Research Article

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Effects of Silver Nanoparticles Synthesized from Phenolic Extract of *Agaricus bisporus* Against Pathogenic Bacteria and Yeasts

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Abstract

Agaricus biosporus is a mushroom known for its use for nutritional and medicinal purposes. This study tested whether silver nanoparticles (AgNPs) synthesized from phenolic compounds extracted from this mushroom have an efficient antimicrobial impact against pathogenic bacteria and yeast isolated from urine, sputum, and vaginal swabs. Different methods were employed in the characterization of AgNPs. The microorganisms of isolated bacteria and yeast were identified according to morphological features, results of biochemical tests, and the VITEK 2 compact system. The susceptibility to antibiotics was determined by the disk method. The bacteria and yeast resisted all antibiotics employed in this research. But the AgNPs had good antimicrobial activity against pathogenic bacteria and yeast. The highest inhibition zone of AgNPs against *Pseudomonas aeruginosa* in a 100 mg/mL concentration was 30 mm, while the minimal inhibition zone of AgNPs was 12 mm against *Escherichia coli* at the same concentration. The antibacterial effect of the synthesized AgNPs was concentration-dependent against Gram-positive and Gram-negative bacteria. With respect to the effect on yeast growth, there was a clear inhibitory effect of the synthesized AgNPs at 100 mg/mL, when the largest zone of inhibition was found against *Candida glabrata* (29 mm), and the minimal inhibition zone was found against *C. guillermondi* and *C. albican* (23 mm). Similarly, the AgNPs synergized with antibiotics were used against pathogenic bacterial and yeast isolates because when antibiotics and AgNPs were combined, the inhibition zones were larger compared to those of the antibiotics alone.

Keywords: silver nanoparticles; phenolic compound; synergistic effect; *Agaricuss bisporus*; pathogenic bacteria; yeasts

Introduction

The fruiting bodies of the macroscopic filamentous fungi *Agaricuss bisporus* are an important nutrient source in humans [1, 2]. Because of their potential health advantages, phenolic compounds are arguably the most researched natural substances, as evidenced by several studies [3, 4], in addition to secondary metabolic products with an aromatic ring with a hydroxyl substituent, most of which are of plant origin. Some carboxylic acids and glycosides are exclusively soluble in organic solvents, while others are water-soluble. Insoluble polymers are different types of phenolic chemicals [5, 6]. Their unique

physicochemical features include physical capabilities, significant broad-spectrum antibacterial and anti-inflammatory action, and cheap manufacturing cost. All of these characteristics provide them with significant benefits in creating alternative products [7]. In addition, the nanoparticles (NPs) showed high antimicrobial activity against most bacteria [8]. Another study considered the synthesis of silver NPs (AgNPs) from *Inonotus hispidus*, which were then used to combat various pathogens such as *Escherichia coli*, *Klebsiella pneumonie*, *Aspergillus niger*, and *Aspergillus flavus* [9]. The current study aims to obtain active substances such as phenol from *A. bisporus* and then manufacture NPs from it in addition to studying the effectiveness of AgNPs made from the active substance of the *A. bisporus* against some pathogenic bacteria and yeast. Finally, the study addresses the potential synergetic effect between AgNPs and some antibiotics used for the treatment of these pathogenic bacteria and yeast.

Experimental Procedures

Specimens collection and isolation

Clinical specimens (urine, sputum, and vaginal swabs) were collected to isolate bacteria and yeast. Seventy-five specimens were collected from Al-Yarmouk Teaching Hospital/Baghdad patients under aseptic conditions.

Identification of bacterial and yeast isolates

Identification of bacterial isolates

All sterile urine, sputum, and vaginal secretions were collected from the patients; four genera of bacteria were isolated from Baghdad's hospitals. The isolates were added to a brain–heart infusion broth and incubated for 18–24 h at 37 °C [10]. Then, they were inoculated onto MacConkey and blood agar plates and incubated at 37 °C for 18–24 h for Gram-negative bacteria. In parallel, Gram-positive bacteria were inoculated onto plates of blood agar and mannitol salt agar with the same conditions.

All specimens were cultured using a loop on MacConkey agar appearing as a pale color; on blood agar, they gave the β or γ type of hemolysis after 18–24 h at 37 °C [11]. VITEK-2 compact system ID-GNB and ID-GPB cards were used to prove the final identification of bacteria.

Identification of yeast isolates

Yeast isolates were isolated from the vagina and then inoculated on dextrose-Sabouraud agar containing chloramphenicol. All dishes were incubated overnight at 37 °C. To identify yeast isolates, the methods used were the serum's germ tube test at 37 °C for 2–3 h, chlamydospore forming test on corn meal agar media, and sugar assimilation test to identify yeasts of *Candida* spp. The morphological and physiological characteristics, according to Ref. [11], were also considered. Then, colonies were tested in the VITEK 2 compact system ID-YST kit for confirmation [12].

Preparation of an aqueous extract of *A. bisporus*

The mushroom was dried and blended, then the powder was added to deionized water at concentration of 100 mg/mL, boiled at 60 °C for 0.5 h, and left covered for another 0.5 h. Next, all residues were removed through gauze and centrifuged at 10 000 r/min for 0.5 h at 4 °C. The supernatants were then filtered through the paper of What-man (No. 1). Finally, the aqueous extract was saved in a refrigerator, according to Ref. [13].

Extraction of phenolic compounds

A. bisporus was crushed, and 1 g of the powder was put into ethanol (10 mL; 80%). The sample mixture was then placed in a water bath at room temperature for 20 min. This was followed by centrifugation at 3 500 r/min for 15 min and filtering with filter paper [14].

Phenolic indicators

Ferric chloride and potassium ferric cyanide reagents were used to detect available phenols. This test was prepared by taking 2 mL:2 mL of an aqueous solution of the above materials. The formation of blue-green color was an indicator of phenolic compound formation [15].

Biosynthesis of AgNPs

$AgNO_3$ as a stock solution was made ready with sterile deionized water at a concentration of 1 mmol/L. The AgNPs dilutions were prepared according to Ref. [16]. The solution was added to 10 mL of $AgNO_3$ stock solution, saved under room temperature, and then measured at a ultraviolet (UV) of 365 nm. After one day of incubation, the color of the mixture was transformed from shining yellow to

dusky yellow, indicating the formation of AgNPs, also with atomic force microscopy (AFM), X-ray diffraction (XRD), and Fourier transform infrared (FTIR) methods. The AgNPs remained stable at room temperature for over two months because they showed activity against used microorganisms.

Characterization of AgNPs

UV–visible (*UV–Vis*) *spectrophotometer*

The prepared AgNPs were characterized by UV–Vis spectroscopy [1]. 1 mL of AgNPs was added to 4 mL of deionized water at room temperature, using the absorbance of 300–800 nm [2].

AFM assay

Additionally, the surface topography and size of the sample film were assessed with the AFM method [17].

XRD method

Using an X-ray spectrometer, the crystalline structure of phenolic AgNPs was investigated (XRD-6000, Shimadzu, Japan) according to Ref. [18].

FTIR assay

FTIR spectroscopy (FTIR 8400S, Shimadzu, Japan) was employed to investigate the significant modifications in the surface structure and bonding of phenolic AgNPs with the method in Ref. [18].

Antibiotic analysis

This test was performed as in Ref. [19] after diluting McFarland tubes (No. 0.5) using a sterile swab dipped in the organism culture and placed onto Mueller Hinton agar. The plate was let dry for some minutes after the streaking. Next, forceps were used to apply antibiotic disks on the agar surface. Then, dishes were incubated overnight at 37 °C. Seven disks of antibiotics for bacteria were used, including amikacin (30 μg), ticarcillin + clavulanic acid (75/10 μg), ceftriaxone (30 μg), piperacillin (100 μg), imipenem (10 μg), gentamicin (10 μg), and nitrofurantoin (300 μg).

The five commonly used antifungal agents used for the *Candida* isolates included nystatin (100 μg), ketoconazole (50 μg), clotrimazole (10 μg), miconazole (50 μg), and itraconazole (30 μg). Following that, the diameter of the inhibitory zone (in mm) was measured using a ruler, and the findings were recorded and compared with Clinical and Laboratory Standards Institute (CLSI) [20].

Antimicrobial activity of AgNPs against pathogenic bacteria and yeasts

The antimicrobial potency of AgNPs was verified by the good test [19]. After diluting the pathogenic strains of tested bacteria and yeast into the McFarland tube (No. 0.5), the microorganisms were cultured as described in the above sensitivity test method. The diameter of these wells (8 mm) was cut into the agar's surface and filled with the AgNPs (100, 50, 25, 12.5, and 6.25 mg/mL). All plates received incubation at 37 °C overnight, and the antimicrobial effect was quantified by calculating the inhibition zone's diameter.

Results and Discussion

Identification of bacterial and yeast isolates

The detected bacterial isolates were *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. At the same time, the yeasts were *Candida albican*, *C. glabrata*, *C. guillermondi*, *C. krusei*, and *C. dubliniensis* by cultural and morphological features. After that, all isolates were purified to obtain an isolated colony for the next steps. Then, VITEK-2 compact system ID-GNB and ID-GPB cards were used to prove the final identification of bacteria.

Biosynthesis of AgNPs from a phenolic compound of *A. bisporus*

Color change

The color of the mixture solution changes from light yellow to dark yellow, which indicated the formation of AgNPs, as shown in Fig. 1. The dark yellow color in aqueous solution represents the irritation of surface plasmon vibrations in AgNPs [21].

Characterization of AgNPs from a phenolic compound of *A. bisporus*

UV–Vis spectrophotometer

The reported absorbance at 350 nm of AgNPs is

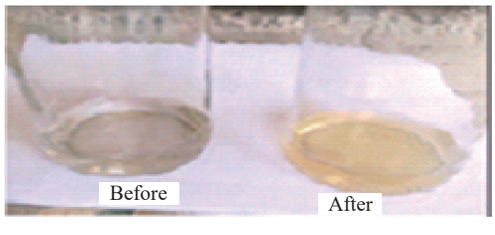

Fig. 1 Color change indicates the formation of nanoparticles.

illustrated in Fig. 2(a). When an increased absorbance peak is observed, the number of AgNPs is formed by reduction. The absorbance of the surface plasmon band in the AgNPs solution is typical at the range of 300–450 nm [22, 23]. Chemical products of the plants, such as alkaloids, terpenoids, flavonoids, coumarone, phenols, polysaccharides, and proteins, are essential for the equilibrium and synthesis of AgNPs [24, 17]. This result is because the phenolic compound in the extract may be caused by the reduction of the Ag^{+} to AgNPs and participation in AgNPs formation. Remarkably, the activity of AgNPs stayed approximately stable for two months (Fig. 2(b)).

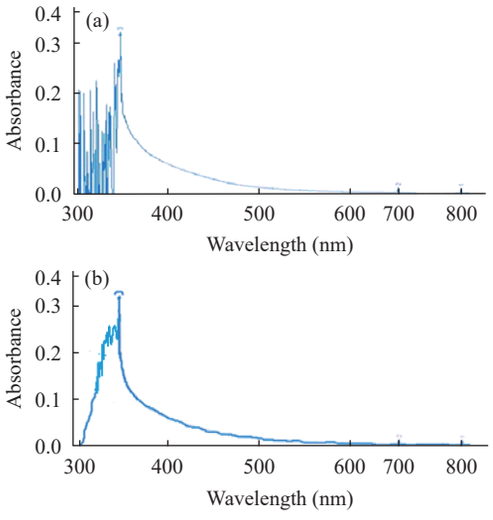

Fig. 2 (**a**) UV–Vis spectrophotometer of synthesis of the AgNPs. (**b**) UV–Vis spectrophotometer of synthesis of the AgNPs after two months.

AFM assay

AFM images showed that the layer roughness of the biologically produced AgNPs was screened using lateral two-dimensional (2D) and three-dimensional (3D) images with the size of 2040.31 nm × 2040.31 nm (Fig. 3). As a result, the average roughness was 4.16 nm, the core roughness depth was 13.5 nm, and the reduced valley depth was 0.472 nm. This study agreed with another one that characterized AgNPs using the AFM test [25, 26].

The AFM method captured the size, shape, and

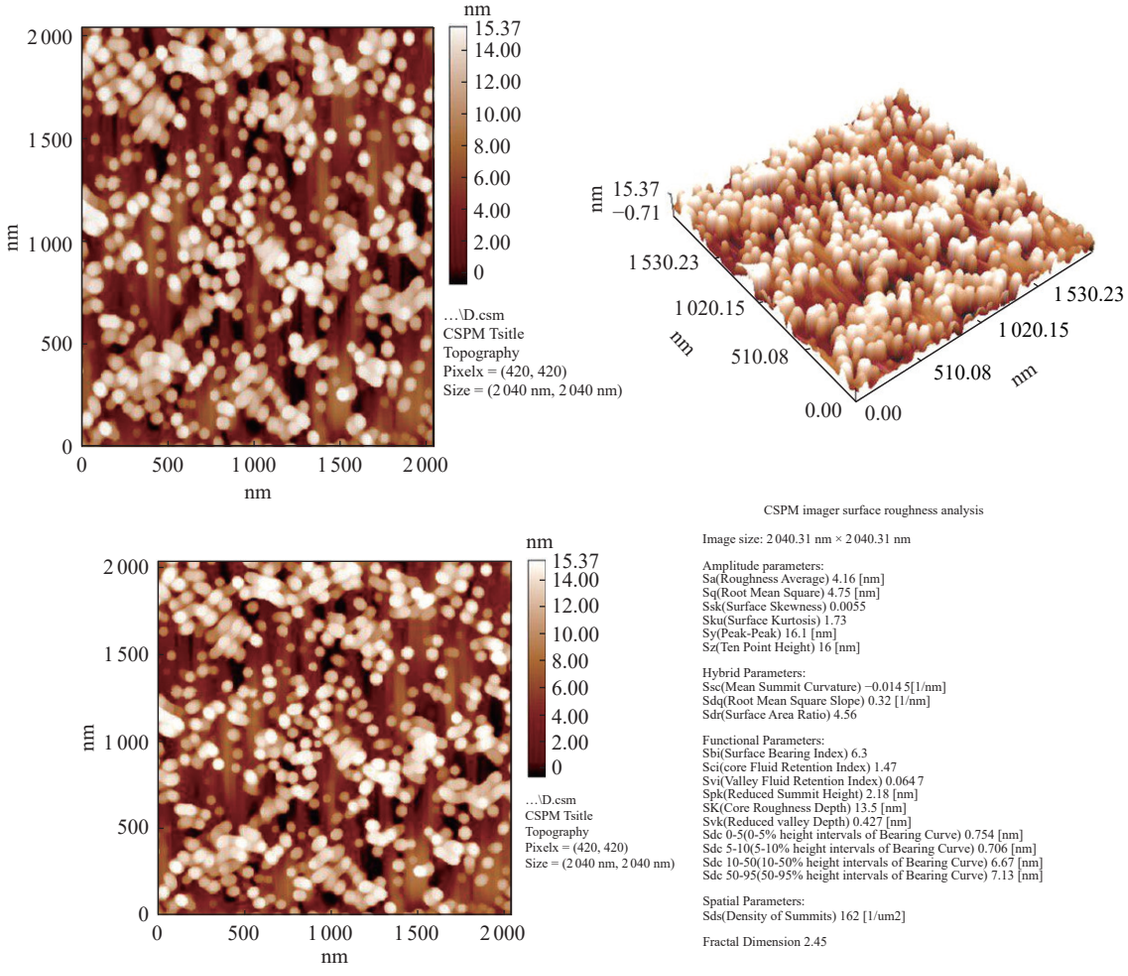

Fig. 3 AFM test of the synthesized AgNPs from phenol of *A. bisporus*.

distribution of AgNPs, which proved the styling of fractions, reaching more than a thousand times the optical deviation limits [27].

XRD method

To illustrate the crystalline character of the AgNPs, X-ray diffraction was performed on a solution containing AgNPs (Fig. 4). The refracting spectra were recorded between 20° and 80° away from the pattern. There were four prominent reflections at 38.48°, 45.36°, 64.75°, and 78.08°. The produced XRD spectrum conformed to the planes of 113, 202, 224, and 315 compared to the standard. These results agreed with the results in Ref. [26].

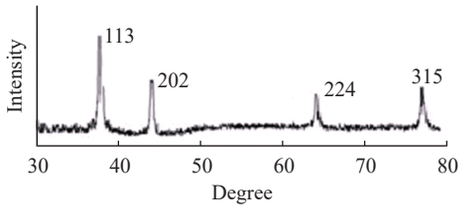

Fig. 4 XRD assay of AgNPs from phenol of *A. bisporus*.

FTIR assay

Figure 5 displays an FTIR spectrum that was used to distinguish among different functional groups in solutions that were in charge of reducing AgNPs and bio-reducing Ag^{+}. The presence of a capping agent with AgNPs was indicated by the transmission bands discovered at the following wavelengths: 3 425, 2 922, 2 856, 1 746, 1 634, 1 452, 1 378, 1 238, 1 046, and 596 cm^{-1} [26].

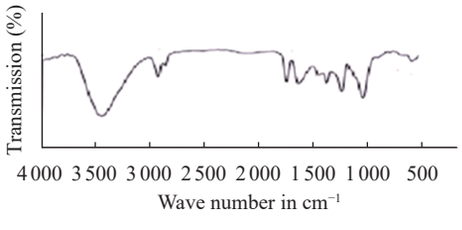

Fig. 5 FTIR results of AgNPs from phenol of *A. bisporus*.

Antibiotic susceptibility test

The four isolates (Gram-positive and Gram-negative) were tested for antibiotics. This test aimed to compare the antimicrobial effects of some of these antibiotics with the synthetic AgNPs from the phenolic extract of *A. bisporus* and demonstrate its antimicrobial properties. The Gram-positive isolates *S. pyogenes* are resistant to amikacin, azithromycin, gentamicin, and penicillin, and sensitive to amoxicillin + clavulanic acid, ceftriaxone, vancomycin, and tetracycline (Table 1). *S. aureus* is a Gram-positive aerobic bacterium resistant to amoxicillin + clavulanic acid, gentamicin, and penicillin, and sensitive to amikacin, azithromycin, and Tat simat simultaneously. The Gram-negative bacteria (*E. coli* and *P. aeruginosa*) are resistant to amikacin, gentamicin, piperacillin, ticarcillin + clavulanic acid, and nitrofurantoin, and sensitive only to amikacin and ceftriaxone. Table 2 compares to another study, which found that the inhibition zones of ciprofloxacin, ticarcillin + clavulanic acid, and cefotaxime were 28, 27, and 25 mm, respectively; these results showed that *P. aeruginosa* resisted the antibiotics used in that study [28]. According to another research, Gram-negative bacteria were more oversensitive to the AgNPs than the Gram-positive bacteria [29]. In this study, different Gram-positive and Gram-negative antibiotics were used because these bacteria have different structures. Gram-negative bacteria have a thin peptidoglycan layer but have an outer membrane, while Gram-positive bacteria have a thick peptidoglycan layer and do not have an outer membrane in their structure. Therefore,

Table 1 The sensitivity test with antibiotic disks against Gram-positive bacteria

Antibiotic disks symbol	Gram-positive bacteria	
	S. pyogenes	*S. aureus*
Amikacin (AK)	S	R
Amoxicillin + clavulanic acid (AMC)	S	S
Azithromycin (ATH)	S	R
Ceftriaxone (CRO)	S	S
Gentamicin (CN)	S	S
Penicillin (P)	R	R
Tetracycline (TE)	R	R

S: sensitive; R: resistant.

Table 2 The sensitivity test with antibiotic disks against Gram-negative bacteria

Antibiotic disk symbol	Gram-negative bacteria	
	E. coli	*P. aeruginosa*
Amikacin (AK)	S	S
Piperacillin (PC)	R	R
Imipenem (IMP)	S	S
Ceftriaxone (CRO)	S	R
Gentamicin (CN)	S	S
Ticarcilin + clavulanic acid (TCC)	R	R
Nitrofurantoin (NIT)	R	R

S: sensitive; R: resistant.

the penicillin group is a good choice for treating Gram-positive because of its work on the peptidoglycan layer and its negative charge, which promotes the interaction with the positive charge of the Ag^+ trapped outside the cell.

Similarly, antifungal activity was assessed, and the current findings demonstrated that the polyene antifungal Nystatin was highly effective on the yeast strains tested in Table 3.

Table 3 The sensitivity test of fungal isolates against different antibiotics

Antibiotic disk symbol	*Candida* spp.				
	C. albicans	*C. glabrata*	*C. guillermondi*	*C. krusei*	*C. dubliniensis*
Nystatin (NS)	S	S	S	S	S
Clotrimazole (CC)	I	S	R	I	I
Itraconazole (IT)	I	S	S	I	R
Ketoconazole (KT)	I	S	S	I	R
Miconazole (MIC)	I	S	S	I	R

S: sensitive; I: intermediate; R: resistant.

Several studies have found that nystatin is the best drug against *Candida* isolated from the vagina. For example, Farhan et al. [30] reported that 97.9% of *Candida* spp. were susceptible to nystatin, and 35% were susceptible to amphotericin B.

As for azole compounds, some *Candida* species demonstrated a significant azole resistance. However, ketoconazole was found to be the most efficient azole in several types of research. According to that study's findings, the sensitivity of *Candida* spp. to antibiotics differs significantly, and *C. dubliniensis* showed a high rate of azole resistance while *C. glabrata* showed no resistance to the drug in this study. Although *C. albicans* has shown no resistance to azoles in *Candida* vaginal isolates in previous investigations conducted in different countries, other species have shown resistance [31]. According to many researchers, increasing the usage of antifungals for the prevention or treatment of recurrent candidiasis is the most frequent danger for azole resistance and prolonged therapy [32]. Furthermore, improper antifungal drug use predisposes the development of antifungal resistance [33].

Antimicrobial effect of the AgNPs phenolic compound of *A. bisporus* against pathogenic bacteria and yeast

A good antimicrobial effect of AgNPs synthesized from the phenolic compound of *A. bisporus* was observed at a concentration of 100 mg/mL at 30 mm. In contrast, at 6.25 mg/mL, the highest effect was 20 mm against *P. aeruginosa.* The lowest effect against *E. coli* at a concentration of 100 mg/mL was 12 mm, while at 6.25 mg/mL, it was 4 mm (Fig. 6).

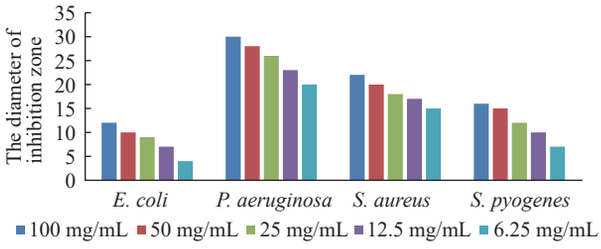

Fig. 6 The antibacterial activity of phenolic AgNPs against Gram-positive and Gram-negative bacteria.

A previous study showed that AgNPs could be used as a strong antibacterial agent against Gram-negative and Gram-positive bacteria, including bacteria highly resistant to antibiotics, such as *P. aeruginosa* and *S. aureus*. In this regard, it has been shown that the mechanism of AgNPs penetrates the bacterial cell wall, then causes changes in the cell membranes' permeability and causes the death of cells [34, 35]. AgNPs appeared very active against *P. aeruginosa* and also toward *C. albicans,* and another study concluded that the bactericidal impact of AgNPs on *E. coli* is significantly higher than amoxicillin. Furthermore, many other antimicrobial compounds can be combined with AgNPs and used as antimicrobial agents against bacterial and yeast infections [36].

On the other hand, research revealed that AgNPs formed from edible mushrooms had a considerable inhibitory effect on *S. aureus, E. coli,* and *P. aeruginosa*, and could be used as a cheap, safe, and effective alternative to antibiotics [37].

According to the results presented in Fig. 7, the test

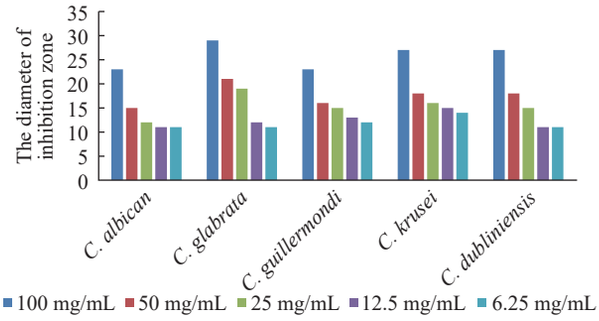

Fig. 7 The antifungal activity of phenolic AgNPs against pathogenic yeasts.

by agar well diffusion method showed a clear inhibitory effect of the AgNPs synthesized from the phenolic compound of *A. bisporus* at a concentration of 100 mg/mL; the largest zone of inhibition was found against *C. glabrata* (29 mm), followed by *C. krusei* and *C. dubliniensis* (27 mm), and the smallest inhibition zone was found against *C. guillermondi* and *C. albicans* (23 mm).

Antifungal activity in extracts produced from *A. bisporus* mushrooms has been described in the literature, suggesting that AgNPs have a significant effect and can become an antifungal agent. Öztürk et al. [38] showed an antifungal activity of methanolic extracts of *A. bisporus*, *A. bitorquis*, and *A. essettei* against *C. albicans* and *C. tropicalis*. Despite this, Barros et al. [39] found no evidence of *A. bisporus* action against *C. albicans*.

The basic mechanisms of AgNP antifungal activity include interactions with microbial membrane proteins and DNA. As Kim and colleagues reported, the interaction of AgNPs with the protein of the fungus surface causes the denaturation of pores of the membrane that materials pass through it. Consequently, this causes a cell's disintegration by rupturing the membrane, destroying microorganisms. In addition, silver ions can create cross-links with the DNA bases of fungi, according to the observation of Feng et al. [40], and then substitute hydrogen bonds close to nitrogen in bases. To achieve the impact of fungi killing, it can alter the DNA structure and disrupt its replication capacity. Furthermore, Dibrov et al. [41] stated that AgNPs have a very small diameter (10–100 nm) and can permeate the cell wall than inside the bacterial cell. The silver ion can bind to the (—SH) groups of the enzyme, disabling them and causing of miss out on the cell split and reduplicate.

Synergistic effect of AgNP phenolic compound of *A. bisporus* with antibiotics

This test was done by utilizing the disk diffusion method to detect if there was a synergistic effect with AgNPs from the phenolic compound of *A. bisporus* with antibiotics in the lowest concentration (6.25 mg/mL), which was used in this study for bacteria and yeast. Estimating the interaction between antibiotics and AgNPs, and combining antibiotics with AgNPs against microorganisms increased the diameters of inhibition zones compared to antibiotics alone (Figs. 8−10).

Studies have shown that the effectiveness against *B. pseudomallei* of antibiotics such as ceftazidime, imipenem, meropenem, and gentamicincan can be boosted by combining them with AgNPs. Each antibiotic combined with AgNPs exhibited bactericidal concentrations and inhibiting value ranges of 0.312–0.75 μg/mL and 0.252–0.625 μg/mL, respectively [42]. Additionally, the findings imply that AgNPs may be employed as a substitute therapy against bacteria. Thus, the findings of this research support the literature findings showing considerable antibacterial activity for the bio-prepared NPs by increasing their activity with ampicillin for *P. aeruginosa* and *S. flexneri*, and vancomycin for *S. aureus* and *S. pneumoniae* [43]. Although the combination of sub-minimum concentration of antibiotics and AgNPs synthetized from the phenolic compound of *A. biosporus* increased the killing of bacteria, antibiotics and AgNPs tend to be used alone [16]. The combination of antibiotics with AgNPs is still undervalued and the mechanisms underlying the synergistic need to be elucidated. According to this study, the synergistic antimicrobial effect of antibiotics with AgNPs was increased probably because antibiotics and AgNPs compromise the

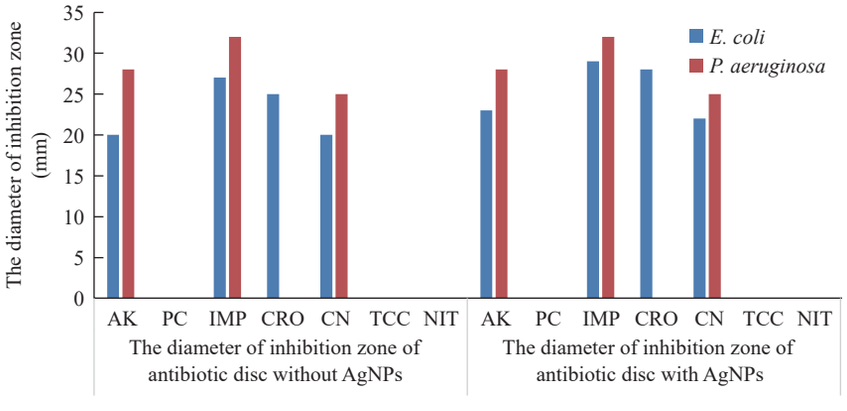

Fig. 8 The inhibition zones (in mm) of different antibiotics alone or in combination with phenolic AgNPs against Gram-negative bacteria.

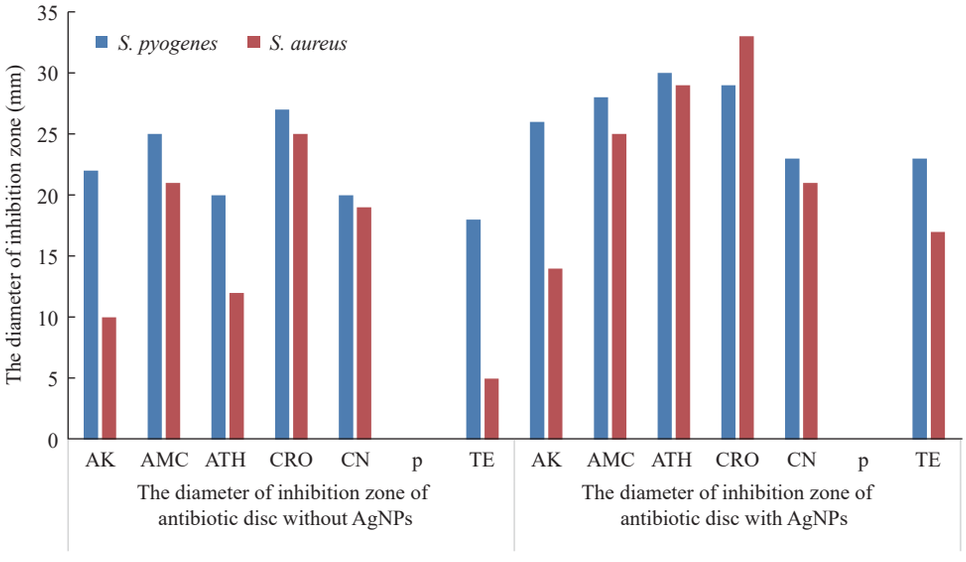

Fig. 9 The inhibition zones (in mm) of different antibiotics alone or in combination with phenolic AgNPs against Gram-positive bacteria.

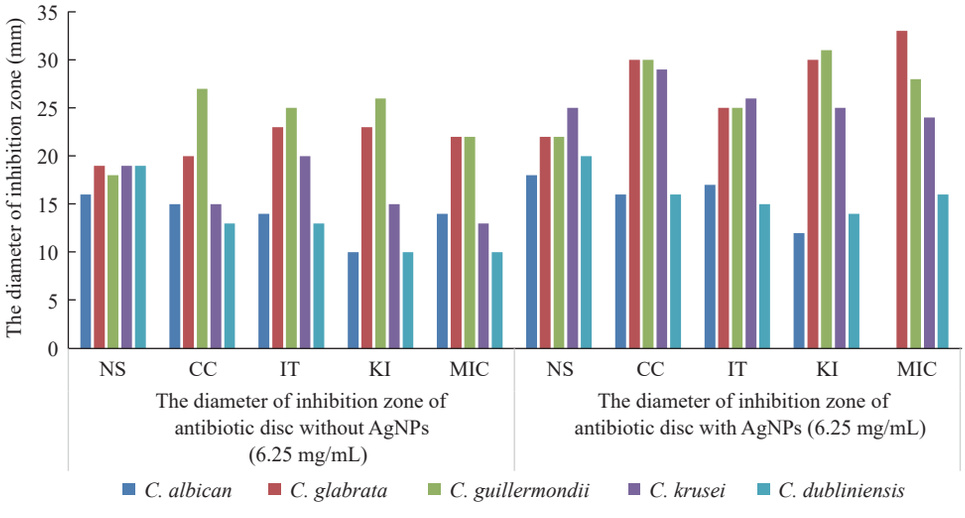

Fig. 10 The inhibition zones (in mm) of different antibiotics alone and in combination with phenolic AgNPs against pathogenic yeasts.

integrity of the same targets, i.e., the cell wall, cell membrane, and DNA.

The inhibition zones for the antibiotics nystatin, clotrimazole, itraconazole, ketoconazole, and miconazole in *C. dubliniensis* were 19, 13, 13, 10, and 10 mm, respectively. It is naturally resistant to the antibiotics itraconazole, ketoconazole, and miconazole, while AgNPs demonstrated a good efficacy against fungal isolates. The inhibition zones for *C. dubliniensis* with antibiotics nystatin, clotrimazole, itraconazole, ketoconazole, and miconazole were 20, 16, 15, 14, and 16 mm, respectively.

The findings revealed that fungi became more sensitive, with an increase in the zone inhibition of antibiotics with AgNPs. The synergistic activity was better for *C. glabrata* and *C. guillermondii*.

The inhibition zones of itraconazole, ketoconazole, and miconazole were larger against fungal isolates.

Multidrug resistance is the most serious issue created by several microorganisms toward chemical antibacterial drugs. As a result, an alternative method for overcoming multidrug resistant bacteria is badly needed, particularly in medical settings [42]. This study corroborates a report [44] showing that AgNPs have activity against *C. albicans*. These are potentially important findings because treating fungal infections with antibiotics such as amphotericin B and nystatin is a serious problem for people with renal and liver dysfunction [45]. Furthermore, another

study demonstrated that AgNPs have antifungal activity against *Aspergillus* spp. from hospitals [46].

Conclusion

AgNPs synthesized from the phenolic compound of *A. biosporus* had a considerable antimicrobial potency against multidrug resistant Gram-negative and Gram-positive pathogenic bacteria and yeast, mainly *Candida* spp. Furthermore, AgNPs demonstrated a synergetic effect with antibiotics by enhancing their effect against bacteria and yeast. These results could have important applications in the clinical field as they suggest that it should be possible to reduce the doses of antibiotics and AgNPs, *in vitro* and *in vivo*. Validation studies are needed.

CRediT Author Statement

E.G. Sweedan: Design of project idea, writing and editing of paper, methods design, supervising of research, practical participation. **S.M. Abdul Majeed**: Writing of paper, practical participation, shapes design.

Conflict of Interest

The authors declare that they have no conflicts of interest to disclose.

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