Mycosynthesis of AgNPs from Candida albicans and its antagonistic activity against pathological factors the urinary and reproductive system in women

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Abstract

This study was conducted in the Department of Life Sciences of the College of Education for Pure Sciences/ University of Thi Qar, Iraq. During (February - September 2022). To study the inhibitory effect of silver nanoparticles manufactured from yeast (Candida albicans) on the fungal and bacterial pathogens that infect the system urinary and genital in women. Four species of yeast belonging to the genus Candida (C. albicana, C.glabrata, C.tropicalis and C. krusei) and five species of bacteria (Escherichia coli, Proteus spp, Klebsiella spp, Staphylococcus aureus and Streptococcus spp) were tested. Five variants of a solution of silver nanoparticles synthesized from Candida albicans were used in the study (0 control, 25%, 50%, 75%, and 100) concentration. Results confirmed that there are significant differences between Candida spp. and concentrations used for silver nanoparticles in the experiment. C. krusei were recorded the highest value for the diameter of growth inhibition 24.26 mm, and 100% concentration were recorded the highest inhibition rate 23.98 mm while 25% concentration were recorded lowest inhibition value 13.70 mm. Also, results confirmed that there are significant differences between species and genuses of bacteria and concentrations used for silver nanoparticles in the experiment. Staphylococcus aureus were recorded highest value of growth inhibition diameter 12.18mm, and 100% concentration were recorded the highest inhibition rate 15.82 mm.

Keywords: *silver, nanoparticles, yeast, genus, pathogens.*

1. INTRODUCTION

Nanoparticles are a group of materials that contain particles that have dimensions of at less than 100 nm (Tiwari et al., 2012). Studies confirm that the synthesis of nanoparticles by chemical and physical methods is not environmentally friendly and expensive, so biosynthesis methods were used in the production of environmentally friendly and economical nanoparticles. Nanoparticles are divided into: organic nanoparticles such asgraphene, fullerenes, carbon nanotubes (CNTs), drug delivery systems, carbon nanofibers, and carbon black (Anu Mary Ealia and Saravanakumar, 2017); inorganic (metal) nanoparticles such as aluminum , cobalt , cadmium ,copper, gold, iron, lead, silver and zinc (Anu Mary Ealia and Saravanakumar, 2017; Kim amd kim, 2019). Silver nanoparticles have received a lot of attention as they have the ability to control the growth of microorganisms (Guilger-Casagrande et al., 2019; Sathiyaseelan et al., 2020). The characteristics of silver nanoparticles through shape, size, and morphology helped them interact with various microorganisms, animals,

and plants and show good potential against phytopathogenic insects, fungi, bacteria, and viruses (Kim, 2004). By producing types of free radicals and active oxygen that cause protein denaturation, DNA destruction and cell wall damage, thus cell death (Khan and Jamil, 2017). The study aims to know the effect of silver nanoparticles manufactured from yeast (Candida albicans) on the fungal and bacterial pathogens that infect the system urinary and genital in women.

2. Materials and Methods

2.1. Fungal and bacterial samples

The study was conducted in the Department of Life Sciences of the College of Education for Pure Sciences/ University of Thi Qar, Iraq. During February - September 2022. To study the inhibitory effect of silver nanoparticles manufactured from yeast (Candida albicans) on the fungal and bacterial pathogens that infect the system urinary and genital in women. Pure fungal and bacterial colonies were brought from the microbiology laboratory, College of Education for Morphological Sciences, University of Thi Qar. It included four species of yeast belonging to the genus Candida (C. albicana, C.glabrata, C.tropicalis and C. krusei) and five species of bacteria (Escherichia coli, Proteus spp, Klebsiella spp, Staphylococcus aureus and Streptococcus spp.).

2.2. Biosynthesized silver nanoparticles

Dissolved 0.0421 gm of silver nitrate in 100 ml of distilled water to obtain 1 mM, then we were placed it in the refrigerator in a glass container covered with cellophane to prevent oxidation of the silver nitrate from light until used. For the biosynthesis of silver nanoparticles, the extract prepared from the biomass of Candida yeast grown in liquid Sabouraud Dextrose Broth (SDB) 250 ml was used. Culture medium containing the yeast biomass was placed in a sterile glass vial and then placed in a shaking incubator for 24 hours at a temperature of 30°C. Then the resulting solution was filtered by filter papers and placed in test tubes in a centrifuge (3000 rpm), then the resulting extract was kept in a glass vial covered with cellophane and placed it in the refrigerator at a temperature of 4 °C until used. Then adding 10 ml of the yeast extract to 90 ml of 1 mM silver nitrate solution and placing it on a shaking incubator (220 rpm) for 72 hours at a heating temperature of 70 °C in the dark room. A reaction was observed in the solution, where the color of the solution changed from transparent yellow to brown and then to dark brown which indicates the process of reducing silver particles by yeast extract. Then, five concentrations were prepared by diluting the prepared AgNPs with distilled water (0, 25, 50, 75 and 100) %.

2.3. Characteristics of silver nanoparticles

For knowledge of physical and chemical properties of silver nanoparticles were used a range of analytical techniques (Scanning Electron Microscopy (SEM), X-ray diffraction (XRD) and UV-Visible Spectrophotometer).

2.3.1. SEM analysis

For the purpose of analyzing the structural, morphological and size characteristics of the silver particles used in the experiment, the Nova Nano SEM 450 scanning electron microscope was used in the Department of Physics in the College of Science, University of Basrah, Iraq. Samples were prepared according method (Vanmathi Selvi and Sivakumar, 2012). 10 µL was taken from solution of silver nanoparticles and filtered by sterile filter papers (0.2 µm (Millipore), then 5 µL was taken and placed on the electron microscope stand consisting of gold cover and carbon, then the samples were left to dry at room temperature, then the readings were taken by the device through multiple magnification powers.

2.3.2. X-Ray analysis

X-ray diffraction properties of the thin films prepared from a solution of silver nanoparticles were studied using the Nova Nano SEM 450 device, which is the same scanning electron microscope that contains an X-ray diffraction reading attachment equipped from the USA.

2.3.3. UV Vis. analysis

5 ml were put in a centrifuge at (800 rpm), and then put 1 ml from solution in spectrophotometer and the samples were measured at lengths Waveforms (200 - 800) to define absorption bands. (Bharathidasan and Panneerselvam, 2012).

2.4. Effect of silver nanoparticles on fungal and bacterial species under study.

Cultures medium were inoculated by (loop) with a fungal and bacterial suspension separately on (SDA and nutrients agar) medium, then cultures medium were left to dry at room temperature. Diffusion method was used by etching using a sterile cork punch to make the drillings and transfer 100 microliters of each concentration of AgNPs and poured in drillings. The cultures medium were incubated in the incubator at a temperature of 27 ℃ for 7 days for fungi and 37 °C for 24 hours for bacteria. The diameters of the inhibition zones (mm) were measured around each hole to know the effect of the concentrations used on inhibiting the growth of fungi and bacteria used in this study (Balouiri et al., 2016).

2.5. Statistical analysis

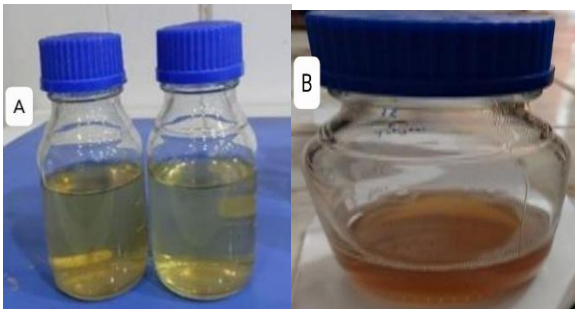
Experiment was carried out using the Complete Randomized Design (CRD) and repeated three times. The results of the experiment were analyzed statistically by estimating the averages for the inhibition diameters of the silver nanoparticles and standard error using the SPSS version 20 statistical analysis program, where the two-way ANOVA test was used with LSD test at < 0.05 (Al-Rawi and Khalaf, 2000).

3. Results and discussion

3.1. Biosynthesized silver nanoparticles

Results of the current study confirmed that adding 10 ml of the yeast extract to 90 ml of 1 mM silver nitrate solution and placing it on a shaking incubator (220 rpm) for 72 hours at a heating temperature of 70 °C in the dark room. A reaction was observed in the solution, where the color of the solution changed from transparent yellow to brown and then to dark brown (Figure 1), which indicates the process of reducing silver particles by fungal filtrate. The reason for the difference in the color change is due to the effect on the surface plasmon resonance (SPR) (Chan and Don 2013), which is a phenomenon that occurs when the elements transform to their nano sizes, the color change of the solution is one of the methods for detecting the reduction of nanoparticles by Biosynthesis (Huang et al., 2007).

Fig. 1. A. yeast extracts C. albicans B. AgNPs



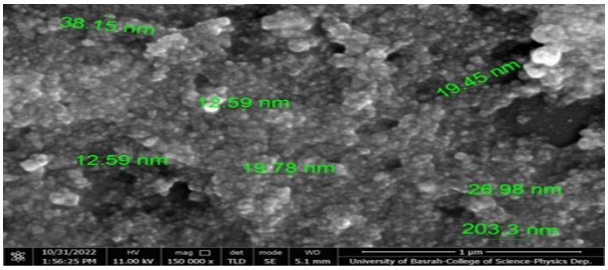
3.2. Characteristics of silver nanoparticles

3.2.1. SEM analysis

SEM images of silver nanoparticles revealed that they take different shapes of particles in the form of agglomerations of different shapes and sizes (oval, spherical, and irregular) ranging in size between 12.59 and 38.15 nm (figure 2), which is close to the results of

studies Rahimi et al., (2016), where it was found that the results of the SEM images of silver nanoparticles are equal to 42nm, while the studies Qader et al., (2019) showed that there is a significant difference between the sizes of silver nanoparticles produced by ivy leaf extract and aloe vera extract, as the results were between (25.68 - 141.17 nm) and between (27.2 - 109.8nm), respectively.

Fig. 2. SEM image under 150000 magnification, size and shape of silver nanoparticles synthesized from C. albicana yeast

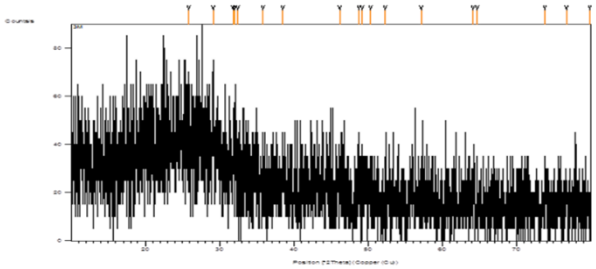


Studies indicate that the morphology of nanoparticles is highly variable and includes spherical, rod-like, decahedral, triangular, and various sheet shapes. This difference may be attributed to the correlation of the absorption spectrum with individual silver nanoparticles (Mock et al., 2002).

3.2.2. X-Ray analysis

In figure (3), XRD graph showed sharp peaks at a number of angles and the patterns perfectly showed that the silver nanoparticles were synthesized by reduction of metal ions by candidiasis yeast extract. Sharp peaks, it clearly showed that the particles were in the nanoscale system according to the international X-ray diffraction database for silver particles JCPDS NO: 04-0783, it was recorded the highest peak has an intensity of 100% at the angle of 31° using the Debye-Scherrer equation s $D=0.94\lambda/\beta\ cos\ \theta$ (Bykkam et al., 2015).

Fig. 3 X-ray analyses of silver nanoparticles manufactured from C. albicana yeast.

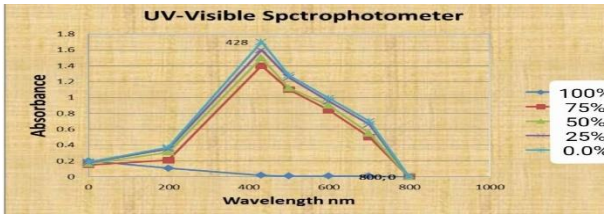


These results agree with the results of the studies Saxena et al., (2016) on silver nanoparticles made from white rot fungus (Sclerotinia sclerotiorum) and also agreed with the results of a study (Rigopoulos et al., 2019) on silver nanoparticles made from banana peel extract. The reason for this is the reflection of X-ray light on any crystal which leads to the formation of many diffraction patterns that reflect the physical and chemical patterns and properties of crystal structures (Das et al., 2014).

3.2.3. UV-Vis. analysis

UV-Vis spectrophotometer readings were recorded when measuring the wavelengths of samples of silver nano-solutions manufactured from Candida yeast in the study (0% control, 25%, 50%, 75%, and 100%) and the absorption bands at 428nm were (0.02, 1.40, 1.5, 1.6, and 1.7), respectively, and this indicates the presence of silver particles in the solution compared to the yeast extract (control), which did not record any absorption peaks at the mentioned wavelengths. The method for detecting the absorption bands of silver particles by spectrophotometer is a widespread method (Chan and Don, 2013). Previous researches confirmed that wavelengths between (800-200nm) are the absorption peaks of silver nanoparticles (figure 4).

Fig. 4. UV-Vis. of silver nanoparticles manufactured from C. albicans yeast



The results of this study agreed with previous studies conducted by Al-Bayati (2021) when it was found that the reading of the absorption bands of silver particles manufactured from cinnamon plant extract was 431 nm. Also, results agreed with the results of Al-Khafaji (2014), who found that the absorbance of silver particles had different absorption peaks and specific wavelengths depending on the extracts obtained from 4 types of fungi that he used in his study (420 nm) C. tuberculata, (420 nm) M phaseolina, (430 nm) N. sphaerica, (430 nm) H. grisea. The reason for the difference between the results of one study and another is attributed to the reason for the effect on the surface plasmon resonance (SPR) (Chan and Don 2013). Visible and ultraviolet spectroscopy is very commonly used to find information about the plasmonic resonance of nanoparticles. The position of the detected plasmonic band on metallic nanoparticle solutions depends on several factors such as: size, shape, and polydispersion of the particles, the narrower the band the greater the uniformity index of the silver nanoparticle size distribution (Becaro et al. 2015).

3.3. Effect of silver nanoparticles on fungal and bacterial species under study

3.3.1. Effect of silver nanoparticles on Candida spp.

Results in table (1) indicate that there are significant differences between the types of the genus Candida and the concentrations used for silver nanoparticles in the experiment at the level of 5%, C. krusei was recorded the highest value for the growth inhibition diameter (24.26 mm), while C. albicana was recorded lowest inhibition value and the highest resistance to the effectiveness of silver nanoparticles compared to the rest of the types, as it recorded a value (12.99 mm). Also, results confirmed in table (1) that the 100% concentration was recorded highest inhibition rate for the effectiveness of the nanoparticles (32.98 mm) compared to 0% concentration (control) that maintained the value of the initial drilling diameter (0.00 mm), while the 25% concentration was recorded lowest inhibition value and the highest resistance to the activity of nanoparticles amounted (13.70 mm) (figure 5). The results of the current study agree with the results of the researchers' study (Panáček et al., 2009 ; Jalal et al., 2018) when they found that silver nanoparticles which synthesized from the biosynthesis of some types of the yeast genus Candida spp. were effective in inhibiting some species of Candida spp and pathogenic bacteria. The reason that the type C. albicana had a lower value of inhibition compared to the rest of the Candida species used in the study is due to the formation of biofilm as well as the possession of many diverse mechanisms through which it can survive and expand as hydrolytic enzymes, metabolic flexibility, resistance to strong stress response and formation of germination tube (Mayer et al., 2013; Nicholls et al., 2011). The virulence factors in Candida spp. are attributed to a number of extracellular enzymes such as proteinase, phospholipase, lipase, hemolysin, chondroitinase, and hyaluronidase, which play an important role in its pathogenicity. Where it was found that silver nanoparticles inhibit the production of the enzyme phospholipase hemolysin, as the enzyme phospholipase works on the ability of Candida to adhere, stimulate germ tubes, move from yeast to thread forms, penetrate and infect tissues (Jalal et al., 2019). These results are consistent with the results of studies Al-Khafaji, (2014) when silver nanoparticles manufactured from some soil fungi were used

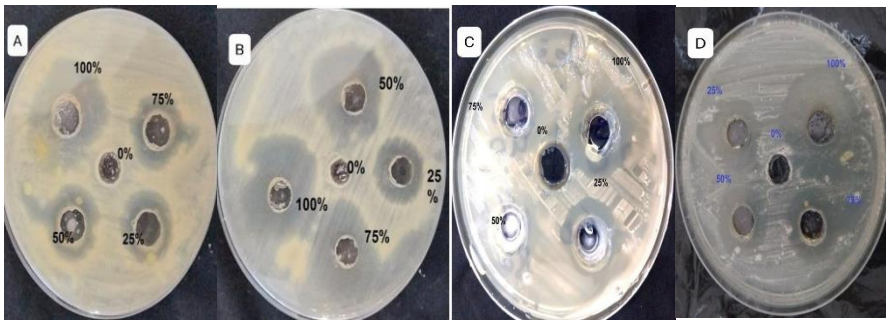
on some pathogenic bacteria, where it was found that high concentrations of nanoparticles were effective in inhibiting bacterial growth. Also, results agreed with the results of Al-Bayati,(2016) research when he used silver nanoparticles manufactured from the biosynthesis of Cinnamomum extract, where the silver nanoparticles showed inhibitory effectiveness at concentrations of 25, 50, 75, and 100% against P. aeruginosa by diffusion method, as well as consistent with the results of the researchers (Mansoor et al. al., 2021; Sunkar and Nachiyar, 2013; Jain et al., 2009).

Table 1. Effect of concentrations of silver nanoparticles to inhibition diameters (mm) on Candida spp.

Candida spp.	% of AgNPs					Mean
	0	25	50	75	100	
C. albicana	0.00	9.03±2.27	12.39±0.86	15.20±3.28	23.35±4.70	12.99±6.81
C.glabrata	0.00	14.50±2.97	22.03±4.71	26.05±5.92	36.41±0.86	20.80±11.32
C.tropicalis	0.00	9.49±6.44	27.06±1.19	28.94±0.71	33.86±0.88	20.87±11.98
C. krusei	0.00	21.80±4.72	24.96±3.79	31.25±4.16	38.32±1.53	24.26±11.80
Mean	0.00	13.70±6.65	21.61±6.42	25.36±7.29	32.98-6.37	19.73±11.32

*±=standard error

Fig. 5. Effect of concentrations of silver nanoparticles to inhibition diameters (mm) on Candida spp. (A=C.albicans, B= C.tropicalis, C= C.glabrata, D= C. krusei)



3.3.2. Effect of silver nanoparticles on five bacteria under study

Diffusion test method was used to determine the inhibitory activity of silver nanoparticles on five types of bacteria under study, Escherichia coli and Proteus spp . Klebsiella pneumoniae., Staphylococcus aureus, and Streptococcus spp. Results in table (2) confirm that there are significant differences between the bacteria species and the concentrations used for silver nanoparticles in the experiment at 5%, Staphylococcus aureus bacteria were recorded highest value of growth inhibition diameter (12.18mm), followed by Streptococcus spp. (10.19 mm), while Klebsiella spp were recorded the lowest inhibition value and highest resistance to the effectiveness of silver nanoparticles (8.07 mm). Also, results in table (2) were confirmed that 100% concentration was recorded the highest inhibition rate for the effectiveness of nanoparticles by 15.82mm compared to the concentration (0%control), while 25% concentration was recorded lowest value of inhibition and highest effective resistance of nanoparticles (7.61mm)(figure 6). Results of the current study agree with the results of the researchers' study Waghmar et al., (2015), where the antibacterial activity of silver nanoparticles manufactured by the cellular biomass of Candida utilis was tested, and its

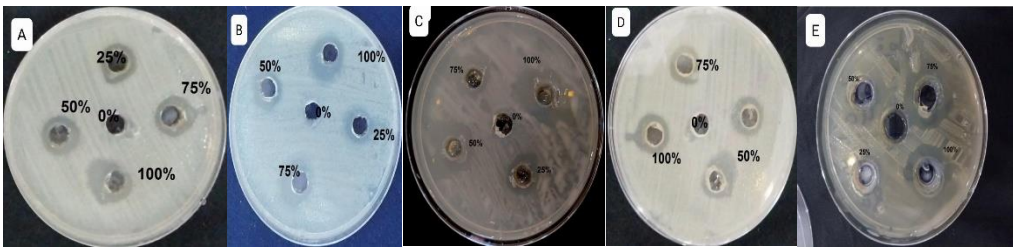
effectiveness were tested on three types of bacteria Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli.). The silver nanoparticles showed more antibacterial activity against Gram-negative bacteria such as P. aeruginosa and E. coli than against Gram-positive bacteria S. aureus. Also, the results of the current study agreed with the studies of Jalal et al., (2019) when they found that silver nanoparticles manufactured from the seeds of jamon trees (Syzygium cumini) gave significant results in inhibiting the growth of fungal colonies (C. albicans, C. tropicalis, C. dubliniensis, C. parapsilosis and C. krusei) for Candida and bacterial growths at high concentrations of 500-1000 µg/ml. The cause of the death of the bacterial colonies is attributed to the adhesion of the nanoparticles to the cell membranes and thus the permeability and disruption of the enzymatic process inside the bacterial cell, which leads to the breakdown of DNA and the inhibition of DNA synthesis (Klasen et al., 2000).

Table 2. Effect of concentrations of silver nanoparticles to inhibition diameters (mm) on five genuses of bacteria

Genuses of bacteria	% of AgNPs					Mean
	0	25	50	75	100	
Escherichia coli	0.00	6.23±0.13	8.39±1.41	12.20±2.31	17.35±1.10	9.83±4.73
Protesu spp.	0.00	6.70±0.51	9.63±0.39	12.05±1.64	16.41±0.86	9.96±4.19
Klebsiella pneumoniae	0.00	5.49±1.32	7.06±1.19	8.94±0.71	13.86±0.88	8.07±3.38
Staphylococcus aureus	0.00	11.00±1.39	12.96±1.09	15.25±1.62	16.72±1.12	12.18±4.31
Streptococcus spp.	0.00	8.62±2.85	9.78±0.62	12.80±0.62	14.77±1.15	10.19±3.69
Mean	0.00	7.61±2.47	9.56±2.20	12.25±2.48	15.82±1.62	10.05±4.23

*±=standard error

Fig. 6. Effect of concentrations of silver nanoparticles to inhibition diameters (mm) on five genuses of bacteria(A= Proteus spp., B= Escherichia coli, C= Streptococcus spp., D= Klebsiella spp., E= Staphylococcus aureus)



4. Conclusions

Results confirmed that there are significant differences between Candida spp. and concentrations used for silver nanoparticles in the experiment. C. krusei were recorded the highest value for the diameter of growth inhibition 24.26 mm, and 100% concentration were recorded the highest inhibition rate 23.98 mm while 25% concentration were recorded lowest inhibition value 13.70 mm. Also, results confirmed that there are significant differences between species and genuses of bacteria and concentrations used for silver nanoparticles in the experiment. Staphylococcus aureus were recorded highest value of growth inhibition diameter 12.18mm, and 100% concentration were recorded the highest inhibition rate 15.82 mm.

5. Acknowledgment

The authors thank all colleagues, researchers, and staff at the College of Education for Pure Sciences, Bint Al-Huda Hospital in Nasiriyah city, Health Ministry of Iraq to assist them in completing these research papers.

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