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The antimicrobial effect of silver nanoparticles coated with silica against human pathogenic bacteria and fungi

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***Abstract*---**The antibacterial property of silver (Ag) has been known since ancient time. It is reported in the literature that silver nanoparticles (AgNPs) exhibit improved antibacterial and antifungal properties in comparison to silver ions of equivalent metallic Ag concentration.A simple method has been used based on solenoid soles for the synthesis of nanoparticles of silver coated on silica. AgNPs were compared positively with silver nitrate solution and ampicillin standard antibiotics at a concentration of 50µg / mL (Table 1 and 2). AgNPs mentioned in the silica activity exhibited more than standard silver nitrate and antibiotics. AgNPs were fairly toxic to *Salmonella sp.*, *Neisseria sp.*, *Klebsiella sp.*, and *Pseudomonas sp.* with an inhibition zone 21, 24, 16, 23 mm respectively. AgNPs exhibited strong antifungal activity against fungal strains (*Candida sp.*, *Aspergillus fumigatus* and *Aspergillus flavus*) in different concentrations such as 30-50 and 60 µg/ml were examined for antifungal activity. AgNPs revealed the highest antifungal activity with the inhibition zone 26, 25 and 22 mm respectively. Results of the present study prevealed that AgNPs have a remarkable potential as antimicrobial agent in treating infectious diseases.

***Keywords*---**Silver nanoparticles, Antimicrobial effect, Bacteria, Fungi.

Introduction

Silver is a transitional metal with an atomic number 47 and an atomic mass of 107.87. It has been widely used as an antimicrobial agent for centuries because it is a non-selective toxic biocide (Rajeshkumar *et al.*, 2019). During ancient times, silver metal pots and cups were used to store water to prevent pollution. Before the advent of antibiotics, colloidal silver was used as a bactericidal agent (Soulé *et al.*, 2016).

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Silver-based biocides were used as preservatives, antiseptics, antifungal, antimicrobial, and antiviral agents. Silver salts are known to treat skin infections; silver nitrate has been used to treat eye infections, such as neonatal optics for more than 100 years(Park *et al.*, 2016). Many silver salts, such as silver acetate, are formulated in lotions and creams for the treatment of eye infections. (Misran *et al.*, 2018).

It was suggested to use silver nanoparticles (AgNPs) to get rid of microbial infections. Extensive antimicrobial activities of Ag were reported against pathogens, including both Gram-positive and Gram-negative bacteria. Ag has been installed with other materials to give them antibacterial properties(Yan *et al.*, 2018). For example, antimicrobial activity of nylon fibers saturated with AgNPs was assessed against *Staphylococcus aureus* and *Candida albicans* due to wound dressing. AgNPs were also used to treat *E. coli* because of their ability to damage bacterial cell walls (Xu *et al.*, 2017) Currently, AgNPs are used in creams, wound dressings, Ag-coated medical devices such as catheters, vascular grafts, tracheal tubes to prevent and treat bacterial infections(Hjerrild *et al.*, 2016). Also, in new years, resistance to tradely fungicides by fungi has been rising and has be a significant problem (Goffeau, 2008). So, the search for modern fungicides and alternate is of fundamental value to conflict recently emerging resistant strains of fungal pathogens (Kanhed *et al.*,2014). One resolution would be nanotechnology that promote the antimicrobial activity of nanoparticles in contrast to their salts is belong to their unique characteristics i.e large surface area to volume ratio(Kanhed *et al.*,2014). So, this study aimed to detect the antimicrobial effect of silver nanoparticles coated with silica against some of the human pathogenic bacteria and fungi isolates.

Materials and Methods

Ag- Nanoparticles

A simple method has been used based on solenoid soles for the synthesis of nanoparticles of silver coated with silica .Silver (Ag) Nanoparticles 200 pH 7 (Ag Si NP 200 pH 7):120 mg of silver nitrate was dissolved in DI water. The magnetic stirrer was mixed at 400 rpm. After completely dissolving the silver nitrate salt in the water, 600 liters of tetraethylurithosilicate (TEOS) (silica silicate precursors) were added to the silver nitrate solution ground under continuous stirring conditions. Allow the above solution to be mixed for 5 minutes(Misran *et al.*, 2018). Turn the prepared mixture over a magnetic stirrer for 24 hours at room temperature. After 24 hours, a colorless transparent mixture was obtained in pH 7, thus forming silica Ag nanoparticle (AgSiNP / NG) 200 in pH 7 solution.The nanoparticles were distinguished by UV-Vis spectroscopy(Soulé *et al.*, 2016).

Antibacterial activity of AgSiNPs

Antibacterial activity of AgSiNPs against the following human pathogenic bacteria has been tested by a well diffusion method: *Salmonella sp. , Neisseria sp., Klebsiella sp. and Pseudomonas sp.* The diameter of the clearance areas was measured in millimeters using the ruler scale and compared with the standard ampicillin tablet (positive control) and silver nitrate solution (negative

control)(Park *et al.*, 2016). The experiment was performed on three copies of each pathogenic bacteria and compared with the standard antibiotic sensitivity scheme(Rajeshkumar *et al.*, 2019).

Antifungal activity of AgSiNPs

The antifungal activity of AgNPs was determined to be synthesized using a well-agar screening method. Stock cultures of human pathogenic fungi : *Candida sp.* , *Aspergillus fumigatus* and *Aspergillus flavus* were prepared and maintained at Dextrose Agar at 4°C. The plates were examined for evidence of an inhibition zone, which appears as a clear area around wells(Sun *et al.*, 2016; Yan *et al.*, 2018; Hafsan et al.,2022). The diameter of these areas was measured by inhibition using a meter ruler. The mean value was calculated by conducting experiments in three copies.

Results and Discussions

AgNPs coating in silica

AgNPs are known to show a brown color in the water that arises due to the stimulation of surface plasma vibrations in metallic nanoparticles. AgNPs were synthesized using UV spectroscopy and subjected to antimicrobial activity.Silica acts as a reducing agent. The proposed silica data reduced silver ions to elemental silver, resulting in the formation of nanoparticles that were embedded in the silica matrix. Ethanol also helps reduce silver salts. As no ethanol is added as solvent in this formula, ethanol from TEOS may play a role in facilitating the formation of silver nanoparticles. The spectra also showed the presence of silica around silver nanoparticles, as silica was observed when the analysis was performed on the surface of silver nanoparticles, indicating that silica had attached silver nanoparticles in a matrix(Wong *et al.*, 2017; Bokov et al.,2022).

Antibacterial activity

Antibacterial activity was performed using four different strains, *Salmonella sp.*, *Neisseria sp.*, *Klebsiella sp.* and *Pseudomonas sp.* The results of the investigation showed that AgNPs coated with silica have antibacterial activity against pathogenic bacteria at a concentration of 10µg/ml. AgNPs were compared positively with silver nitrate solution and ampicillin standard antibiotics at a concentration of 50µg / mL (Table 1and 2). AgNPs coated with the silica exhibited activity more than standard silver nitrate and antibiotics. AgNPs at concentration (60 µg /mL) were fairly toxic to *Salmonella sp.*, *Neisseria sp.*, *Klebsiella sp.* and *Pseudomonas sp.* with an inhibition zone of 21, 24,16 and 23 mm respectively.

Attach AgNPs to sulfur-containing proteins in the cell membrane, causing membrane damage and depletion of ATP levels within cells of microorganisms. Silver can also interact with the DNA of microorganisms, which inhibits cell proliferation(Zhang *et al.*, 2017; Huldani et al.,2022).

Zarei *et al.*(2014) studied the antibacterial effect of silver nanoparticles (chemical synthesis) against four foodborne pathogens (*Listeria monocytogenes*, *Escherichia*

coli, *Salmonella typhimurium* and *Vibrio parahaemolyticus*), which showed that nanoparticles have a highly effective antimicrobial effects on four pathogens .Also, the antibacterial effect of nanoparticles (chemical synthesis) detected by (Park *et al.*, 2016; Zadeh et alk.,2022) .

Table 1
Comparing the effect of Ag-nanoparticles coated with silica and ampicillin on different human pathogenic bacterial species

Organism	Concentration(µg)	Zone of inhibition (mm)		
		Control	Antibiotic	AgSiNps
Salmonella sp.	50	-	18	17
Neisseria sp.	50	-	20	22
Klebsiella sp.	50	-	13	15
Pseudomonas sp.	50	-	23	21

Table 2
Effect of different Ag nanoparicles coated with Silica on different bacterial species

Organism	Zone of inhibition (mm) at different Concentrations (µg/ml)						
	5	10	20	30	40	50	60
Salmonella sp.	1	3	6	9	10	17	21
Neisseria sp.	-	2	9	15	19	22	24
Klebsiella sp.	-	2	8	10	12	15	16
Pseudomonas sp.	-	2.3	7	11	18	21	23

Anti-fungal activity

Antifungal activity of AgSiNPs against human pathogenic fungi *Candida sp.* , *Aspergillus fumigatus* and *Aspergillus flavus* were investigated using antifungal-nystatin as a similar control. AgNPs exhibited strong antifungal activity against fungal strains in different concentrations such as 30-50 and 60µg/ml that were examined for antifungal activity. AgNPs at concentration (60 µg/ml) revealed the highest antifungal activity against with the inhibition zone 26, 25 and 22 mm respectively (Table 3,4).

Kim *et al.* (2007) reported that AgNPs have shown strong activity against egg candidiasis compared to commercially available antifungal agents. The treatment of fungal infections becomes a frantic problem due to serious side effects such as impaired renal and liver function associated with amphotericin B and nystatin.

Ag + is also composed of complexes that have bases contained in DNA and is a potent inhibitor of innate DNAase. The rate of bio-synthesis of nanoparticles from seaweed is cost-effective and does not use toxic chemicals. It is well known that antimicrobial activity in nanoparticles is likely to be well associated with decreased size and shape due to increased surface area with increased antimicrobial effect(Rajeshkumar *et al.*, 2019; Ansari et al.,2022).

Table 3
Comparing the effect of Ag-nanoparticles coated with silica and nystatin on different human pathogenic fungal species

Organism	Concentration (mg)	Zone of inhibition (mm)		
		Control	Antibiotic	AgSiNPs
Candida sp.	50	-	14	16
Aspergillus fumigatus	50	-	19	17
Aspergillus flavus	50	-	18	14

Table 4
Effect of different Ag nanoparticales coated with Silica on different pathogenic fungal species

Organism	Zone of inhibition (mm) at different Concentrations (µg/ml)						
	5	10	20	30	40	50	60
Candida sp.	-	-	7	18	20	23	26
Aspergillus fumigatus	-	-	7	11	15	20	25
Aspergillus flavus	-	-	5	14	18	19	22

Conclusions

It was probable to synthesize the silver nanoparticles coated with silica. The procedure used to keep track of time constancy, UV spectra, was appropriate. The outcome gained from the susceptibility test exhibited that the AgNPs were active against bacterial and fungal growth in contrast to the controls. So, they have noteworthy potency as antimicrobial agents in healing infectious disease.

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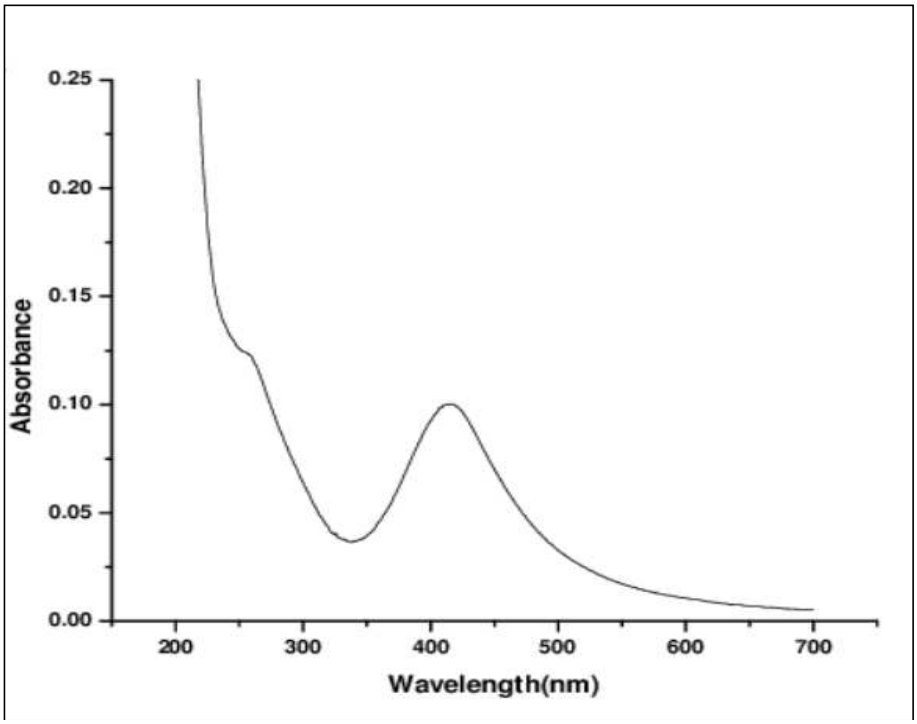

Figure 1: UV-visible spectra of silver nanoparticles

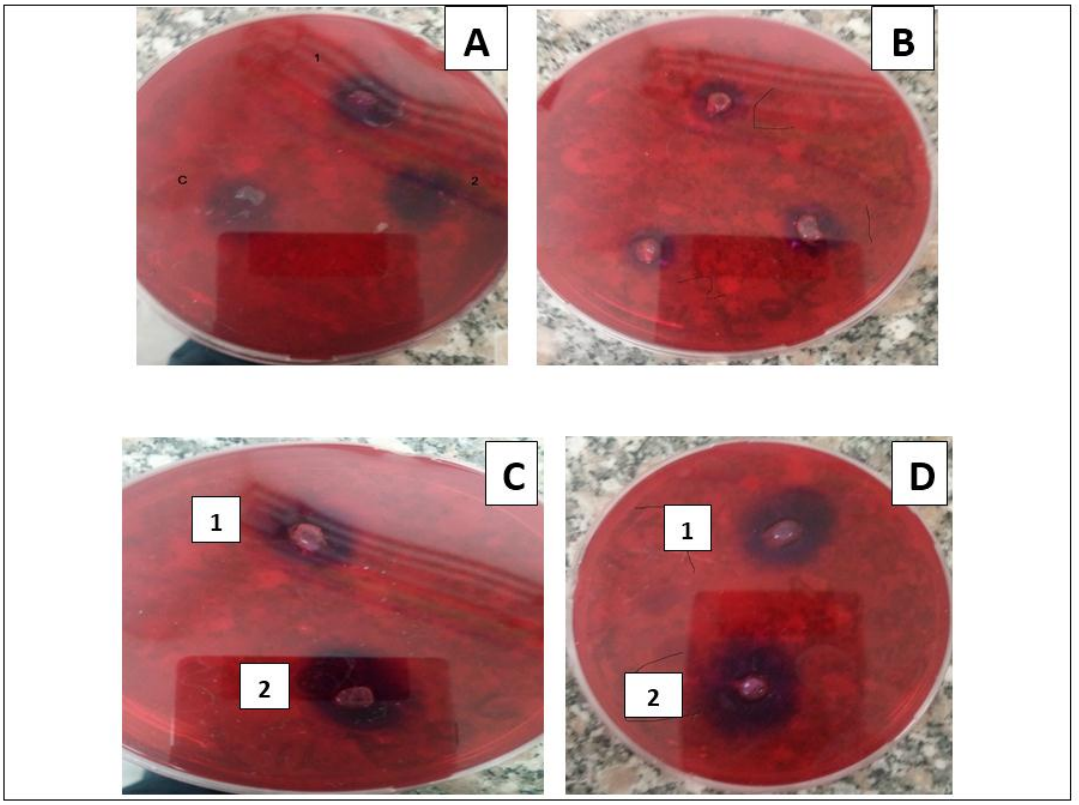

Figure 2: The antibacterial effect of Ag nanoparticles coated with silica

A: *Salmonella sp*. While C. control with ampicillin; 1: 50 µg/ml of Ag nanoparticles coated with silica, 2: 60 µg/ml of Ag nanoparticles coated with silica

B: *Klebsiella sp*. While C. control with ampicillin, 1: 50 µg/ml of Ag nanoparticles coated with silica, 2: 60 µg/ml of Ag nanoparticles coated with silica

C: *Neisseria* sp;1: 50 µg/ml of Ag nanoparticles coated with silica, 2: 60 µg/ml of Ag nanoparticles coated with silica

D: *Pseudomonas sp*,1: 50 µg/ml of Ag nanoparticles coated with silica ,2: 60µg/ml Ag nanoparticles coated with silica

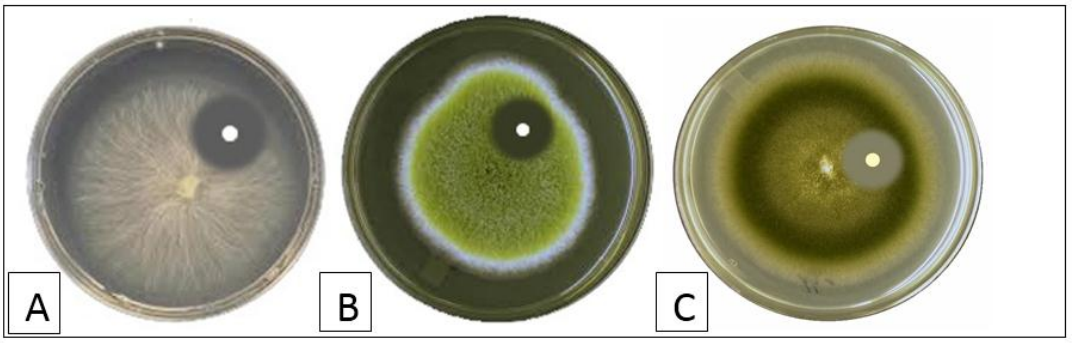

Figure 3: The antifungal effect of Ag nanoparticles coated with silica

A. *Candida sp* with 60 µg/ml Ag nanoparticles coated with silica
B. *Aspergillus flavus* with 60 µg/ml Ag nanoparticles coated with silica
C. *Aspergillus fumigatus* with 60 µg/ml Ag nanoparticles coated with silica