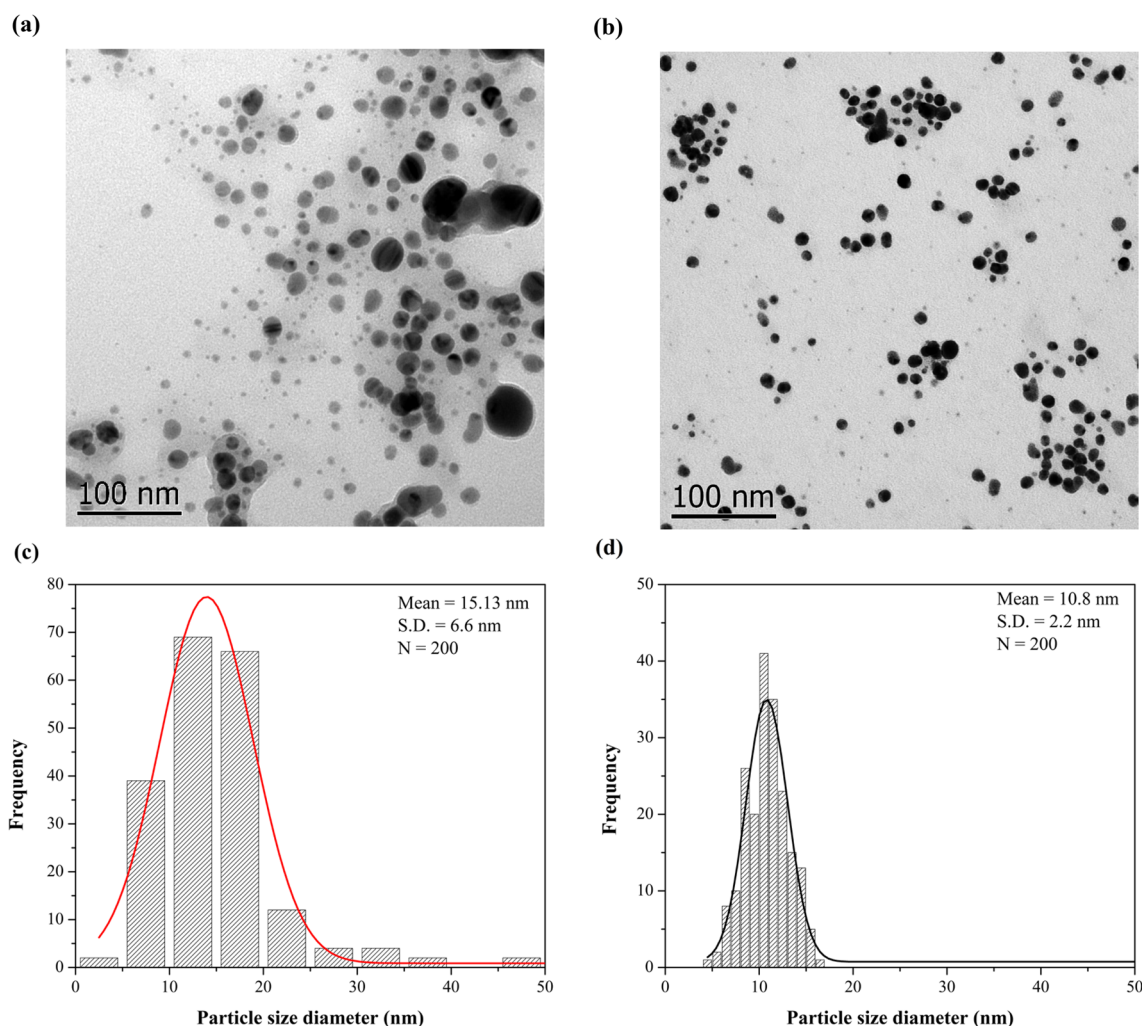
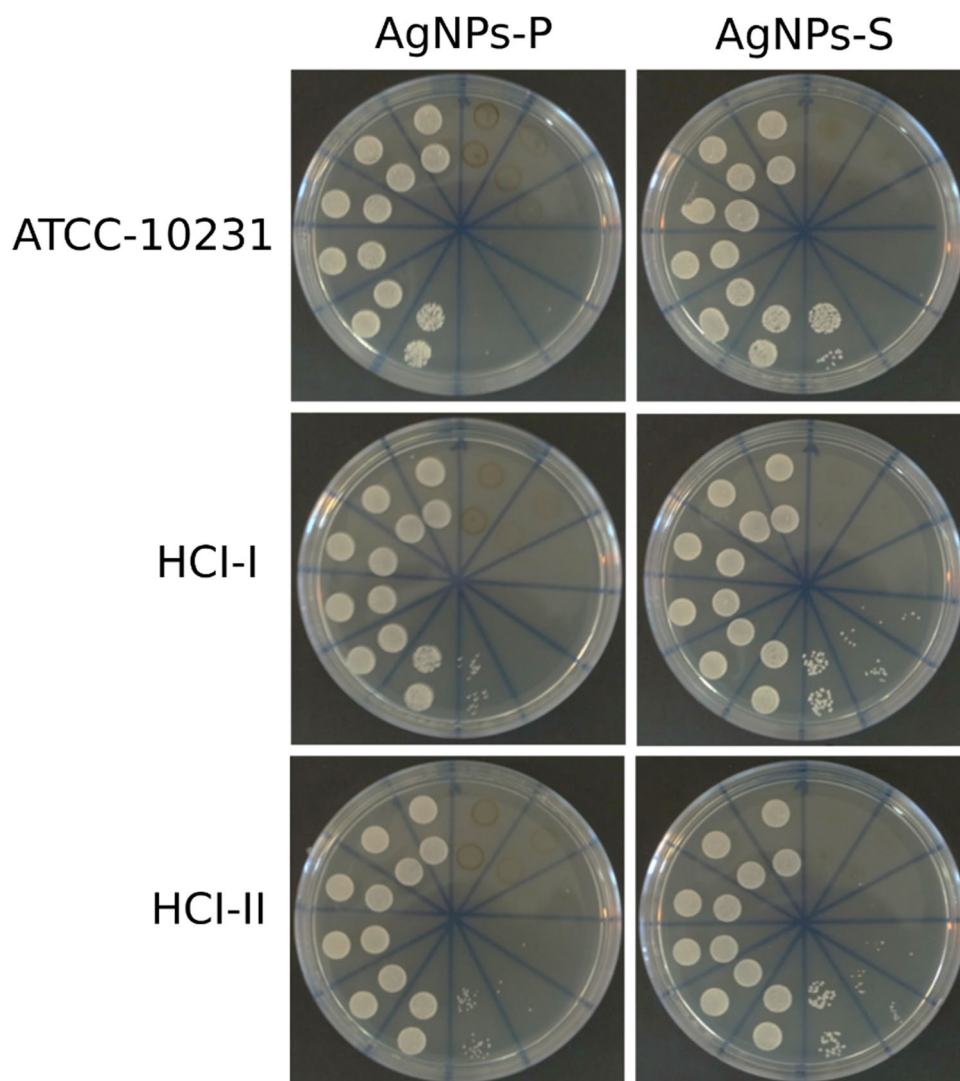


Fig. 3 Representative TEM images of AgNPs-P and AgNPs-S (a and b) and their respective particle size histogram (c and d)

HCI-II, the resistance patterns were more notorious, as indicated by higher MFuC and MFC values. For the HCI-I isolate, the MFuC and MFC values were 6.25 and 12.5 $\mu\text{g/mL}$ for AgNPs-P and 25 and 50 $\mu\text{g/mL}$ for AgNPs-S (Table 1). These results pointed out that the clinical isolate HCI-I is less susceptible to AgNPs synthesized with the aqueous extract of *S. molle*. On the other hand, HCI-II was the most resistant since the MFuC was 12.5 and 25 for AgNPs-P and AgNPs-S, respectively. In this context, similar MIC/MFC values have been reported by different researchers [35–37]. In general, AgNPs-P were more effective because a low concentration was always required to produce the fungistatic/fungicidal effect. These results suggest that the phytochemical composition of *P. koidzumii* may enhance the antifungal activity of the as-prepared AgNPs-P, as reflected in the consistency of lower MFuC and MFC values attained (Table 1). It has been reported that the *Pyracantha* genus contains compounds such as quercetin, rutin, apigenin, and hyperoside, which exhibit significant antimicrobial activity [38].

Moreover, nanoparticle concentration provides a more detailed understanding of the relationship between NPs dosage and antifungal efficacy. For the ATCC-10231 strain, AgNPs-P required a notably lower particle concentration to achieve the MFC and MFuC compared to AgNPs-S (Table 1). For instance, AgNPs-S required 3.09×10^{10} particles/mL to achieve the MFC, and AgNPs-P required only 1.76×10^{10} particles/mL (1.75-fold decrease). Furthermore, for the clinical isolates HCI-I and HCI-II, the differences in NPs concentration were even more pronounced. Thus, for the HCI-I isolate, the MFC value found when using AgNPs-P (3.52×10^{10} particles/mL) was significantly lower than that for AgNPs-S (1.24×10^{11} particles/mL), a 3.5-fold decrease. Similarly, for the HCI-II isolate, the MFC value attained when using AgNPs-P was 7.04×10^{10} particles/mL, while AgNPs-S required nearly a double concentration (1.24×10^{11} particles/mL). These results indicate that AgNPs-P achieved similar or superior antifungal effects at lower nanoparticle concentrations. These findings underscore the

Fig. 4 Illustrative SDA plates showing the different *C. albicans* treated with AgNPs-P and AgNPs-S. An aliquot of each well was dropped following a clockwise direction. HCI = human clinical isolate. AgNPs-P and AgNPs-S = silver nanoparticles synthesized using *P. koidzumii* and *S. molle* aqueous extracts, respectively



importance of reporting this parameter in addition to the MIC, MFuC, and MFC values. Therefore, this parameter

provided a more comprehensive understanding of nanoparticle efficacy and could help establish optimal dosages for

Table 1 Comparison of the MIC, MFuC, and MFC values of the AgNPs against the tested *C. albicans*

	Treatment	MIC		MFuC		MFC	
		($\mu\text{g/mL}$)	(particles/mL)	($\mu\text{g/mL}$)	(particles/mL)	($\mu\text{g/mL}$)	(particles/mL)
ATCC-10231	Nystatin	1.95a	-	7.81c	-	15.62c	-
	AgNPs-P	1.56b	4.4×10^9	3.12e	8.80×10^9	6.25e	1.76×10^{10}
	AgNPs-S	1.56b	3.86×10^9	6.25d	1.54×10^{10}	12.5d	3.09×10^{10}
HCI-I	Nystatin	1.95a	-	7.81c	-	15.62c	-
	AgNPs-P	1.56b	4.4×10^9	6.25d	1.76×10^{10}	12.5d	3.52×10^{10}
	AgNPs-S	1.56b	3.86×10^9	25a	6.18×10^{10}	50a	1.24×10^{11}
HCI-II	Nystatin	1.95a	-	7.81c	-	15.62c	-
	AgNPs-P	1.56b	4.4×10^9	12.5b	3.52×10^{10}	25b	7.04×10^{10}
	AgNPs-S	1.56b	3.86×10^9	25a	6.18×10^{10}	50a	1.24×10^{11}

Means with different letters in the same column are statistically different (Kruskal–Wallis, $p < 0.05$)

Abbreviations: MIC minimum inhibitory concentration, MFuC minimum fungistatic concentration, MFC minimum fungicidal concentration, HCI human clinical isolate

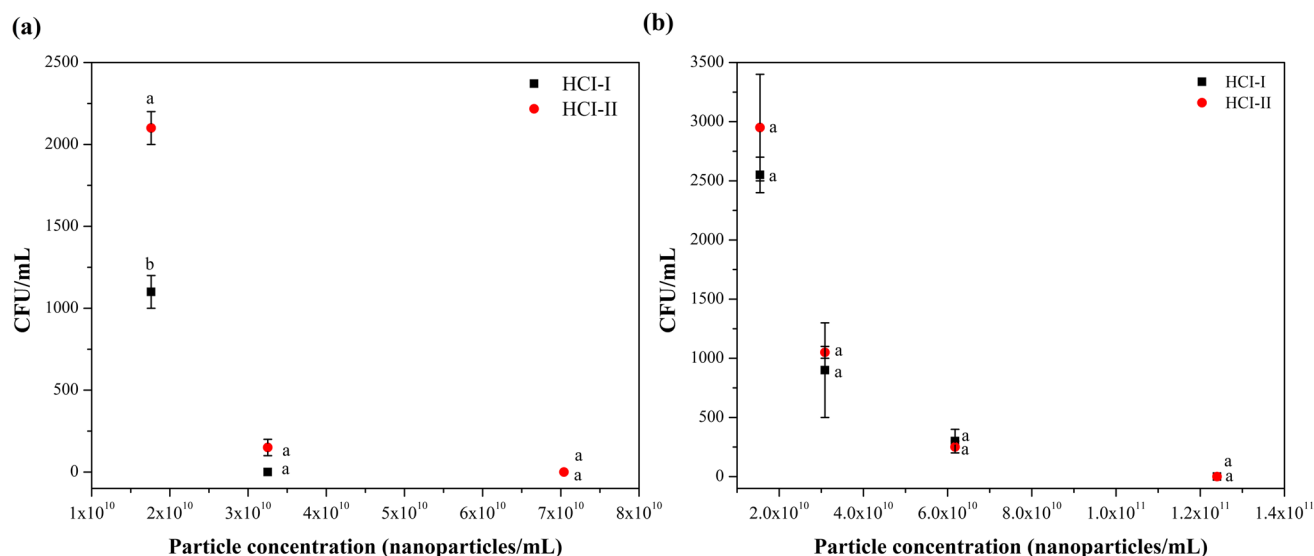


Fig. 5 Nanoparticle concentration vs CFU/mL after the treatment of the two clinical isolates with (a) AgNPs-P and (b) AgNPs-S. Mean $\pm$ standard error, HCl = human clinical isolate. Means with different

letters at the same nanoparticle concentration are statistically different (Kruskal–Wallis, $p < 0.05$)

therapeutic applications while minimizing potential toxicity effects.

3.4 The Relationship Between the Concentration of AgNPs and the CFU

Figure 5 illustrates the relationship between the concentration of NPs (particles/mL) and the CFU/mL for the two clinical isolates (HCl-I and HCl-II).

For the two tested isolates, CFU/mL post-treatment significantly decreased as the concentration of AgNPs increased, demonstrating a clear dose–response relationship. In general, when clinical isolates were treated with AgNPs-P (Fig. 5a), a significant reduction in CFU count was observed, particularly at 1.7×10^{10} particles/mL for HCl-I. In contrast, HCl-II showed a more moderate response, with a higher CFU/mL value, confirming that HCl-II is more resistant to AgNPs-P. On the other hand, for AgNPs-S (Fig. 5b), no significant differences were detected among concentrations for either isolate, as CFU/mL values remained statistically similar across all tested doses.

In this context, is it possible to establish a mathematical association between the nanoparticle concentration corresponding to the MFC (number of particles/mL) and the fungal load (CFU/mL). For this purpose, we assume that 1.25×10^6 CFU/mL remains constant in all wells, the theoretical average CFU found in each well when using the 0.5 McFarland standard [19]. Thus, the following simplified equation can be proposed to know the particle efficiency index (PEI):

$$PEI = \frac{\text{Number of particles}}{\text{CFU}} \quad (2)$$

The PEI is defined as the number of particles required to eliminate one CFU. Its biological significance lies in the fact that a lower value indicates greater efficiency of the nanoparticle system. The results demonstrated that PEI was significantly lower for AgNPs-P across all *C. albicans*, indicating a superior antifungal efficacy compared to the AgNPs-S (Fig. 6). For the HCl-I isolate, AgNPs-P showed a PEI of 2.8×10^4 NPs/CFU, 3.5 times lower than that for AgNPs-S (9.9×10^4 NPs/CFU), implying that fewer NPs are required to completely eradicate the CFU. The lower PEI values attained for AgNPs-P suggest that the formulation enhances their bioavailability and fungicidal activity. This effect could also be attributed to the *P. koidzumii* aqueous extract, which contains a wide variety of phytochemicals [39], producing with the AgNPs a synergistic effect.

3.5 Cell Wall Integrity

Fluorescence microscopy studies also demonstrated that the synthesized AgNPs at the MIC value ($1.56 \mu\text{g/mL}$) induced notable damage to the cell wall. This observation was evidenced by the penetration of the propidium iodide dye and its subsequent binding to DNA, resulting in red fluorescence in *C. albicans* cells, as shown in Fig. 7.

Moreover, the percentage of cells with wall damage was estimated and the results are presented in Fig. 8.

In general, the ATCC strain exhibited the highest cell wall damage due to AgNP exposure, with damage levels reaching

Fig. 6 Number of particles (AgNPs-P or AgNPs-S) required to eliminate a single CFU of *C. albicans*. HCI = human clinical isolate. Means with different letter are statistically different (Kruskal–Wallis, $p < 0.05$)

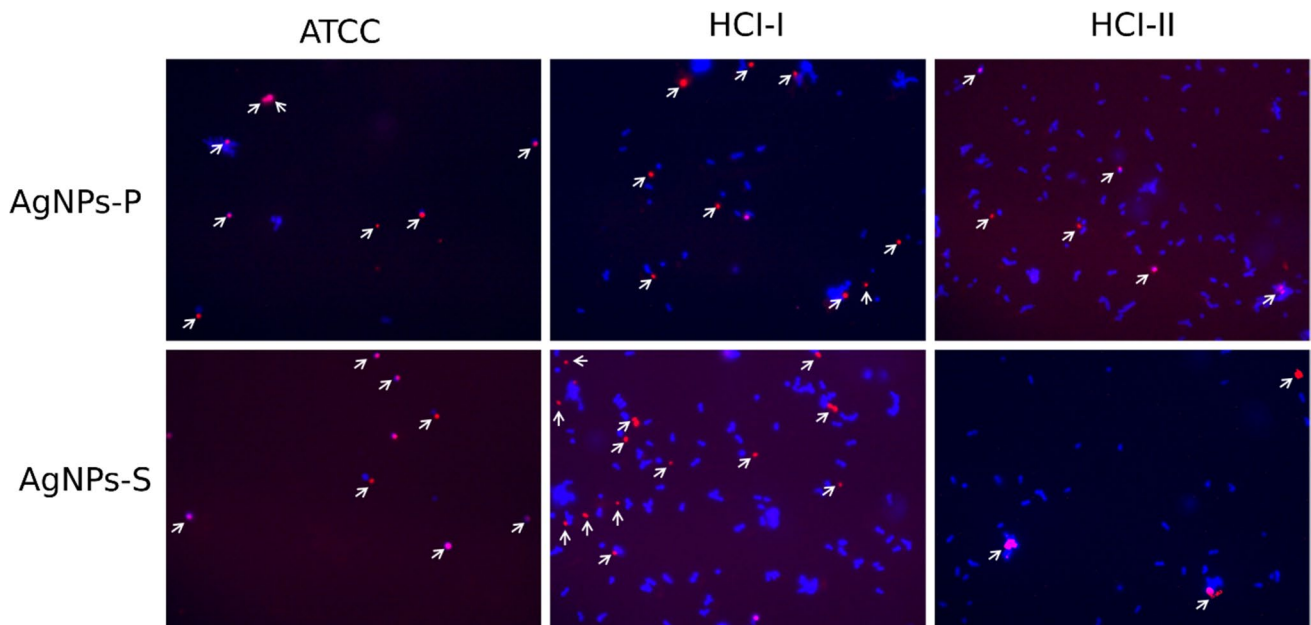
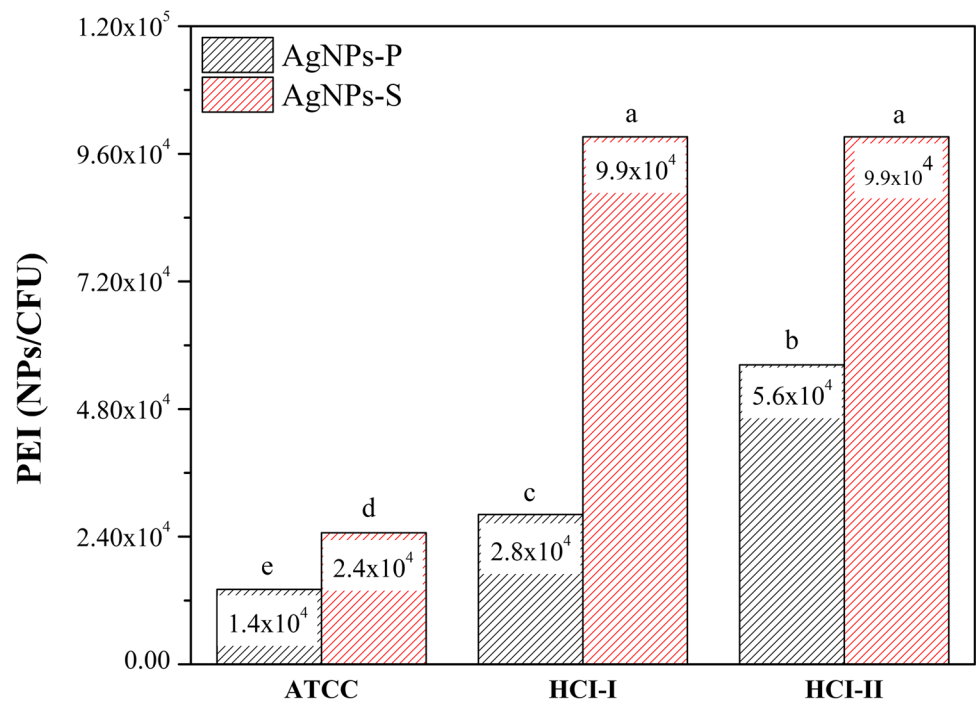


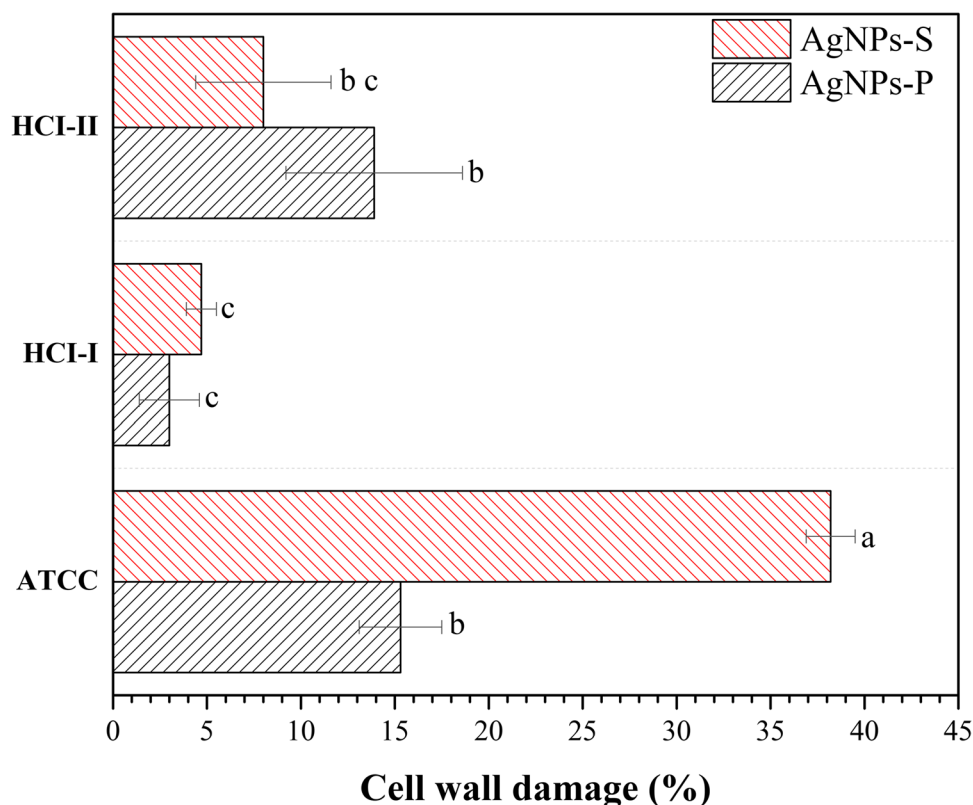
Fig. 7 The effect of AgNPs on the cell wall integrity of *C. albicans* measured with calcofluor white and propidium iodide dyes. The white arrows show cells with wall damage. HCI = human clinical iso-

late. AgNPs-P and AgNPs-S = silver nanoparticles synthesized using *P. koidzumii* and *S. molle* aqueous extracts, respectively

15.3% for AgNPs-P and 38.2% for AgNPs-S, respectively. Among the clinical isolates, HCI-I showed the lowest effect, possible due to mutations that induce adaptations in the cell wall [40]. In contrast, the HCI-II exhibited cell wall damage up to 13.9% and 8% when the isolate was exposed to AgNPs-P and AgNPs-S, demonstrating a differential response

between the two clinical isolates tested. Interestingly, the two HCI exhibited hyphal formation after 24 h of incubation. However, after the treatment with AgNPs, hyphal structures were absent (Fig. S3). The morphological transition of *C. albicans* from yeast to hyphae is a key virulence factor that facilitates tissue invasion and biofilm formation [41].

Fig. 8 Percentage of cells with wall damage in *C. albicans* after treatment with the synthesized AgNPs. HCI = human clinical isolate. AgNPs-P and AgNPs-S = silver nanoparticles synthesized using *P. koidzumii* and *S. molle* aqueous extracts, respectively. Means with different letters are statistically different (Kruskal–Wallis, $p < 0.05$)



Our findings align with previous studies demonstrating that AgNPs can effectively inhibit hyphal development. In this regard, Lara et al. [42] observed that AgNPs (0.48 $\mu\text{g/mL}$) induced significant structural changes in *C. albicans*, limiting the apparition of true hyphae, which is in accordance with our results.

3.6 Protease Activity

Proteases are enzymes that play a crucial role in the virulence and pathogenicity of various microorganisms by degrading host proteins and evading immune responses [43]. In *C. albicans*, secreted aspartic proteases are key virulence factors that facilitate tissue invasion and immune evasion [44]. Assessing protease activity before and after treatment with AgNPs is essential to understand their potential

in mitigating fungal infections. The results of the protease activity assay are shown in Table 2.

According to the results, *C. albicans* can be classified with high enzymatic activity prior to the treatment with AgNPs ($P_z = 0.38–0.56$). Notably, AgNPs-S was the only treatment that reduced the P_z value in the HCI-I, from 0.56 to 0.73, changing their enzymatic activity from high to moderate. This phenomenon can be attributed to the fact that the concentration required to achieve the MFuC in AgNPs-S was 6.18×10^{10} particles/mL, which is 3.5 times higher than that for AgNPs-P (1.76×10^{10} particles/mL). This excess of AgNPs-S could potentially lead to a significant decrease in enzymatic activity through various pathways, such as the suppression of genes associated with virulence and biofilm formation [44]. The observed reduction in the protease activity of the HCI-I upon treatment with AgNPs-S suggests a potential interference with the proteolytic

Table 2 Protease activity index (P_z) of *C. albicans* after treatment with the biogenically synthesized AgNPs

	ATCC		HCI-I		HCI-II	
	AgNPs-P	AgNPs-S	AgNPs-P	AgNPs-S	AgNPs-P	AgNPs-S
Without treatment	0.42	0.38	0.54	0.56	0.49	0.48
MFuC	0.51	0.48	0.62	0.73	0.52	0.52

$P_z = 1$, non-enzymatic activity; 0.9–0.99, very weak activity; 0.8–0.89, weak activity; 0.70–0.79, moderate activity, and <0.69, high activity

Abbreviations: HCI human clinical isolate, MFuC minimum fungistatic concentration

mechanisms. Thus, the inhibition of these enzymes could potentially attenuate the pathogenicity of the *C. albicans* isolate, making it more susceptible to host defenses and antifungal treatments [45]. While our findings demonstrate promising in vitro antifungal activity, further in vivo studies are necessary to confirm the therapeutic potential and safety of the synthesized AgNPs under physiological conditions.

4 Conclusion

This study describes the biogenic synthesis of AgNPs in aqueous media through a simple, cost-effective, and eco-friendly route. The AgNPs were successfully synthesized using *P. koidzumii* and *S. molle* aqueous extracts, which contain various phytochemicals that act as reducing and stabilizing agents. Notably, this study emphasized that the MIC visually determined is not always equivalent to the MFuC or MFC. This finding underscores that the determination of MIC alone may not adequately reflect the antifungal potency of AgNPs. This discrepancy, although rarely reported in the literature, pointed out the importance of considering these additional parameters when evaluating the antifungal efficacy of metallic NPs. In general, AgNPs-P demonstrated superior performance with lower MFuC and MFC values compared to AgNPs-S, even when their MIC values were equivalent. AgNPs-P showed a superior PEI because a lower particle concentration was needed to achieve the MFC compared with AgNPs-S. This enhanced efficacy could be attributed to the phytochemical composition of the *P. koidzumii* aqueous extract, which promotes the formation of nanoparticles with optimal physicochemical properties and bioactivity. Fluorescence microscopy analysis revealed that AgNPs induced cell wall damage and inhibited hyphal formation. Furthermore, only AgNPs-S reduced the protease activity in one of the tested clinical isolates (HCI-I), indicating their potential to attenuate fungal virulence factors. Although this study provides valuable in vitro evidence, further in vivo validation and mechanistic studies at the molecular level will be essential to confirm the therapeutic potential of these nanoparticles. Additional research is required to assess their efficacy in clinically relevant settings, including evaluations of their effects on other key virulence factors such as biofilm formation, morphological transitions, and enzymatic activity. Moreover, studies involving a broader range of clinical *C. albicans* isolates, as well as cytotoxicity and immunogenicity assessments in mammalian systems, are necessary to fully evaluate their safety and clinical applicability. Ongoing research on these topics is currently being conducted in our laboratories.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethics Approval Not applicable.

Consent for Publication The authors gave their consent for publication of the research results.

Conflict of interest The authors declare no competing interests.

Research Involving Humans and Animals Statement None.

Informed Consent None.

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References

- Corzo-Leon, D. E., Alvarado-Matute, T., Colombo, A. L., Cornejo-Juarez, P., Cortes, J., Echevarria, J. I., Guzman-Blanco, M., Macias, A. E., Nucci, M., Ostrosky-Zeichner, L., Ponce-de-Leon, A., Queiroz-Telles, F., Santolaya, M. E., Thompson-Moya, L., Tiraboschi, I. N., Zurita, J., & Sifuentes-Osornio, J. (2014). Surveillance of *Candida* spp bloodstream infections: Epidemiological trends and risk factors of death in two Mexican tertiary care hospitals. *PLoS ONE*, 9(5), e97325. <https://doi.org/10.1371/journal.pone.0097325>
- Reyes-Montes, M. D. R., Duarte-Escalante, E., Martínez-Herrera, E., Acosta-Altamirano, G., & Frías-De León, M. G. (2017). Current status of the etiology of candidiasis in Mexico. *Revista Iberoamericana de Micología*, 34(4), 203–210. <https://doi.org/10.1016/j.riam.2017.05.001>

3. Whaley, S. G., Berkow, E. L., Rybak, J. M., Nishimoto, A. T., Barker, K. S., Rogers, P. D. (2017). Azole antifungal resistance in *Candida albicans* and emerging non-*albicans* *Candida* Species. *Frontiers in Microbiology* 7. <https://doi.org/10.3389/fmicb.2016.02173>
4. Arendrup, M. C., & Patterson, T. F. (2017). Multidrug-resistant candida: Epidemiology, molecular mechanisms, and treatment. *Journal of Infectious Diseases*, 216, S445–S451. <https://doi.org/10.1093/infdis/jix131>
5. Parambath, S., Dao, A., Kim, H. Y., Zawahir, S., Izquierdo, A. A., Tacconelli, E., Govender, N., Oladele, R., Colombo, A., Sorrell, T., Ramon-Pardo, P., Fusire, T., Gigante, V., Sati, H., Morrissey, C. O., Alfenaar, J. W., Beardsley, J. (2024). *Candida albicans*—A systematic review to inform the World Health Organization Fungal Priority Pathogens List. *Medical Mycology* 62(6). <https://doi.org/10.1093/mmy/myae045>
6. Rodríguez-de la Garza, P., Cruz-de la Cruz, C. D. L., Bejarano, J. I. C., Romo, A. E. L., Delgado, J. V., Ramos, B. A., Neira, M. N. M., Rodríguez, D. S., Rodríguez, H. M. S., & Selvera, O. A. R. (2024). A multicentric outbreak of *Candida auris* in Mexico: 2020 to 2023. *American Journal of Infection Control*, 52(12), 1384–1389. <https://doi.org/10.1016/j.ajic.2024.07.012>
7. Ettadili, H., & Vural, C. (2024). Current global status of *Candida auris* an emerging multidrug-resistant fungal pathogen: Bibliometric analysis and network visualization. *Brazilian Journal of Microbiology*, 55(1), 391–402. <https://doi.org/10.1007/s42770-023-01239-0>
8. Arshad, F., Naikoo, G. A., Hassan, I. U., Chava, S. R., El-Tanani, M., Aljabali, A. A., & Tambuwala, M. M. (2024). Bioinspired and green synthesis of silver nanoparticles for medical applications: A green perspective. *Applied Biochemistry and Biotechnology*, 196(6), 3636–3669. <https://doi.org/10.1007/s12010-023-04719-z>
9. Hosseiniinasab, S. S., Naderifar, M., Akbarizadeh, M. R., Rahimzadeh, M., Soltaninejad, S., Makarem, Z., Satarzadeh, N., Sadeghi Dousari, A. (2024). The use of phages for the biosynthesis of metal nanoparticles and their biological applications: A review. *Journal of Cellular and Molecular Medicine* 28 (11). <https://doi.org/10.1111/jcmm.18383>
10. Fahim, M., Shahzaib, A., Nishat, N., Jahan, A., Bhat, T. A., Inam, A. (2024). Green synthesis of silver nanoparticles: A comprehensive review of methods, influencing factors, and applications. *JCIS Open* 16. <https://doi.org/10.1016/j.jciso.2024.100125>
11. Ghramh, H. A., Mahyoub, J. A., & Alhag, S. K. (2018). Synthesis of silver nanoparticles from *schinus molle* extract and its larvicidal activity against *Aedes aegypti* (L.), the vector of dengue fever in kingdom of Saudi Arabia. *Research Journal of Biotechnology*, 13(5), 63–70.
12. Hailan, W. A., Al-Anazi, K. M., Farah, M. A., Ali, M. A., Al-Kawmani, A. A., Abou-Tarboush, F. M. (2022). "Reactive oxygen species-mediated cytotoxicity in liver carcinoma cells induced by silver nanoparticles biosynthesized using *Schinus molle* extract." *Nanomaterials* 12(1). <https://doi.org/10.3390/nano12010161>
13. Erenler, R., Chaoui, R., Yildiz, I., Genc, N., Gecer, E. N., Temiz, C., Akkal, S. (2023). Biosynthesis, characterisation, and antioxidant activity of silver nanoparticles using *Schinus molle* L. *Trends in Sciences* 20 (10). <https://doi.org/10.48048/tis.2023.6105>
14. Gupta, Stuti, Jasrotia, Shivani, Kandwal, Anuj, Rawat, Rashmi, & Purohit, M. C. (2023). Green synthesis of silver nitrate nanoparticles using L. of *Pyracantha crenulata* and its anticancer activity on liver cancer. *Journal of Mountain Research*, 18(2), 273–283. <https://doi.org/10.51220/jmr.v18i2.29>
15. Sharmin, S., Rahaman, M. M., Sarkar, C., Atolani, O., Islam, M. T., Adeyemi, O. S. (2021). Nanoparticles as antimicrobial and antiviral agents: A literature-based perspective study. *Heliyon* 7(3). <https://doi.org/10.1016/j.heliyon.2021.e06456>
16. Odds, F. C., & Bernaerts, R. (1994). CHROMagar *Candida*, a new differential isolation medium for presumptive identification of clinically important *Candida* species. *Journal of Clinical Microbiology*, 32(8), 1923–1929. <https://doi.org/10.1128/jcm.32.8.1923-1929.1994>
17. CLSI. (2017). Reference method for broth dilution antifungal susceptibility testing of yeasts—Fourth edition: M27. Clinical and laboratory standards institute. Wayne.
18. CLSI. (2022). M27 M44S Ed3. Performance standards for antifungal susceptibility testing of yeasts. Clinical and Laboratory Standards Institute. Wayne.
19. Johnson, E. M., Espinel-Ingroff, A. V., Pfaller, M. A. (2022). Susceptibility test methods: Yeasts and filamentous fungi. In *Manual of Clinical Microbiology: Tenth Edition*, 2020–2078.
20. Rivera-Yañez, C. R., Terrazas, L. I., Jimenez-Estrada, M., Campos, J. E., Flores-Ortiz, C. M., Hernandez, L. B., Cruz-Sanchez, T., Garrido-Fariña, G. I., Rodríguez-Monroy, M. A., Canales-Martínez, M. M. (2017). Anti-*Candida* activity of *Bursera moreletensis* Ramirez essential oil and two compounds, α -pinene and γ -terpinene—An in vitro study. *Molecules (Basel, Switzerland)* 22(12). <https://doi.org/10.3390/molecules22122095>
21. Vidotto, V., Pontón, J., Aoki, S., Quindós, G., Mantoan, B., Pugliese, A., Ito-Kuwa, S., & Nakamura, K. (2004). Differences in extracellular enzymatic activity between *Candida dubliniensis* and *Candida albicans* isolates. *Revista Iberoamericana de Micología*, 21(2), 70–74.
22. Ombrelli, A. M., Racca, L., & Ramos, L. (2008). Proteases and phospholipases activities of *Candida albicans* isolated from vaginal secretions with different pH. *Revista Iberoamericana de Micología*, 25(1), 12–16. [https://doi.org/10.1016/s1130-1406\(08\)70004-4](https://doi.org/10.1016/s1130-1406(08)70004-4)
23. Marambio-Jones, C., & Hoek, E. M. V. (2010). A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *Journal of Nanoparticle Research*, 12(5), 1531–1551. <https://doi.org/10.1007/s11051-010-9900-y>
24. Glišić, S., Cvetković, D., Cvetinović, M., Stanojević, L., Danilović, B., & Cakić, K. (2018). Green synthesis, characterization and antimicrobial activity of silver nanoparticles produced from *Fumaria officinalis* L. plant extract. *Colloid Journal*, 80(6), 803–813. <https://doi.org/10.1134/S1061933X18070013>
25. Siddiqi, K. S., Husen, A., Rao, R. A. K. (2018). A review on biosynthesis of silver nanoparticles and their biocidal properties. *Journal of Nanobiotechnology* 16(1). <https://doi.org/10.1186/s12951-018-0334-5>
26. Lima, A. K. O., Souza, L. M. D. S., Reis, G. F., Junior, A. G. T., Araújo, V. H. S., Santos, L. C. D., Silva, V. R. P. D., Chorilli, M., Braga, H. D. C., Tada, D. B., Ribeiro, J. A. D. A., Rodrigues, C. M., Nakazato, G., Muehlmann, L. A., Garcia, M. P. (2024). Synthesis of silver nanoparticles using extracts from different parts of the *Paullinia cupana* Kunth plant: Characterization and in vitro antimicrobial activity. *Pharmaceuticals* 17(7). <https://doi.org/10.3390/ph17070869>
27. Gangaram, S., Naidoo, Y., Dewir, Y. H., Singh, M., Lin, J., Nicole Daniels, A., Mendler-Drienyovszki, N. (2024). Biosynthesis of silver nanoparticles using *Barleria allostellata* C.B. Clarke leaves and stems: Antibacterial and cytotoxic activity. *Applied Sciences (Switzerland)* 14 (18). <https://doi.org/10.3390/app14188331>
28. Rheder, D. T., Guilger, M., Bilesky-José, N., Germano-Costa, T., Pasquoto-Stigliani, T., Gallep, T. B. B., Grillo, R., Carvalho, C. S., Fraceto, L. F., Lima, R. (2018). Synthesis of biogenic silver nanoparticles using *Althaea officinalis* as reducing agent: Evaluation of toxicity and ecotoxicity. *Scientific Reports* 8 (1). <https://doi.org/10.1038/s41598-018-30317-9>
29. Pérez-de León, A., Plasencia, J., Vázquez-Durán, A., & Méndez-Albores, A. (2020). Comparison of the in vitro antifungal and

- anti-fumonigenic activities of copper and silver nanoparticles against *Fusarium verticillioides*. *Journal of Cluster Science*, 31(1), 213–220. <https://doi.org/10.1007/s10876-019-01638-0>
30. Lai, M. J., Huang, Y. W., Chen, H. C., Tsao, L. I., Chang Chien, C. F., Singh, B., Liu, B. R. (2022). Effect of size and concentration of copper nanoparticles on the antimicrobial activity in *Escherichia coli* through multiple mechanisms. *Nanomaterials* 12 (21). <https://doi.org/10.3390/nano12213715>
 31. Delattin, N., Cammue, B. P., & Thevissen, K. (2014). Reactive oxygen species-inducing antifungal agents and their activity against fungal biofilms. *Future Medicinal Chemistry*, 6(1), 77–90. <https://doi.org/10.4155/fmc.13.189>
 32. Fukushima, K., Liu, S., Wu, H., Engler, A. C., Coady, D. J., Maune, H., Pitera, J., Nelson, A., Wiradharma, N., Venkataraman, S., Huang, Y., Fan, W., Ying, J. Y., Yang, Y. Y., Hedrick, J. L. (2013). Supramolecular high-aspect ratio assemblies with strong antifungal activity. *Nature Communications* 4. <https://doi.org/10.1038/ncomms3861>
 33. Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy*, 48(SUPPL. 1), 5–16. https://doi.org/10.1093/jac/48.suppl_1.5
 34. Jadhav, K., Dhamecha, D., Bhattacharya, D., & Patil, M. (2016). Green and ecofriendly synthesis of silver nanoparticles: Characterization, biocompatibility studies and gel formulation for treatment of infections in burns. *Journal of Photochemistry and Photobiology B: Biology*, 155, 109–115. <https://doi.org/10.1016/j.jphotobiol.2016.01.002>
 35. Panáček, A., Kolář, M., Večeřová, R., Pucek, R., Soukupová, J., Kryštof, V., Hamal, P., Zbořil, R., & Kvítek, L. (2009). Antifungal activity of silver nanoparticles against *Candida* spp. *Biomaterials*, 30(31), 6333–6340. <https://doi.org/10.1016/j.biomaterials.2009.07.065>
 36. Arsène, M. M. J., Viktorovna, P. I., Alla, M., Mariya, M., Nikolaevitch, S. A., Davares, A. K. L., Yurievna, M. E., Rehailia, M., Gabin, A. A., Alekseevna, K. A., Vyacheslavovna, Y. N., Vladimirovna, Z. A., Svetlana, O., Milana, D. (2023). Antifungal activity of silver nanoparticles prepared using Aloe vera extract against *Candida albicans*. *Veterinary World* 16(1): 18–26. <https://doi.org/10.14202/vetworld.2023.18-26>
 37. Harine, A., Ranjani, S., Hemalatha, S. (2025). Antifungal efficacy of Citrusfusion mediated silver nanoparticles in *Candida* species. *BMC Biotechnology* 25(1). <https://doi.org/10.1186/s12896-025-00952-y>
 38. Chauhan, A., & Banerjee, R. (2025). Evaluation of traditional uses, phytochemical constituents, therapeutic uses and future prospects of *Pyracantha* genus: A systematic review. *Natural Product Research*, 39(4), 922–934. <https://doi.org/10.1080/14786419.2024.2319660>
 39. Lin, R. D., Chen, M. C., Liu, Y. L., Lin, Y. T., Lu, M. K., Hsu, F. L., & Lee, M. H. (2015). New whitening constituents from Taiwan-native *Pyracantha koidzumii*: Structures and tyrosinase inhibitory analysis in human epidermal melanocytes. *International Journal of Molecular Sciences*, 16(12), 28598–28613. <https://doi.org/10.3390/ijms161226115>
 40. Czajka, K. M., Venkataraman, K., Brabant-Kirwan, D., Santi, S. A., Verschoor, C., Appanna, V. D., Singh, R., Saunders, D. P., Tharmalingam, S. (2023). Molecular mechanisms associated with antifungal resistance in pathogenic *Candida* species. *Cells* 12 (22). <https://doi.org/10.3390/cells12222655>
 41. Sharma, A., Solis, N. V., Huang, M. Y., Lanni, F., Filler, S. G., Mitchell, A. P. (2023). "Hgc1 independence of biofilm hyphae in *Candida albicans*. *mBio* 14(2). <https://doi.org/10.1128/mbio.03498-22>
 42. Lara, H. H., Romero-Urbina, D. G., Pierce, C., Lopez-Ribot, J. L., Arellano-Jiménez, M. J., Jose-Yacamán, M. (2015). Effect of silver nanoparticles on *Candida albicans* biofilms: An ultrastructural study. *Journal of Nanobiotechnology* 13(1). <https://doi.org/10.1186/s12951-015-0147-8>
 43. Ramírez-Larrota, J. S., Eckhard, U. (2022). An introduction to bacterial biofilms and their proteases, and their roles in host infection and immune evasion. *Biomolecules* 12(2). <https://doi.org/10.3390/biom12020306>
 44. Bras, G., Satala, D., Juszczak, M., Kulig, K., Wronowska, E., Bednarek, A., Zawrotniak, M., Rapala-Kozik, M., Karkowska-Kuleta, J. (2024). Secreted aspartic proteinases: Key factors in *Candida* infections and host-pathogen interactions." *International Journal of Molecular Sciences* 25(9). <https://doi.org/10.3390/ijms25094775>
 45. Macias-Paz, I. U., Pérez-Hernández, S., Tavera-Tapia, A., Luna-Arias, J. P., Guerra-Cárdenas, J. E., & Reyna-Beltrán, E. (2023). *Candida albicans* the main opportunistic pathogenic fungus in humans. *Revista Argentina de Microbiología*, 55(2), 189–198. <https://doi.org/10.1016/j.ram.2022.08.003>

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