

# Assay of Antibacterial and Antifungal Activity of Silver Nanoparticles Accompanying Different Impurities

**Mohsen A. Sayed**

Cairo University

**Asmaa A. H. El-Bassuony** (✉ [asmaa@sci.cu.edu.eg](mailto:asmaa@sci.cu.edu.eg))

Cairo University <https://orcid.org/0000-0003-0201-7538>

**H. K. Abdelsalam**

Higher Institute of Applied Arts 5th Settlement

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## Research Article

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## Abstract

No doubt that antimicrobial compounds such as antibiotics are the basic tools in clinical medicine. Increase in antimicrobial resistance may cause several human diseases. The potential of drugs and other agents can be improved by using nanoparticles. Wide use of antimicrobial compounds resulted in formation of resistance in microbes. There is demand to look for novel agents for therapy. Novel silver nanoparticles accompanied different impurities were assayed against *Streptococcus pyogenes* and *Staphylococcus aureus* (Gram-positive bacteria), *Pseudomonas aeruginosa* and *Proteus mirabilis* (Gram-negative bacteria). In addition to *Aspergillus niger*, *A. terreus*, *Penicillium cluclauni* and *Rhizopus stolonifer* fungi. Four *Candida* spp. were also assayed: *C. krusei*, *C. famata*, *C. parapsilosis* and *C. utilis*. The method which applied in assaying these microorganisms was diffusion method. Dilution method was obtained for the tested nanoparticles to find the minimum inhibitory concentration MIC. The annealing temperature of the tested nanoparticles affected on antifungal and antibacterial activities. It was found that the preparation of nanoparticles at room temperature gave higher activities against tested antimicrobial than that at high temperatures.

## Key Points:

The synthesized nanoparticles showed promising antimicrobial activity.

This article contains seven Tables and one Figure.

## Introduction

Pathogenic bacteria that are antibiotic resistant cause great challenges in health worldwide (Mantravadi et al. 2019). Bacteria can resist old and newly discovered antibiotics through inherent and acquired mechanisms (Abusaiba and AL-Harmoosh 2020). Fluconazole was the primary therapeutic agent for treatment of fungal infections. Now it is only a fungistatic agent against *Candida* spp. where both inherent and acquired resistance has been reported (Berkow and Lockhart 2017). Resistance includes alteration in the target of the drug, increase in efflux of the drug, and presence of different pathways for target sterol production (Jarvis 1995). The spread of drug-resistant microorganisms represents huge challenges in healthcare worldwide (Sharafutdinov et al. 2020). It was reported that *Aspergillus* sp had the lowest antifungal activity in comparison to caspofungin and triazoles (Lalitha et al. 2007). Generally, the preparation of nanoparticles has a great effect on the antimicrobial study. There are two methods to prepare different nanoparticles materials, solid state and wet methods. In the solid-state method, bulk nanoparticles are prepared; while in the wet method, nanoparticles are prepared (Nadaroglu et al. 2017; El-Bassuony and Abdelsalam 2017, 2018 (1, 2, 3, 4), 2019 (1, 2), 2020 (1); El-Bassuony 2020 (1); Maklad et al. 2014). To synthesize novel antimicrobial drugs, researchers in nanotechnology and biological science should be cooperated to improve the drug efficacy. (Ventola 2015; Lu et al. 2017). Nanoparticles show simultaneous multiple mechanisms against the resistance of microbes (Singh et al. 2014; Cavassin et al. 2015). Such mechanisms include release of metal ions, oxidative stress, and non-oxidative stress. This makes resistance difficult to develop (Wang et al. 2017). Finally, silver nanoparticles are applied in different fields including antimicrobial activity, water purification, cancer treatment, magnetic targeting, and biomedical applications (Paula et al. 2009; Nangmenyi et al. 2011; Abdelhamid et al. 2015; El-Bassuony 2017, 2018 (1, 2), 2020 (2); Abdelsalam 2018; Ateia 2017; Sayed et al. 2020(1, 2)).

The novelty of this work is the assaying of silver nanoparticles accompanying different impurities against different opportunistic fungal and bacterial spp. It is reported from previous studies of silver nanoparticles accompanying different impurities showed fascinating physical properties that can be used as alternative drug nanomaterials (El-Bassuony and Abdelsalam 2020 (2, 3)). Thus, the aim of the present work is based on the study of antimicrobial effect on a novel synthesized silver nanoparticle accompanied different impurities against opportunistic bacterial and fungal species.

## Materials And Methods

## Synthesis of silver nanoparticles accompanied different impurities

Silver nanoparticles were prepared flash method (an easy and low cost method). The materials used in the preparation were: chromium ( $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ), silver ( $\text{AgNO}_3$ ), nickel ( $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ), and iron III ( $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ ) nitrates (Fisher Company). All materials were mixed in distilled water; urea was added drowsily. The mixture was heated to 250 °C till and the samples were collected to be ground for 0.5 h. Finally, some samples were annealed at 400 °C and were ground also for half an hour. Thus, the prepared samples were Ag nanoparticles accompanied iron oxide impurities  $\alpha$ ,  $\gamma$ -  $\text{Fe}_2\text{O}_3$  (Ag-Fe), Ag nanoparticles accompanied iron and nickel oxide impurities  $\alpha$ ,  $\gamma$ -  $\text{Fe}_2\text{O}_3$ , NiO (Ag-Fe-Ni), Ag nanoparticles accompanied delafossite and chromite (Ag-D-C), Ag nanoparticles accompanied delafossite (Ag-D) as-prepared and at 400 °C.

## Biological study

### Materials

Bacterial and fungal species were supplied via Microanalytical Center, Faculty of Science, Cairo University.

### Identification and growth of micro-organisms

Fungi had grown on potato dextrose agar (PDA) media and incubated for seven days at 25 °C (Adwic, El-Nasr chemical Co. Egypt). The fungal species were then identified based on some characters such as: shape of conidia/spores, colony shape, colony pigmentation and presence of septa (Moubasher 1993; Maadon et al. 2018; Raper and Fennell 1965). Bacteria had grown on nutrient agar and blood agar plates. The colonies had been recognized biochemically and morphologically (Holt et al. 1994).

### Antimicrobial study of silver nanoparticles accompanied different impurities

The antimicrobial activity of silver nanoparticles accompanied different impurities were tested against *Aspergillus niger*, *A. terreus*, *Penicillium cluclauni* and *Rhizopus stolonifer* fungal species. Also, the nanoparticles were tested against *Candida* spp.: *C. famata*, *C. parapsilosis*, *C. krusei* and *C. utilis*, fungal species. The tested fungi were added to fresh PDA media before solidification at concentration  $10^6$  colony forming unit/ml (CFU/ml). Then, 100 mg/ml of the investigated nanoparticles was added into the surface of agar plates which were incubated for 5 days at 27 °C. The standard antifungal drug which used in the present work is 100 mg/ml of Fluconazole however 100 mg/ml of Ampicillin was used as a control (Saadabi et al. 2012). The tested bacteria *Staphylococcus aureus* and *Streptococcus pyogenes* (Gram positive bacteria), *Proteus mirabilis* and *Pseudomonas aeruginosa* (Gram-negative bacteria), were added at concentration  $10^6$  (CFU/ml) before solidification. Then, 100 mg/ml of silver nanoparticles accompanied different impurities was added into the surface of the agar plates which were incubated for 2 days at 35 °C.

### Estimation of minimum inhibitory concentration

MIC (minimum inhibitory concentration) was determined by Pershin method (Persin 1971). The inhibitory effect of minimum concentration of silver nanoparticles accompanied different impurities on bacterial and fungal growth was obtained by Serial Dilution method using bifold dilution concentrations from 6.25, 12.5, 25, 50, and 100 mg/ml. The dimethyl sulfoxide (DMSO) solvent was used to dissolve the tested nanoparticles. The culture media for bacteria is Mueller–Hinton broth liquid medium however that for fungi is and Sabouraud liquid media. For bacteria the test inoculums are  $5 \times 10^4$  CFU/ml bacteria per ml however for fungi the test inoculums are  $10^3$  CFU/ml fungal spores per ml. Then, the tested compounds were added onto agar surface after inoculation with the test organism with incubation of plates at 30 °C. Finally, the lowest concentration that showed no growth (MIC) was obtained after incubation o 2- 7 days.

### Statistical analysis

Statistical analysis was obtained using SPSS (Statistical Package of the Social Sciences) version 22. The homogeneity of different treatments was examined using Duncan's test. The significant results were at  $P < 0.05$ , whereas the insignificant results were at  $P > 0.05$ . The reported data is (mean  $\pm$  standard deviation (SD)) (Tahir et al. 2009; Duncan 1955; Maadon et al. 2018; Holt et al. 1994).

## Results

### Assay of synthesized silver nanoparticles accompanied different impurities against fungal species

Tables 1 & 2 showed the highest antifungal effect against tested fungal species while Tables 4 & 5 showed the lowest MIC values for Ag-D-C and Ag-D synthesized at 30 and 400 °C in comparison with the other tested nanosamples that gave lesser antifungal activity and higher MIC values.

### Assay of synthesized silver nanoparticles accompanied different impurities against bacterial species

One can obtain from the results of antibacterial activity, that the sensitivity of Gram-positive bacteria to the investigated samples was greater than that of Gram-negative bacteria. Table 3 showed that Ag-Fe, followed by Ag-D and Ag-D-C, showed the highest antibacterial effect against the tested bacterial species and the lowest MIC values with respect to the remaining tested nanosamples that showed lesser antibacterial activity and high MIC values as shown in Table 6.

### Structural properties

It is reported from previous studies in details the physical and magnetic properties of silver nanoparticles accompanied by different impurities (El-Bassuony AAH, Abdelsalam HK 2020(2, 3)). Table 7 showed the crystallite size and the particle size estimated from X-ray diffraction pattern (XRD), field emission scanning electron microscopy (FESEM) and atomic force microscopy (AFM). These analyses emphasize the nanosize of the investigated samples. Moreover, the crystallite size increased by increasing the annealing temperature as shown from Table (7).

## Discussion

The great demand from many researchers to find novel drugs to overcome the microbial resistance problem that caused health problems all over the world (El-Bassuony and Abdelsalam 2018 (1, 2); Abusaiba and AL-Harmoosh 2020; Berkow and Lockhart 2017). Silver nanoparticles compounds have various applications in many fields especially in biomedical field. The antimicrobial activities were studied for many nanoparticles such as titanium dioxide (Panáč'ek et al. 2009; Gutierrez et al. 2010), zinc oxide (Wani and Shah 2012), magnesium oxide (Kanhed et al. 2014), and copper (Ezema et al. 2010). Nanoparticles can be prepared by many methods however flash technique gave very fine nanoparticles and low cost method which was chosen in preparation of the investigated samples. The annealing temperature is an important parameter that affect the particle size of the nanosamples. Thus, the more efficient annealing temperature is low temperature that gives fine nanoparticles which could be able to penetrate the membrane of the microbial cell and destroy the internal molecules causing the death of the microbes (Hafiz et al. 2015; Mallika et al. 2015; Talal and Ali 2015; Bharamagoudar et al. 2018). The inhibition zone diameter increased by increasing the concentration of the investigated samples. One can obtain that the investigated samples had an effect against antibacterial activities (Gram positive and Gram negative). Moreover, when adding the investigated samples to the tested microbes caused DNA, protein and lipid damage and caused a death to the microbes (Clinical and Laboratory Standards Institute 2013). Thus, authors strongly recommended to use the investigated samples as an alternative antibiotic.

There is another publication to study cytotoxicity assay with concentrations 6.25, 12.5, 25, 50, and 100 mg/ml. Furthermore, it is required to study the investigated samples *in vivo* using animal models to study the treatment of fungal and bacterial infections.

To summarize, in the present study, novel silver nanoparticles accompanied different impurities were synthesized and showed that Ag-D and Ag-D-C had high *in vitro* antifungal effect, while Ag-Fe was the most powerful compound against bacteria. The best activities was shown for compounds that synthesized at 30°C. Finally, Ag-D, Ag-D-C and Ag-Fe are promising compounds that could be used as an alternative drug in the future.

## Declarations

### Authors' contributions

MAS and AAH conceived and designed research. MAS and HAK conducted experiments. AAH contributed new reagents. AAH and HAK analyzed data. MAS and AAH wrote the manuscript. All authors read and approved the manuscript.

### Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by the authors.

### Conflict of interest

The authors declare that they have no conflict of interest.

### Compliance with Ethical Standards

This article does not contain any studies with human participants or animals performed by any of the authors.

### Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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## Tables

Table 1

Antifungal activity of the synthesized silver nanoparticles accompanied different impurities against filamentous fungi

Compounds	Temp. of synthesis °C	<i>Aspergillus niger</i>		<i>A. terreus</i>		<i>Penicillium cluclauni</i>		<i>Rhizopus stolonifer</i>	
		Inhibition zone diameter (mm)	Relative activity (%)	Inhibition zone diameter (mm)	Relative activity (%)	Inhibition zone diameter (mm)	Relative activity (%)	Inhibition zone diameter (mm)	Relative activity (%)
Fluconazole (control)	—	20	100	18	100	19	100	22	100
Ag-D-C	30	25	125	24	133.3	16	84.2	14	63.6
Ag-D	400	20	100	22	122.2	18	94.7	17	77.3
Ag-Fe	30	13	65	16	88.9	14	73.7	14	63.6
Ag-Fe	400	3	15	0	0	2	10.5	0	0
Ag-Fe-Ni	30	12	60	8	44.4	12	63.2	10	45.5
Ag-Fe-Ni	400	3	15	0	0	2	10.5	0	0

Data is represented as mean ± standard deviation.

In the same raw, the values marked with the same superscript letter are similar ( $P > 0.05$ ), whereas those marked with different ones are significantly different ( $P < 0.05$ ).

Table 2

Antifungal activity of the synthesized silver nanoparticles accompanied different impurities against *Candida* sp.

Compounds	Temp. of synthesis °C	<i>Candida famata</i>		<i>C. parapsilosis</i>		<i>C. krusei</i>		<i>C. utilis</i>	
		Inhibition zone diameter (mm)	Relative activity (%)	Inhibition zone diameter (mm)	Relative activity (%)	Inhibition zone diameter (mm)	Relative activity (%)	Inhibition zone diameter (mm)	Relative activity (%)
Fluconazole (control)	—	22	100	20	100	20	100	18	100
Ag-D-C	30	31	140.9	30	150	25	125	28	155.6
Ag-D	400	23	104.5	20	100	21	105	21	116.7
Ag-Fe	30	14	63.6	10	50	10	50	12	66.7
Ag-Fe	400	5	22.7	0	0	0	0	3	16.7
Ag-Fe-Ni	30	15	68.2	10	50	12	60	12	66.7
Ag-Fe-Ni	400	6	27.3	4	20	0	0	2	11.1
Data is represented as mean ± standard deviation.									
In the same raw, the values marked with the same superscript letter are similar (P > 0.05), whereas those marked with different ones are significantly different (P < 0.05).									

Table 3

Antibacterial activity of the synthesized silver nanoparticles accompanied different impurities against Gram + ve and Gram - ve bacteria

Compounds	Temp. of synthesis °C	Gram + ve Bacteria				Gram -ve Bacteria			
		<i>Staphylococcus aureus</i>		<i>Streptococcus pyogenes</i>		<i>Proteus mirabilis</i>		<i>Pseudomonas aeruginosa</i>	
		Inhibition zone diameter (mm)	Relative activity (%)	Inhibition zone diameter (mm)	Relative activity (%)	Inhibition zone diameter (mm)	Relative activity (%)	Inhibition zone diameter (mm)	Relative activity (%)
Ampicillin (control)	—	30	100	31	100	27	100	28	100
Ag-D-C	30	10	33.3	8	25.8	6	22.2	6	21.4
Ag-D	400	0	0	0	0	0	0	0	0
Ag-Fe	30	40	133.3	38	122.6	27	100	30	107.1
Ag-Fe	400	0	0	0	0	0	0	0	0
Ag-Fe-Ni	30	8	26.7	5	16.1	2	7.4	0	0
Ag-Fe-Ni	400	2	6.7	3	9.7	0	0	0	0
Data is represented as mean ± standard deviation.									
In the same raw, the values marked with the same superscript letter are similar (P > 0.05), whereas those marked with different ones are significantly different (P < 0.05).									

Table 4

Minimum inhibitory concentration (MIC mg/ml) of the synthesized silver nanoparticles accompanied different impurities against tested filamentous fungi

Compounds	Temp. of synthesis °C	<i>Aspergillus niger</i> ,	<i>A. terreus</i> ,	<i>Penicillium cluclauni</i>	<i>Rhizopus stolonifer</i>
Fluconazole (Control)	—	25	25	25	25
Ag-D-C	30	12.5	12.5	50	50
Ag-D	400	25	25	50	50
Ag-Fe	30	50	50	50	50
Ag-Fe	400	> 100	> 100	> 100	> 100
Ag-Fe-Ni	30	> 100	> 100	> 100	> 100
Ag-Fe-Ni	400	> 100	> 100	> 100	> 100

Table 5

Minimum inhibitory concentration (MIC) of the synthesized silver nanoparticles accompanied different impurities against tested *Candida* sp.

Compounds	Temp. of synthesis °C	<i>Candida famata</i>	<i>C. parapsilosis</i>	<i>C. krusei</i>	<i>C. utilis</i>
Fluconazole (Control)	—	50	50	50	50
Ag-D-C	30	25	25	50	25
Ag-D	400	50	50	50	50
Ag-Fe	30	100	100	100	100
Ag-Fe	400	> 100	> 100	> 100	> 100
Ag-Fe-Ni	30	> 100	> 100	> 100	> 100
Ag-Fe-Ni	400	> 100	> 100	> 100	> 100

Table 6

Minimum inhibitory concentration (MIC50) of the synthesized silver nanoparticles accompanied different impurities against Bacteria

Compounds	Temp. of synthesis °C	Gram + ve Bacteria		Gram -ve Bacteria	
		<i>Staphylococcus aureus</i>	<i>Streptococcus pyogenes</i>	<i>Proteus mirabilis</i>	<i>Pseudomonas aeruginosa</i>
Ampicillin (Control)	—	25	25	25	25
Ag-D-C	30	100	100	100	100
Ag-D	400	50	50	100	100
Ag-Fe	30	> 100	> 100	> 100	> 100
Ag-Fe	400	> 100	> 100	> 100	> 100
Ag-Fe-Ni	30	> 100	> 100	> 100	> 100
Ag-Fe-Ni	400	> 100	> 100	> 100	> 100

Table 7

Values of Crystallite size from X-ray analysis (XRD), particle size estimated from field emission scanning electron microscopy (FESEM) and particle size estimated from atomic force microscopy (AFM) of the synthesized silver nanoparticles accompanied different impurities

Compounds	Temp. of synthesis °C	Crystallite size from XRD (nm)	Particle size obtained from FESEM (nm)	Particle size obtained from AFM(nm)	References
Ag-D-C	30	85.4	110.2	—	39
Ag-D	400	88.9	112.3	—	39
Ag-Fe	400	42.5	116.4	107.76	38
Ag-Fe-Ni	400	31.4	137.8	139.09	38

## Figures

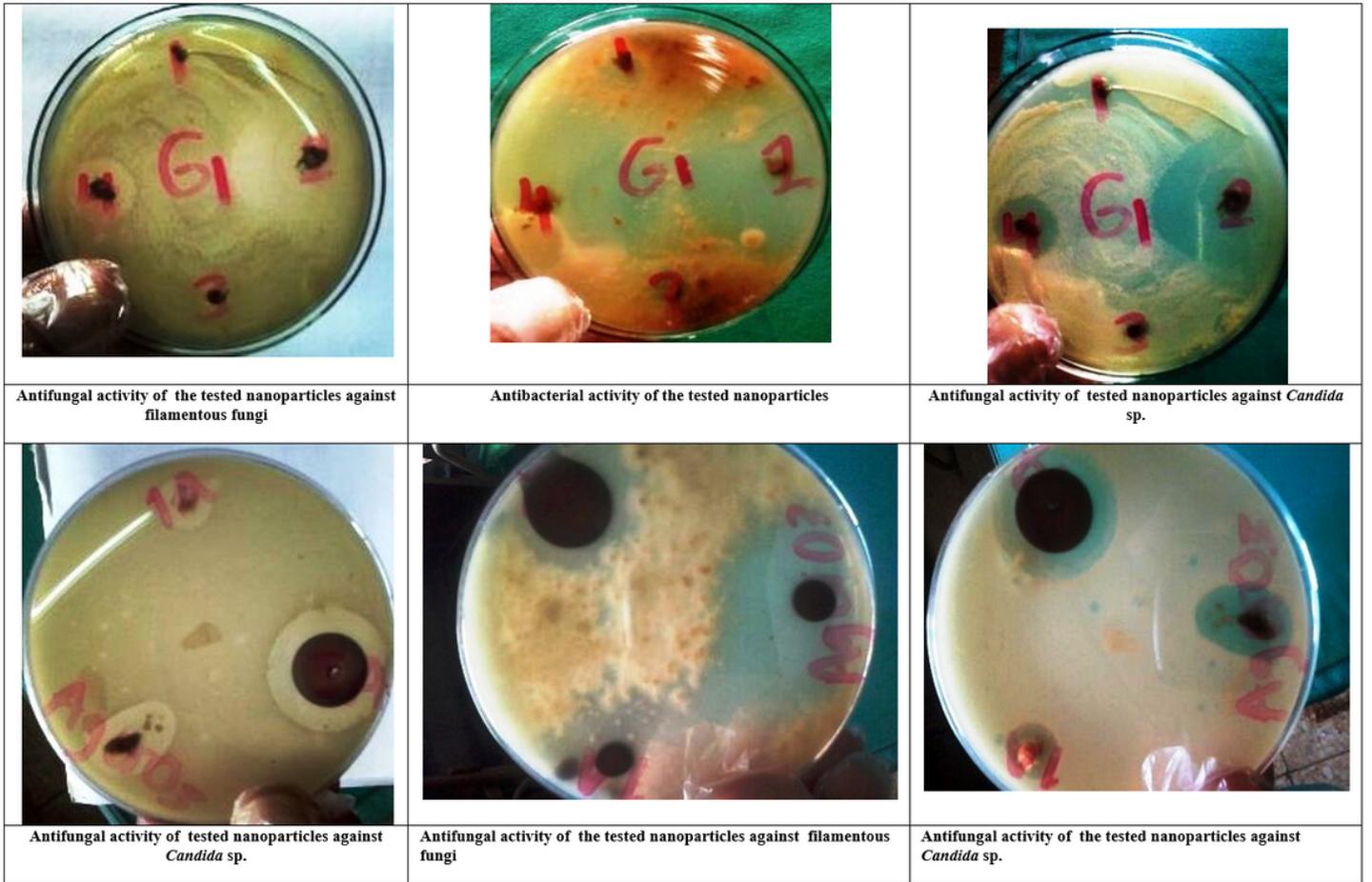


Fig.1

## Figure 1

Representative photos showing the antimicrobial activity of the tested nanoparticles against opportunistic microorganisms