



Biological Evaluation of Selenium Nanoparticles Biosynthesized by *Fusarium semitectum* as Antimicrobial and Anticancer Agents

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NANOTECHNOLOGY is considered one of the most frontiers in the scientific sector, which increasingly attracted researchers' interest due to its variable and valuable applications in all areas. Recently, green nanotechnology is serving as an essential technique that making the processing more friendly, safe and clean. Selenium is one of the most important dietary supplements in the human diet. It is incorporated into the synthesis of many antioxidant enzymes. Therefore, selenium has become antagonistic to many diseases relying on oxidative stress such as cancer, brain and heart illness. In this study, biogenic selenium nanoparticles (Bio-SeNPs) physicochemical characteristics such as formation, morphology and biological activity of Bio-SeNPs as antimicrobial, antioxidant and anticancer were investigated. Transmission electron microscopy (TEM) micrograph of biosynthesized Bio-SeNPs by *Fusarium semitectum* showed an average length of 61.18 ± 15.85 . Antioxidant activity in vitro had showed higher (2,2'-azino-bis(3-rthylbenzothiazoline-6-sulfonic acid) ABTS radical scavenging ability and powerful antioxidant potential (5.656 ± 0.12 mmol trolox/g) than (1,1-diphenyl-2-picrylhydrazyl) DPPH (1.362 ± 0.09 mmol trolox/g), as well as Bio-SeNPs, possess potent antibacterial potential against burns wound infections and selective anticancer potentials against colon, skin and lung cancers without effect on human normal cells. These findings demonstrate the positive effects of Bio-SeNPs as a promising candidate for future therapeutic applications.

Keywords: Bio-SeNP; Mycosynthesis; *Fusarium semitectum*; antimicrobial; anticancer; antioxidant.

Introduction

Patients with serious burn damage are susceptible to many bacterial infections which may lead to death. The main causes of the infections are normal flora of patients or external factors like hospital atmosphere or wound bandages. Several sets of microorganisms associated with burn wounds such as *Pseudomonas aeruginosa*, Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Citrobacter* sp., *Coagulase-negative Staphylococci*, *Enterobacter*

sp. and *Escherichia coli* [1]. The antibacterial susceptibility tests are showing the continuous resistance of these microorganisms which face challenges in treating severe burn infections [2].

Metal nanoparticles have been commonly used in several fields such as biomedicine and material sciences [3]. Recently, Biogenic metal nanoparticles have attracted more attention due to the low toxicity, economically feasible and eco-friendly compared to chemically synthesized metal nanoparticles [4,5]. Various kinds of algae, fungi, plants extracts, also, Flavonoids that known for

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Received 3/8/2019; Accepted 25/9/2019

DOI: 10.21608/ejchem.2019.15618.1945

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their pharmacological activities [6-10], are efficient and environmentally friendly green nano-factories that has been widely used as bio-reductants for the synthesis of NPs. The production of NPs using microorganisms is considered a perfect source for the biosynthesis of nanomaterials. Fungi are considered as a good source for the biosynthesis of metallic NPs, rely on their properties such as large surface areas, resistance to toxicity, easy in handling and scaling up the process compared to other microorganisms [11].

Selenium is a vital trace element in living organisms. It plays an important role in antioxidant defense, immune regulatory and antitumor for human health [12-15]. Bio-SeNPs are relatively new in this important field. They have a great advantage, especially when compared to metal nanoparticles due to their high degradability, low toxicity and ability to clear from the body.

Herein, our objective is biosynthesis of Bio-SeNPs using fungus *Fusarium* sp. and their employment as antimicrobials against severe burn infections along with the study of antioxidants and antitumor properties. Also, the study aimed at investigating the toxicity of Bio-SeNPs on normal liver and kidney cells.

Material and Methods

Isolation and Identification of fungi

A cultivated *Moringa oleifera* plant in a greenhouse of Center of studies and applied research of medicinal plants, Giza, Egypt suffers from Wilt diseases. A yellowish of leaves, branches and soften rot of main root as well as the mycelium of fungal growth were observed. Samples from softening root and tissues cut into small square parts of 0.5cm then sterilized by 5% of sodium hypochlorite and washed in sterile distilled water several times then plated on Potato Dextrose Agar (PDA) at $25\pm 2^\circ\text{C}$ for 5 days. Fungi purified and identified under a light microscope [16]. Fungi purified and identified under light microscope according to Nelson *et al.*, [17], and Barnett and Hunter [18].

Biosynthesis of Selenium Nanoparticles by *Fusarium semitectum*

100 ml of Malt extract Glucose Yeast extract Peptone (MGYP) Broth inoculated with 5mm discs taken from *Fusarium* (7 days-old) and incubated at $25\pm 2^\circ\text{C}$ for 5 days. For Selenium nanoparticles biosynthesis, the fungi were filtrated using Whatmann filter paper No.1. Then, 50 ml of fungal culture filtrate (a) mixed with 50 ml of

deionized water contains 0.2 g of sodium selenite and incubated for 3 days at $28\pm 2^\circ\text{C}$. In another set of experiments, 20 g of fungal biomass washed and mixed with deionized water and incubated for 72 h at $28\pm 2^\circ\text{C}$. After incubation cell filtrates (b) were obtained by passing through Whatmann filter paper No.1 and took 50 ml of *Fusarium* sp. filtrates mixed with 50 ml of deionized water contains 0.2 g of sodium selenite. Flasks were incubated for 3 days at $28\pm 2^\circ\text{C}$ (Adapted [19]).

Characterization of Biosynthesized Selenium Nanoparticles (Bio SeNPs)

The reduction of selenium ions by *Fusarium semitectum* was checked by using UV-VIS Spectra Analysis (UV-VIS (Specord Plus 210, analytic Jena, Germany) Plant chemistry Lab, NODCAR). The possible biomolecules responsible for the reduction, capping, and stabilization of selenium nanoparticles were detected by Fourier transform Infrared Spectroscopy (FT-IR, AKX0901119012A0607, genesis series Nicolet IS-10 F, thermofisher scientific company) and size distribution of Bio-SeNPs in the colloids were measured using a Nano ZS zeta sizer system (Malvern Instruments in the Egyptian Petroleum Research Institute (EPRI). Measurement parameters were as follows: a laser wavelength of 633 nm (He-Ne), a scattering angle of 173° (fixed—without changing possibility), a measurement temperature of 25°C , a medium viscosity of 0.8872 mPa.s and a medium refractive index of 1.330, and the material refractive index of 1.59.

Transmission electron microscope (TEM, JEOL 1010, Japan) at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University) was used to determine the morphology, size, and structure of Bio-SeNPs. Bio-SeNPs were dropped cast on carbon-coated copper grids and dried under vacuum desiccation and TEM micrographs of the sample were taken using the JEOL1010TEM device operated at an accelerating voltage of 200 kV.

Collection of Pathogenic Bacteria

Twenty isolates were collected from the microbiology unit of some Egyptian hospitals. Wound swabs were cultured onto blood agar then incubated at 37°C for 18-24 h. Colonies on the blood and MacConkey agar were gram stained and tested with indole and citrate, Voges-Proskauer test and Triple Sugar Ion test (TSI), urease and oxidase were performed to identify which bacteria species were present.

Antimicrobial susceptibility test

The antimicrobial sensitivity of twenty isolates was detected by using agar - disk diffusion method. Bacterial suspensions (0.5 McFarland standard) were inoculated on Mueller-Hinton Agar (Oxoid, Basingstoke, UK). The disks (6-mm diameter) of different antibiotics such as ampicillin (10 μ g), piperacillin (100 μ g), penicillin 10 units, amoxicillin / clavulanic acid 20/10 μ g, Piperacillin / tazobactam 100/10 μ g, cefepime (30 μ g), cefotaxime (30 μ g), imipenem (10 μ g), meropenem (10 μ g), gentamicin (10 μ g), amikacin (10 μ g), tobramycin (10 μ g), ciprofloxacin (5 μ g), levofloxacin (5 μ g), Oxacillin (1 μ g), Tetracycline (30 μ g), Erythromycin (15 μ g), teicoplanin (30 μ g), clindamycin (2 μ g), Rifampin (5 μ g), linezolid (30 μ g), ceftiofur (30 μ g), aztreonam (30 μ g) and trimethoprim/sulfamethoxazole (1.52/2375 μ g) placed on plates and incubated at 37°C for 16-18 h.

These antimicrobial agents approved by the US Food and Drug Administration for clinical use for testing and reporting on Non-fastidious Organisms. Based on Clinical and Laboratory Standards Institute (CLSI) guidelines and the diameter of inhibition zones monitored the Resistant (R), Intermediate (I) and Sensitive (S) isolates [20].

Antimicrobial analysis

The antimicrobial activity of the synthesized Bio-SeNPs was assessed against different pathogenic bacteria. The assay was performed by plating the tested pathogenic bacteria on Muller-Hinton Agar plates. Wells were cut in the plates using a sterile cork-borer and 100 μ l of Bio-SeNPs solution was dispensed in each well. The plates were incubated at 37°C 24 h. The zone diameter of inhibition was measured in mm [21].

Determination of radical scavenging activity

Free radical scavenging capacity (DPPH, ABTS) of extracts were determined according to Hwang and Do Thi [22].

Anticancer Activities of Bio-SeNPs biosynthesized by Fusarium semitectum

Caco-2 Human Colon cancer cell, SNU16 Stomach gastric cancer cell, A431 Skin cancer cell, THLE2 Normal liver cell and Vero Normal kidney cell lines at concentration of 10 \times 10³ cells/well were seeded in fresh complete growth medium in 96-well microtiter plastic plates at 37°C for 24 h under 5% CO₂ using a water jacketed Carbon dioxide incubator. Media was aspirated, fresh medium (without serum) was

added and cells were incubated either alone (negative control) or with selenium nanoparticles to give final concentration of 0.39, 1.56, 6.25, 25 and 100 μ g/ml. After 48 h of incubation, medium was aspirated, 40 μ l 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) salt (2.5 μ g/ml) were added to each well and incubated for further four hours at 37°C under 5% CO₂. The reaction was stopped and the formed crystals were dissolved by using 200 μ L of 10% Sodium dodecyl sulphate (SDS) to each well and incubated overnight at 37°C. The absorbance was then measured using a microplate multi-well at 595nm and a reference wavelength of 620nm [23].

Statistical Analysis

A statistical significance was tested between samples and negative control (cells with the vehicle) using independent t-test by Statistical Package for the Social science (SPSS 11 program). Percentage of cell viability was calculated using the following formula: (Absorbance of treated cells / Absorbance of negative control) x 100. The lethal concentration of the sample which causes the death of 50% of cells in 48 h (IC₅₀) was detected.

Result and Discussion

The conventional isolation from soften roots and tissues on Potato dextrose medium yielded *Fusarium* sp Heavy white mycelium and pigment is detected after 5 days of incubation at 25 \pm 2°C (Fig.1). *Fusarium* species are known to produce microconidia and macroconidia. A fusiform to ovoid and straight or curved microconidia are characterized by light microscope.

The fusarium identified as *Fusarium semitectum* (currently called *Fusarium incarnatum*) according to Nelson et al. [17] which is totally agreed with the first Egyptian report recorded in infection of *Moringa oleifera* plant by Zeidan et al. [16].

The reaction of selenite ions with fungal culture filtrates (Fig. 2a & b) occurred rapidly and the color of solutions changed with time into dark reddish orange at 24 h and then the red color did not change with 72h. The red orange color is indicator for the occurrence of the reaction and the formation of α -Se. In case of positive control (only fungal culture filtrate of fungal biomass free from culture media (Fig. 2c) and sodium selenite solution as negative control, no change in color was detected.



Fig. 1. *Fusarium semitectum* growth on Potato dextrose agar.

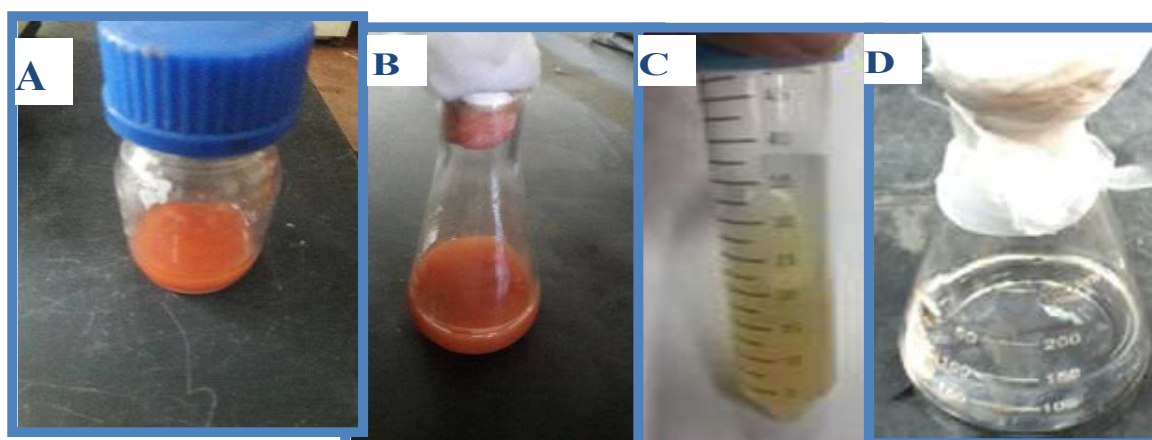


Fig. 2. The conversion of sodium selenite solution into selenium nanoparticles by *Fusarium semitectum* cell free filtrate.

Selenium nanoparticles biosynthesized by fungal culture filtrate (A and B), fungal culture filtrate free from selenium nanoparticles or sodium selenite as positive control (C) and sodium selenite solution as negative control (D).

UV/VIS spectrophotometry analysis showed that maximum absorbance for selenium colloidal suspensions were at 262 nm (Fig. 3). Our result agreed with Gangadoo *et al.* [24] who confirmed the presence of stable selenium nanoparticles by UV-visible spectroscopy for surface plasmon resonance (262 nm). The reducing agent was strong to complete the conversion of the precursor molecules (sodium selenite) into nano-sized selenium particle.

Figure (4) showed that FTIR spectrum of cell free filtrate of *Fusarium semitectum* displays bands at wave number 3445.21, 2088.53, 1636.3, 1461.78, 1161.9, 533.22 and 430 cm^{-1} . SeNPs
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biosynthesized by cell free filtrate of *Fusarium semitectum* exhibiting bands at 3451.15cm^{-1} is attributed to O-H and N-H stretching and bands at 2083.20 and 1634.71 cm^{-1} are assigned to stretching vibration of amide II and amide I respectively, and a characteristic carbon halide at 666.97 cm^{-1} . Data revealed the disappearance of some peaks such as 1461.78 which is attributed to $-\text{CH}_3$ and $-\text{CH}_2$ bending and also 1161.9 which is assigned as carboxylic acids or glycogen and this indicates the interaction between sodium selenite and carboxylic acids or glycogen. The possible mechanism for the reduction of selenium ions of sodium selenite was the presence of multibranched polysaccharide of glucose (glycogen) and this proved by Zhang *et al.* [25] who confirmed the role of different polysaccharides such as chitosan, konjac glucomannan, acacia gum and carboxymethyl cellulose in reduction of selenium.

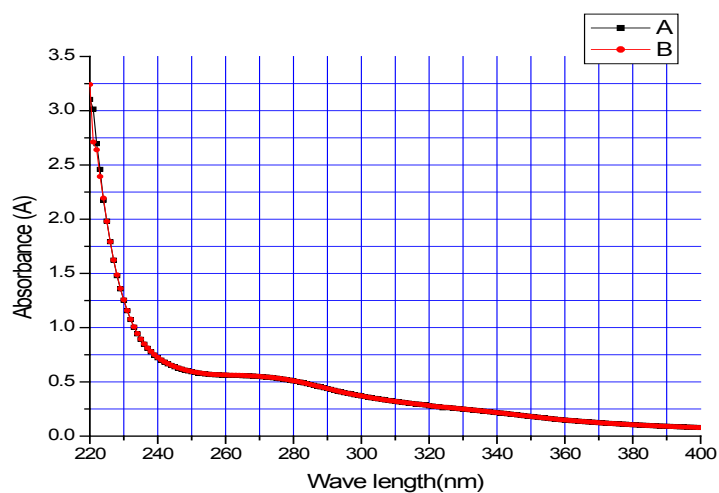


Fig. 3. UV-Vis spectra of Bio-SeNPs biosynthesized by *Fusarium semitectum* culture filtrates. A: fungal culture filtrate and B: filtrate obtained by filtrating the incubated biomasses with deionized water.

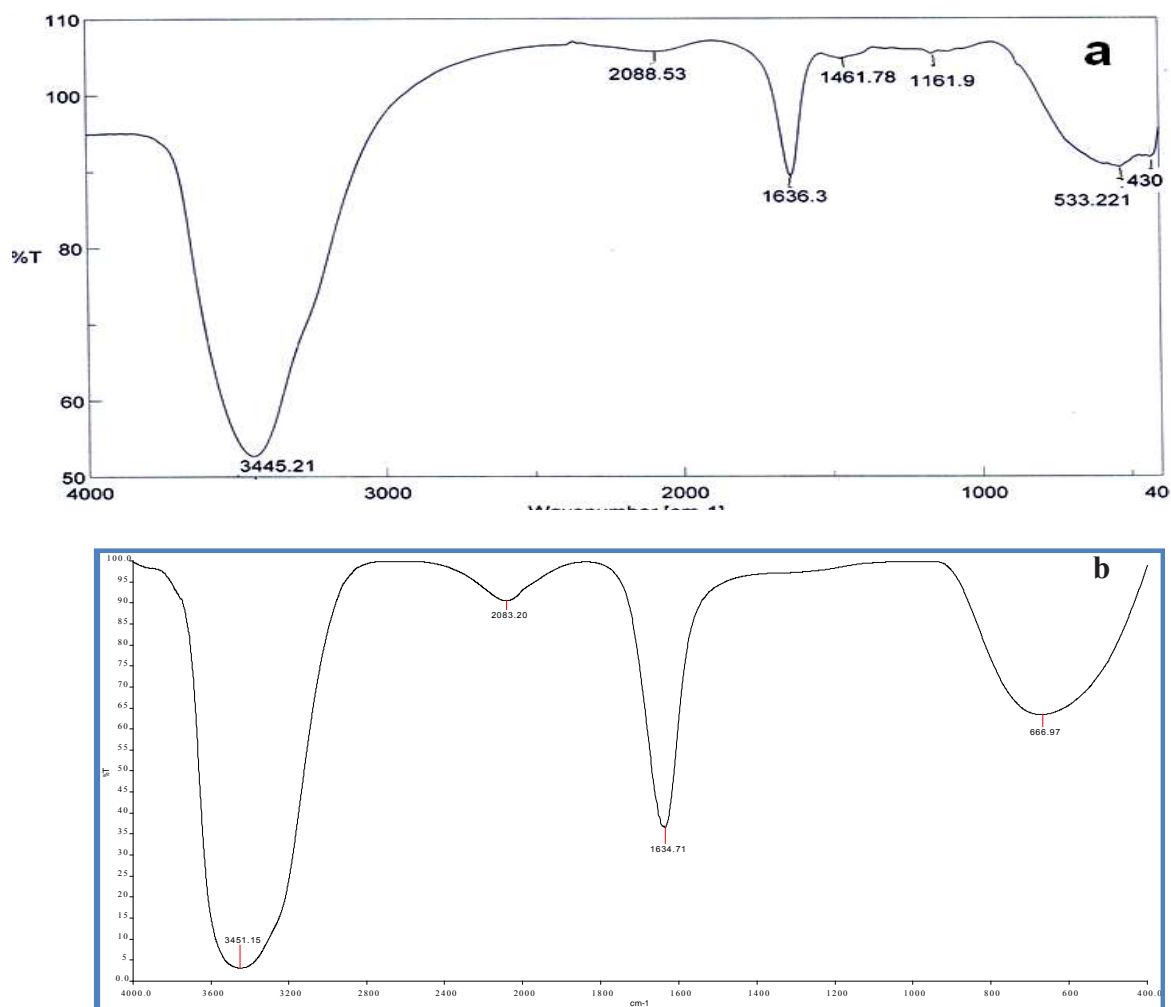


Fig. 4. FTIR recorded from spectra of cell free filtrate of *Fusarium semitectum* (a) and biosynthesized selenium nanoparticles (b).

Particle size of Bio-SeNPs was determined by dynamic light scattering (DLS) measurement. The poly dispersity Index equal (PDI) 0.3 which confirmed the stability of colloidal suspension of Bio-SeNPs. When PDI value is greater than 0.5, it means lower stability of the nanoparticles and their aggregation [26]. Moreover, Bio-SeNPs in the stable colloidal suspension showed an average size of 92.33 ± 48.5 nm for 78.9 % of the sample (Fig. 5- curve A & B).

Transmission electron micrographs confirmed spherical shape and uniform distribution without significant agglomeration (Fig. 6 A & B). The analysis of data from TEM micrograph of Bio-SeNPs showed that the diameter of particles ranged from 32.80 nm to 103.82 nm, with an

average length of 61.18 ± 15.85 . The frequency of particles size ranged from 40-80 nm was 82.96% (Fig. 7). A few reports for production of selenium nanoparticles by fungi were recorded. In Agreement of this study Sarkar *et al.* [19], the size of Bio-SeNPs biosynthesized by *Alternaria alternata* was in the range of 30–150 nm. But the average diameter was of 90 ± 10 nm which differs from our results. However, bacteria were known for production zero valent selenium nanoparticles like Gram-negative *Selenihalanaerobacter shriftii*, *Sulfurospirillum barnesii* [27]. *Klebsiella pneumoniae* [28], and *Pseudomonas aeruginosa* [29]. Besides, gram-positive *Bacillus selenitireducens* produces spherical Bio-SeNPs in their cell envelope [27].

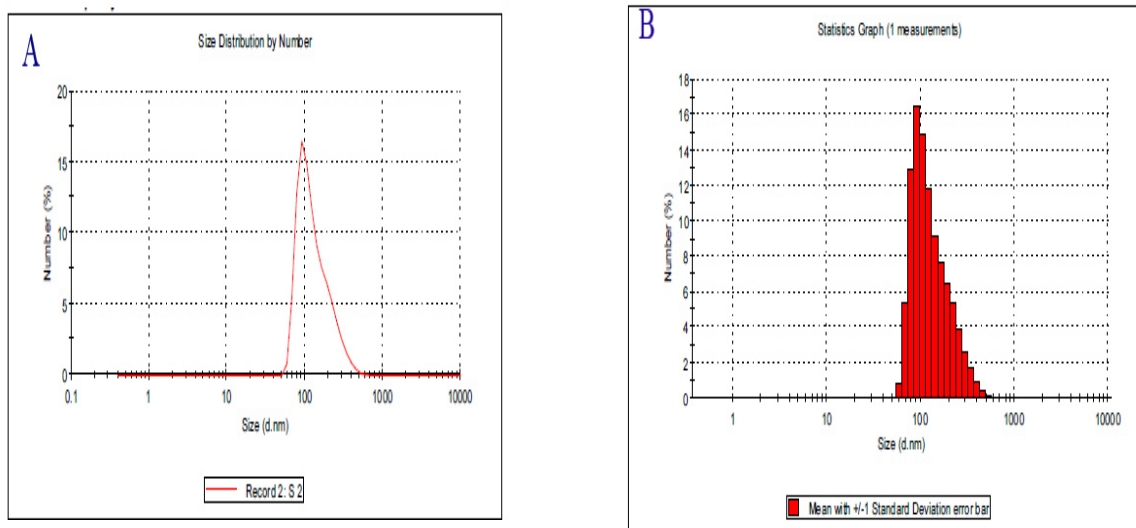


Fig .5. Size distribution of Bio-SeNPs by Dynamic Light Scattering (DLS) method (A) & their statistic graphs (B).

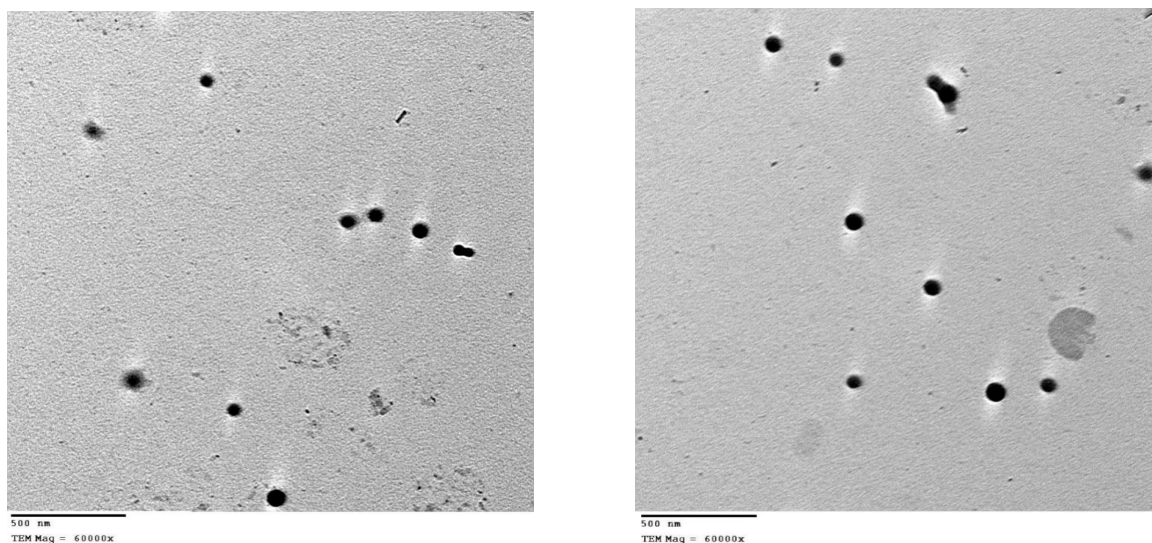


Fig. 6. Transmission electron micrograph of Bio-SeNPs

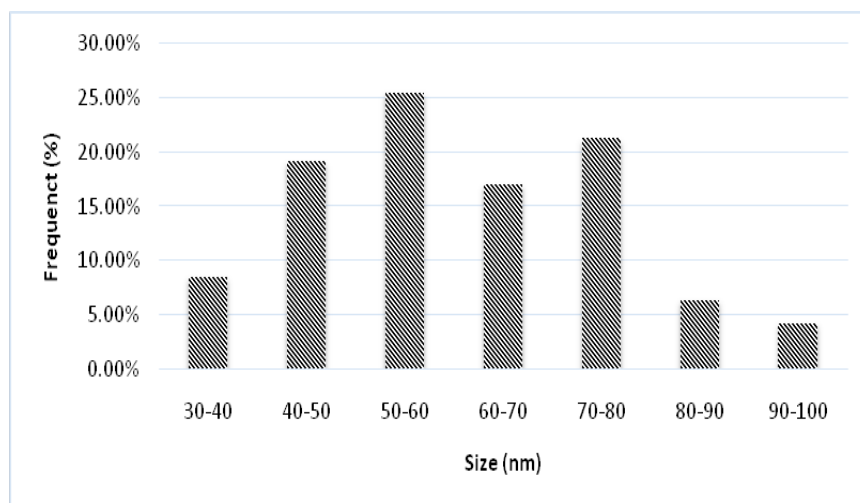


Fig. 7. The frequency of Bio-SeNPs size from TEM images.

Antimicrobial activity

In our study, we try to use biosynthesized SeNPs for continuous resistance of pathogenic bacteria which is the cause of severe burn infections. Twenty pathogenic bacteria isolates were collected. The isolates were gram-negative bacteria 15 (75% Isolate) and 5 (25%) isolates were gram-positive bacteria. Gram-negative Bacteria identified according to the biochemical tests (Table s1). Gram-positive bacteria that give positive coagulase and catalase and β hemolytic on blood agar identified as *Staphylococcus aureus*.

The most predominant pathogenic bacteria were *Pseudomonas aeruginosa* which represent 40% (8 isolates) followed by 5 isolates of *S. aureus* (25%), 3 isolates of *Klebsiella pneumoniae* (15%), 2 isolates of *Acinetobacter baumannii* (10%), 1 isolates of *Escherichia coli* (5%) and 1 isolate of *Proteus vulgaris* (5%). Similarly, Studies carried out by Forson *et al.* [30] & Patil *et al.* [1] revealed the most predominant bacteria isolated from burn wounds were gram-negative bacteria. These gram-negative bacteria include *Pseudomonas sp.*, *Acinetobacter spp.*, *Proteus mirabillis*, *Enterobacter spp.*, *Klebsiella sp.*, *Citrobacter sp.*, *Klebsiella oxytoca* and *Proteus vulgaris*. However, the only gram-positive bacteria isolated were *Staphylococcus aureus*. Moreover, the most prevalence isolates were *Pseudomonas sp.* in burn wounds (33.3%) reported by Lakshmi *et al.* [31] which agreed with our findings.

The antimicrobial susceptibility test was assessed to detect the ability of pathogenic bacteria to resist different antibiotics. Based on CLSI, [20] guidelines, the result in Table (1) shows that 2

isolates of *K. pneumoniae* are resistant while *E. coli* and *P. vulgaris* are sensitive to antibiotics. Three from *P. aeruginosa* from 8 isolates are resistant to antibiotics especially isolate no.5. Moreover, the two isolates of *A. baumannii* are resistant isolates. They were resistant to amikacin, ceftazidime, Levofloxacin, piperacillin/tazobactam, imipenem, Ciprofloxacin, piperacillin, meropenem, trimethoprim/sulfamethoxazole, cefotaxime and Gentamycin.

S. aureus isolates were found to be resistant to oxacillin, erythromycin, amikacin, Clindamycin gentamicin, Levofloxacin, Cefoxitin, Penicillin, tetracycline and Rifampin. The most resistant isolates were isolate no.1as in Table (2). Results are similar to Forson *et al.* [30] who reported that *S. aureus* was resistant to oxacillin, erythromycin, amikacin, and gentamicin. Although *Pseudomonas sp.* showed varying resistance levels to gentamicin, cotrimoxazole and ciprofloxacin, all the *Acinetobacter sp.* were resistant to most of the antibiotics used.

Bio-SeNPs were screened for antibacterial activity of different pathogenic bacteria including *S. aureus*, *P. aeruginosa* isolates, *A. baumannii* and *K. pneumoniae*. Bio-SeNPs exhibited antimicrobial potential against G^+ and G^- bacteria (Fig. 8 and 9) except one isolate (No.5) of *P. aeruginosa*. In agreement of our study, Guisbiers *et al.* [32] reported that Bio-SeNPs have antibacterial activities against both *E. coli* and *S. aureus* bacteria.

Dagmar *et al.* [33] reported the antimicrobial activities of Bio-SeNPs against surface wound infections caused by *Staphylococcus*
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sp., *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, or family Enterobacteriaceae. Moreover, Nguyen *et al.* [34] elucidated that the effect of Bio-SeNPs on food-borne pathogens was bacteriostatic, not bactericidal. They noticed irregular shapes of bacterial cells and shrinkage after treatment of the bacterial cells with Bio-SeNPs.

The probable mechanism for antibacterial activity of metal nanoparticles is electrostatic attraction between the positively charged nanoparticles and negatively charged bacteria. During this attraction, the bacteria get oxidized and die immediately [35]. Mostly, the ions released from nanoparticles react with –SH groups of the proteins existing on the bacterial cell surface which cause the cell decay [36].

Antioxidant activity

Oxidative stress considered the major cause of the propagation of several serious diseases such

as alzheimer and cancer [37]. Researchers have focused their interest on preparing new natural antioxidants which are very potential to manage the oxidative stress and prevent initiation of ailments propagation. Recently, Bio-SeNPs has drawn big attention not only for their antitumor activity but also for their antioxidant potential.

The antioxidant activity of Bio-SeNPs was assessed in terms of DPPH and ABTS scavenging assay by using Trolox as a positive control as presented in Figure (10). The results indicate that Bio-SeNPs had potently active DPPH and ABTS radical scavenging activity (1.362 ± 0.09 and 5.656 ± 0.12 mmol trolox/g), our results coincide with that reported by Cheng *et al.* [38], while our results showed higher antioxidant activity than those reported by Forootanfar *et al.* [39], The reason can be assigned to the different size of Bio-SeNPs was smaller in our study. In general, the smaller size of NPs had stronger antioxidant potential [40, 41].

TABLE S1. Biochemical test for Gram-negative Bacteria.

Gram-stain	TSI	Motility	Urease	Oxidase test	Indole production	Methyl Red	Voges Proskauer	Citrate utilization	Hemolytic Activity	Presumptive Identification	Total no. of Isolates
-Coccobacillus	K/K	Non-Motile	-	-	-	-	-	+	-	<i>Acinetobacter baumannii</i>	2
-Bacilli	A/A	Motile	+	-	-	-	+	+	β Hemolysis	<i>Escherichia coli</i>	1
-Bacilli	A/A	Non-Motile	+	-	-	-	+	+	γ -Hemolysis	<i>Klebsiella pneumoniae</i>	3
-Bacilli	K/A H ₂ S	Motile	+	-	+	+	-	-	γ -Hemolysis	<i>Proteus vulgaris</i>	1
-Bacilli	K/K	Motile	-	+	-	-	-	+	-	<i>Pseudomonas aeruginosa</i>	8

TABLE 1. Antimicrobial Susceptibility test for gram-negative bacteria.

Bacteria Isolates	Diameter of inhibition zones for different Antibiotics (mm) against <i>P. aeruginosa</i>													
	IPM	TZP	ATM	PRL	CAZ	CIP	TOB	LEV	MEM	CN	FEB	AK		
<i>P. aeruginosa</i> (1)	34 (S)	31 (S)	29 (S)	27 (S)	21 (S)	25 (S)	22 (S)	24 (S)	28 (S)	19 (S)	32 (S)	24 (S)		
<i>P. aeruginosa</i> (2)	34 (S)	32 (S)	26 (S)	28 (S)	24 (S)	21 (S)	27 (S)	19 (S)	19 (S)	25 (S)	23 (S)	18 (S)		
<i>P. aeruginosa</i> (3)	10 (R)	27 (S)	0 (R)	26 (S)	0 (R)	0 (R)	21 (S)	0 (R)	11 (R)	11 (R)	0 (R)	19 (S)		
<i>P. aeruginosa</i> (4)	10 (R)	21 (S)	22 (S)	20 (S)	24 (S)	9 (R)	8 (R)	8 (R)	11 (R)	0 (R)	21 (S)	8 (R)		
<i>P. aeruginosa</i> (5)	11 (R)	13 (R)	0 (R)	13 (R)	0 (R)	0 (R)	0 (R)	13 (R)	11 (R)	0 (R)	0 (R)	10 (R)		
<i>P. aeruginosa</i> (6)	16 (I)	31 (S)	23 (S)	27 (S)	20 (S)	23 (S)	17 (I)	20 (S)	19 (S)	17 (S)	28 (S)	21 (S)		
<i>P. aeruginosa</i> (7)	13 (R)	21 (S)	19 (I)	18 (I)	20 (S)	27 (S)	20 (S)	21 (S)	0 (R)	16 (S)	19 (S)	16 (R)		
<i>P. aeruginosa</i> (8)	21 (S)	25 (S)	10 (R)	17 (I)	20 (S)	30 (S)	13 (I)	28 (S)	23 (S)	15 (S)	20 (S)	18 (S)		
Diameter of inhibition zones for different Antibiotics (mm) against <i>Enterobacteriaceae</i>														
Bacteria Isolates	IPM	TZP	AMP	PRL	CAZ	CIP	SXT	AMC	MEM	CN	FEB	AK		
<i>P. vulgaris</i>	23 (S)	29 (S)	23 (S)	29 (S)	29 (S)	21 (S)	20 (S)	25 (S)	25 (S)	22 (S)	32 (S)	21 (S)		
<i>E. coli</i>	21 (I)	21 (S)	19 (S)	31 (S)	29 (S)	31 (S)	22 (S)	21 (S)	24 (S)	19 (S)	35 (S)	21 (S)		
<i>K. pneumoniae</i> (1)	10 (R)	11 (R)	0 (R)	8 (R)	20 (I)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	11 (R)	8 (R)		
<i>K. pneumoniae</i> (2)	11 (R)	13 (R)	0 (R)	9 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	7 (R)	7 (R)	0 (R)		
<i>K. pneumoniae</i> (3)	24 (S)	24 (S)	15 (S)	24 (S)	25 (S)	25 (S)	18 (S)	24 (S)	21 (I)	20 (S)	30 (S)	20 (S)		
Diameter of inhibition zones for different Antibiotics (mm) against <i>A. baumannii</i>														
Bacteria Isolates	IPM	TZP	CTX	PRL	CAZ	CIP	SXT	LEV	MEM	CN	AK			
<i>A. baumannii</i> (1)	15 (R)	11 (I)	0 (R)	11 (R)	8 (R)	0 (R)	0 (R)	12 (R)	12 (R)	0 (R)	14 (R)			
<i>A. baumannii</i> (2)	15 (R)	10 (R)	0 (R)	11 (R)	7 (R)	0 (R)	0 (R)	13 (R)	12 (R)	0 (R)	13 (R)			

(AK) amikacin, (CAZ) ceftazidime, (LEV)Levofloxacin, (TZP) piperacillin/azobactam, (IPM) imipenem, (CIP) Ciprofloxacin, (ATM) Aztreonam, (PRL)piperacillin, (AMP) Ampicillin, (FEB) Cefepime, (AMC) Amoxicillin/clavulanic acid, (MEM)meropenem, (SXT) trimethoprim/sulfamethoxazole, (CTX) cefotaxime and (CN)Gentamycin. (R) Resistant, (I) Intermediate and (S) sensitive.

TABLE 2. Antimicrobial Susceptibility test for *S. aureus*.

Bacteria Isolates	Diameter of inhibition zones for different Antibiotics (mm)											
	OX	LZD	DA	TEC	E	LEV	CN	P	FOX	RD	SXT	TE
<i>S. aureus</i> (1)	0 (R)	30 (S)	0 (R)	15 (R)	0 (R)	10 (R)	0 (R)	0 (R)	0 (R)	16 (R)	14 (I)	0 (R)
<i>S. aureus</i> (2)	0 (R)	30 (S)	0 (R)	13 (I)	0 (R)	10 (R)	0 (R)	0 (R)	0 (R)	14 (R)	20 (S)	0 (R)
<i>S. aureus</i> (3)	0 (R)	30 (S)	0 (R)	14 (S)	0 (R)	11 (R)	0 (R)	0 (R)	0 (R)	15 (R)	21 (S)	0 (R)
<i>S. aureus</i> (4)	0 (R)	31 (S)	0 (R)	10 (R)	0 (R)	7 (R)	0 (R)	0 (R)	0 (R)	16 (R)	26 (S)	0 (R)
<i>S. aureus</i> (5)	0 (R)	28 (S)	0 (R)	13 (I)	0 (R)	7 (R)	0 (R)	0 (R)	0 (R)	14 (R)	17 (S)	0 (R)

(OX) Oxacillin, (P) Penicillin, (LZD) Linezolid, (DA) Clindamycin, (E) Erythromycin, (LEV) Levofloxacin, (SXT) trimethoprim/sulfamethoxazole, (RD) Rifampin, (TEC) Teicoplanin, (FOX) cefoxitin and (CN) Gentamycin. (R) Resistant, (I) Intermediate and (S) sensitive.

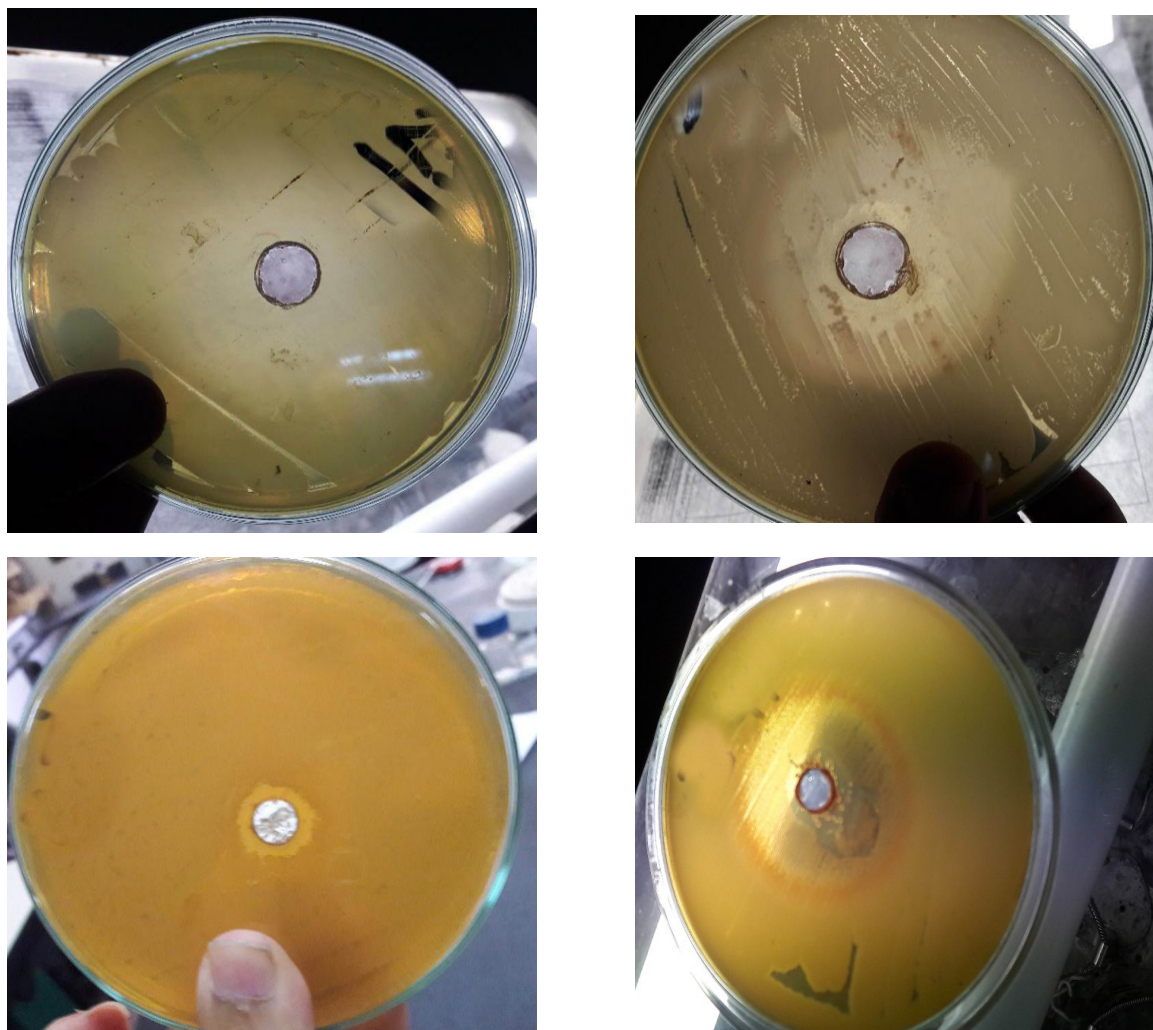


Fig. 8. Antibacterial activities of Bio-SeNPs against different pathogenic bacteria. 1- *Pseudomonas aeruginosa* (1), 2- *Acinetobacter baumannii* (2), 3- *Pseudomonas aeruginosa* (3), and 4- *Staphylococcus aureus* (4).

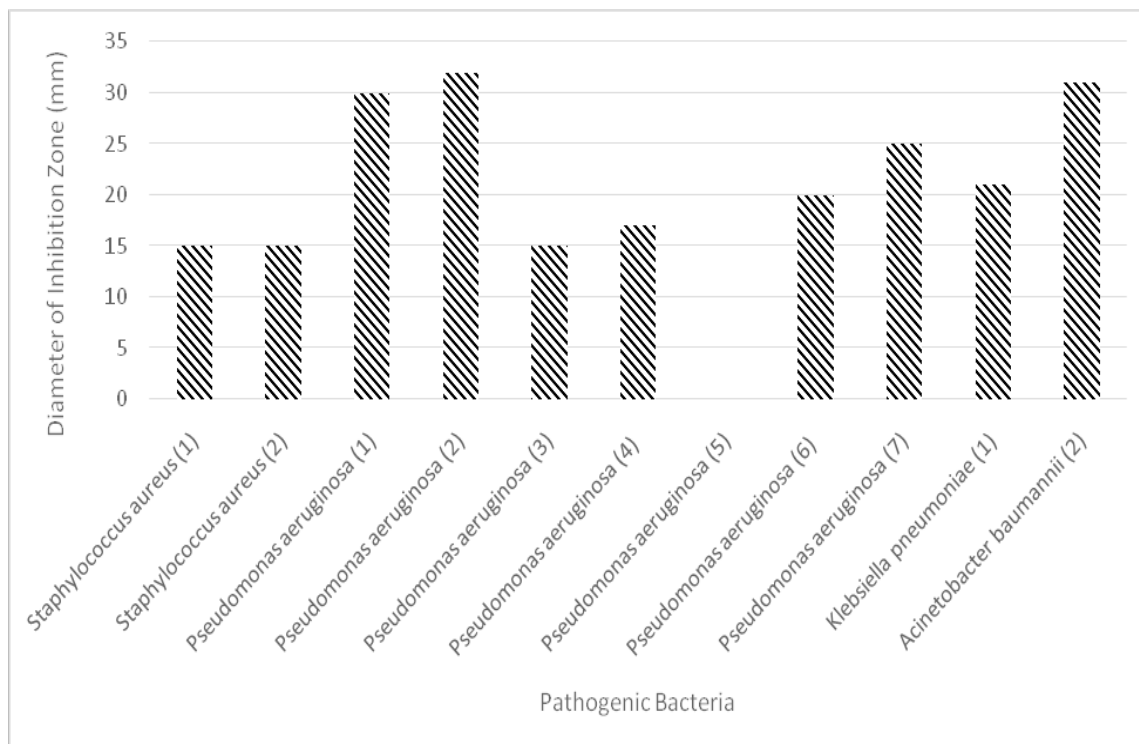


Fig. 9. Effect of Bio SeNPs on different pathogenic bacteria.

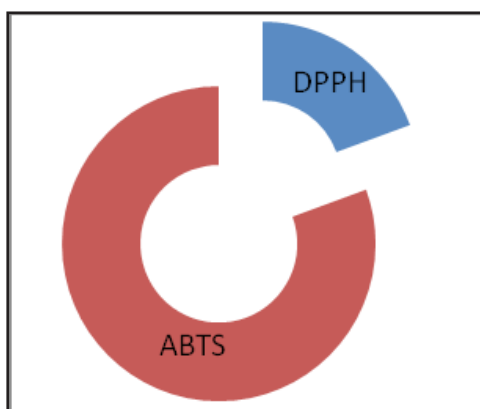


Fig. 10. Antioxidant capacity of Bio-SeNPs.

SeNPs showed had higher ABTS scavenging potential at different levels than DPPH. This feature was due to the effect of the high-water solubility of NPs, which led to the separation of the Se nanoparticle-rich water phase from the free radical rich lipid phase and thus reduced the ability of Se to capture the free radicals.

Unlike DPPH, the high ABTS radical scavenging ability of SeNPs relies on the increase of interaction probability and electron density [42], the DPPH scavenge ability of SeNPs is related to electron-based reaction rather than hydrogen transfer based reaction.

Anticancer activity of Biosynthesized SeNPs (Bio-SeNPs)

Due to Cancer complexity, an effective anticancer drug is essential to be specific for cancer cells. Generally, anticancer drugs showed some side effects and great demands to develop drugs soluble in water have no side effects. However, still, these are major bottlenecks of cancer therapy. To overcome these obstacles biodegradable carrier systems could be considered as a potential solution, Bio-SeNPs have been in the spotlight due to their anticancer activity. At this context, we have studied the antitumor potential of Bio-SeNPs against different cancer cell lines.

Bio-SeNPs showed toxicity to colon, skin and lung cancer (IC_{50} 10.24, 13.27 and 20.44 $\mu\text{g/ml}$). In the same time, it showed no cytotoxic effects on normal liver cells and weak toxicity on normal kidney cells. Therefore, Bio-SeNPs are effective selective anticancer agents with low cytotoxicity to normal cells, (Fig. 11). Thus, the use of Bio-SeNPs as anticancer agent may minimize the damage of chemotherapy by selective delivery to cancer cells without affecting human cells.

Bio-SeNPs can be internalized by cancer cells through endocytosis, which induces apoptotic cancer cell death [43], it is also capable of conjugating with siRNA to achieve enhanced anticancer activity [44]. On the other hand, there is a certain relationship between SeNPs and cell cycle life [45].

Compared to other nanoparticles, SeNPs are superior in many aspects' lower cytotoxicity and antigenicity but higher biocompatibility, cellular uptake, biodistribution and a high degree of biodegradability. Because Selenium is degradable in vivo and can be used as a nutrient for many kinds of normal cells or as an antiproliferative agent for many kinds of cancer cells [46].

Conclusion

In Summary, we have shown high antimicrobial potential of Bio-SeNPs against continuous

resistance of pathogenic bacteria which is the cause of severe burn infections. Bio-SeNPs exhibited antimicrobial potential against G^+ and G^- bacteria except one isolate of *P. aeruginosa*. Bio-SeNPs also had potently active DPPH and ABTS radical scavenging activity (1.362 ± 0.09 and 5.656 ± 0.12 mmol trolox/g). Bio-SeNPs showed anticancer potential toward colon, skin and lung cancer (IC_{50} 10.24, 13.27 and 20.44 $\mu\text{g/ml}$). At the same time, it showed no cytotoxic effects on normal liver cells and weak toxicity on normal kidney cells. Therefore, Bio-SeNPs are effective selective anticancer agents with low cytotoxicity to normal cells. Thus, the use of Bio-SeNPs as anticancer agent may minimize the damage of chemotherapy by selective delivery to cancer cells without affecting human cells. This is a positive factor to encourage the use of biogenic SeNPs as nutraceuticals and as additives in food items.

Acknowledgements

We acknowledge Dr. Amr Basha in Microbiology Lab, NODCAR, Egypt for his help in this study.

Conflict of interest

There is no Conflict of interest to declare.

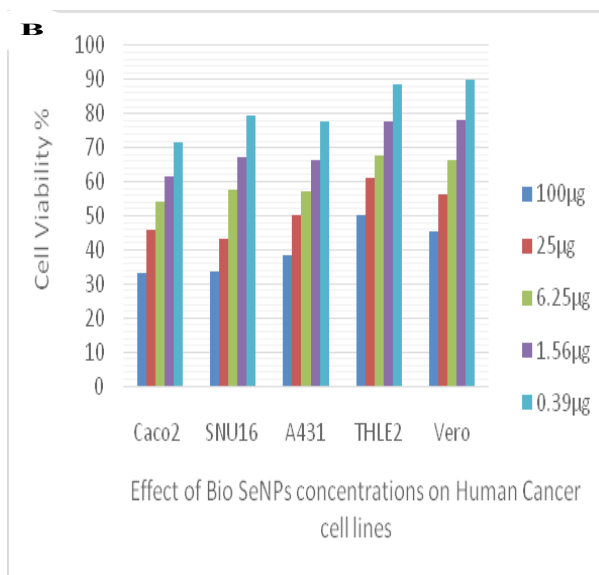
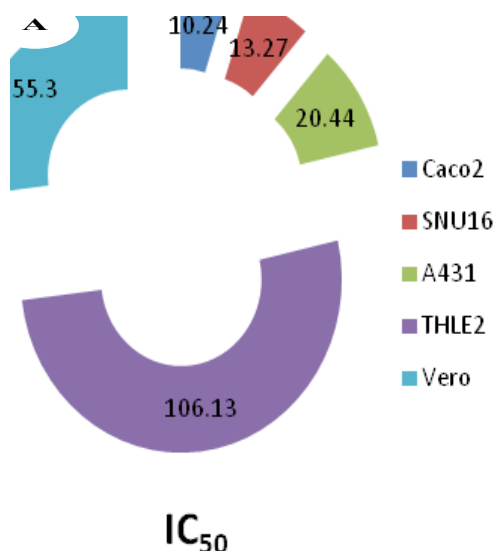


Fig. 11. IC_{50} values (A). Different cell line viability after 24h incubation of different concentration of Bio-SeNPs (0.39, 1.56, 6.25, 100 $\mu\text{g/ml}$) (B).

References

1. Patil, P., Joshi, S. and Bharadwaj, R., Aerobic bacterial infections in a burns unit of Sassoon General Hospital, Pune. *Int J Healthcare Biomed Res*, **3** (3), 106-112(2015).
2. Coban, Y.K., Infection control in severely burned patients. *World journal of critical care medicine*, **1**(4), 94 (2012).
3. Rai, M., Ingle, A.P., Birla, S., Yadav, A. and Santos, C.A.D., Strategic role of selected noble metal nanoparticles in medicine. *Critical reviews in microbiology*, **42**(5), 696-719 (2016).
4. Shakibaie, M., Khorramizadeh, M.R., Faramarzi, M.A., Sabzevari, O. and Shahverdi, A.R., Biosynthesis and recovery of selenium nanoparticles and the effects on matrix metalloproteinase-2 expression. *Biotechnology and applied biochemistry*, **56**(1), 7-15 (2010).
5. Hamadneh, I., Alhayek, H., Al-Mobydeen, A., Abu Jaber, A., Albuqain, R., Alstori, S. and Al Dujaili, A., Green Synthesis and Characterization of Yttrium Oxide, Copper Oxide and Barium Carbonate Nanoparticles Using *Azadirachta Indica* (the Neem Tree) Fruit Aqueous Extract. *Egyptian Journal of Chemistry*, **62**(4), 973-981(2019).
6. El-Gengaihi, S. E., Hamed, M. A., Aboubaker, D. H., and Abdel-tawab, H. M. Flavonoids from sugar beet leaves as hepatoprotective agent. *Int. J. Pharm., Sci*, **8**, 281-286(2016).
7. El-Gengaihi, S., Mossa, A.T.H., Refaie, A.A. and Aboubaker, D., Hepatoprotective efficacy of *Cichorium intybus* L. extract against carbon tetrachloride-induced liver damage in rats. *Journal of dietary supplements*, **13**(5), 570-584(2016).
8. Ibrahim, E.A., Baker, D.H. and El-Baz, F.K., Anti-Inflammatory and Antioxidant Activities of Rhubarb Roots Extract. *Int J Pharm Sci Rev Res*, **17**, 93-99(2016).
9. Mossa, A.T.H., Ibrahim, F.M., Mohafrash, S.M., Abou Baker, D.H. and El Gengaihi, S., Protective effect of ethanolic extract of grape pomace against the adverse effects of cypermethrin on weanling female rats. *Evidence-Based Complementary and Alternative Medicine*, 1-10(2015).
10. Salam, M.A., Ibrahim, B.M., El-Batran, S.E., El-Gengaihi, S.E. and Baker, D.H., Study of the possible antihypertensive and hypolipidemic effects of an herbal mixture on l-name-induced hypertensive rats. *Asian J Pharm Clin Res*, **9**(5), 85-90(2016).
11. Saratale, R.G., Karuppusamy, I., Saratale, G.D., Pugazhendhi, A., Kumar, G., Park, Y., Ghodake, G.S., Bharagava, R.N., Banu, J.R. and Shin, H.S., A comprehensive review on green nanomaterials using biological systems: Recent perception and their future applications. *Colloids and Surfaces B: Biointerfaces*, **170**, 20-35 (2018).
12. Zhuang, C., Yao, D., Li, F., Zhang, K., Feng, Q. and Gan, Z., Study of micron-thick MgB₂ films on niobium substrates. *Superconductor Science and Technology*, **20**(3), 287(2007).
13. Ahsan, U., Kamran, Z., Raza, I., Ahmad, S., Babar, W., Riaz, M.H. and Iqbal, Z., Role of selenium in male reproduction—A review. *Animal reproduction science*, **146**(1-2), 55-62(2014).
14. Messarah, M., Klibet, F., Boumendjel, A., Abdennour, C., Bouzerna, N., Boulakoud, M.S. and El Feki, A., Hepatoprotective role and antioxidant capacity of selenium on arsenic-induced liver injury in rats. *Experimental and toxicologic pathology*, **64**(3), pp.167-174(2012).
15. Schomburg, L., Dietary selenium and human health. *Nutrients*, **9**(1), 22(2017).
16. Ziedan EH, Farrag ES and Mahmoud SY First Report: Pathological Potential of Fungi on *Moringa Oleifera* Lam in Egypt. *International Journal of PharmTech Research* **9**(8):31-35(2016).
17. Nelson, P.E., Toussoun, T.A. and Marasas, W.F.O., *Fusarium species: an illustrated manual for identification*. The Pennsylvania Univ, USA, **189**, 13(1983).
18. Barnett, H.L. and Hunter, B.B., *Illustrated genera of imperfect fungi*. 4th Edition APS Press st. Paul. Minnesota, USA, **218**(1998).
19. Sarkar, J., Dey, P., Saha, S. and Acharya, K., Mycosynthesis of selenium nanoparticles. *Micro & nano letters*, **6** (8), 599-602(2011).
20. CLSI. National Committee for Clinical Laboratory Standards (US), *Performance standards for antimicrobial disk susceptibility tests: approved standards*. National Committee for Clinical Laboratory Standards, (2018).
21. Thomas, R., Viswan, A., Mathew, J. and Radhakrishnan, E.K., Evaluation of antibacterial activity of silver nanoparticles synthesized by a novel strain of marine *Pseudomonas* sp, *Nano Biomed. Eng*, **4**(3), 139-143(2012).
22. Hwang, E.S. and Do Thi, N., Effects of extraction

- and processing methods on antioxidant compound contents and radical scavenging activities of laver (*Porphyra tenera*). *Preventive nutrition and food science*, **19**(1), 40(2014).
23. El-Menshawi, B.S., Fayad, W., Mahmoud, K., El-Hallouty, S.M. and El-Manawaty, M., Screening of natural products for therapeutic activity against solid tumors. *IJEB*, **48**, 258-264(2010).
 24. Gangadoo, S., Stanley, D., Hughes, R.J., Moore, R.J. and Chapman, J., The synthesis and characterisation of highly stable and reproducible selenium nanoparticles. *Inorganic and Nano-Metal Chemistry*, **47**(11), 1568-1576(2017).
 25. Zhang, S.Y., Zhang, J., Wang, H.Y. and Chen, H.Y., Synthesis of selenium nanoparticles in the presence of polysaccharides. *Materials Letters*, **58**(21), 2590-2594(2004).
 26. Masarudin, M.J., Cutts, S.M., Evison, B.J., Phillips, D.R. and Pigram, P.J., Factors determining the stability, size distribution, and cellular accumulation of small, monodisperse chitosan nanoparticles as candidate vectors for anticancer drug delivery: application to the passive encapsulation of [14C]-doxorubicin. *Nanotechnology: science and applications*, **8**, 67(2015).
 27. Oremland, R.S., Herbel, M.J., Blum, J.S., Langley, S., Beveridge, T.J., Ajayan, P.M., Sutto, T., Ellis, A.V. and Curran, S., Structural and spectral features of selenium nanospheres produced by Se-respiring bacteria. *Appl. Environ. Microbiol.*, **70**(1), 52-60(2004).
 28. Fesharaki, P.J., Nazari, P., Shakibaie, M., Rezaie, S., Banoee, M., Abdollahi, M. and Shahverdi, A.R., Biosynthesis of selenium nanoparticles using *Klebsiella pneumoniae* and their recovery by a simple sterilization process. *Brazilian Journal of Microbiology*, **41**(2), 461-466(2010).
 29. Yadav, V., Sharma, N., Prakash, R., Raina, K.K., Bharadwaj, L.M. and Prakash, N.T., Generation of selenium containing nano-structures by soil bacterium, *Pseudomonas aeruginosa*. *Biotechnology*, **7**(2), 299-304(2008).
 30. Forson, O.A., Ayanka, E., Olu-Taiwo, M., Pappoe-Ashong, P.J. and Ayeh-Kumi, P.J., Bacterial infections in burn wound patients at a tertiary teaching hospital in Accra, Ghana. *Annals of burns and fire disasters*, **30**(2), 116(2017).
 31. Lakshmi, N., Ramalakshmi, K., Perala, K.B. and Jayalaxmi, M., Bacteriological profile and antibiogram of burn wound infections in a tertiary care hospital. *Journal of Dental and Medical Sciences*, **14**(10), 1-4(2015).
 32. Guisbiers, G., Wang, Q., Khachatryan, E., Mimum, L.C., Mendoza-Cruz, R., Larese-Casanova, P., Webster, T.J. and Nash, K.L., Inhibition of *E. coli* and *S. aureus* with selenium nanoparticles synthesized by pulsed laser ablation in deionized water. *International journal of nanomedicine*, **11**, 3731(2016).
 33. Dagmar, H.E., Kristyna, C.I., Pavel, K.O., Vojtech, A.D. and Rene, K.I., Selenium nanoparticles and evaluation of their antimicrobial activity on bacterial isolates obtained from clinical specimens. *Nanocon. Oct 14th-16th* (2015).
 34. Nguyen, T.H., Vardhanabhuti, B., Lin, M. and Mustapha, A., Antibacterial properties of selenium nanoparticles and their toxicity to Caco-2 cells. *Food Control*, **77**, 17-24(2017).
 35. Rezaei-Zarchi, S., Javed, A., Javeed Ghani, M., Soufian, S., Barzegari Firouzabadi, F., Bayanduri Moghaddam, A. and Mirjalili, S.H., Comparative study of antimicrobial activities of TiO₂ and CdO nanoparticles against the pathogenic strain of *Escherichia coli*. *Iranian Journal of Pathology*, **5**(2), 83-89(2010).
 36. Zhang, H. and Chen, G., Potent antibacterial activities of Ag/TiO₂ nanocomposite powders synthesized by a one-pot sol-gel method. *Environmental science & technology*, **43**(8), 2905-2910(2009).
 37. van der Vliet, A., Janssen-Heininger, Y.M. and Anathy, V., Oxidative stress in chronic lung disease: From mitochondrial dysfunction to dysregulated redox signaling. *Molecular aspects of medicine*, (2018).
 38. Cheng, Y., Xiao, X., Li, X., Song, D., Lu, Z., Wang, F. and Wang, Y., Characterization, antioxidant property and cytoprotection of exopolysaccharide-capped elemental selenium particles synthesized by *Bacillus paralicheniformis* SR14. *Carbohydrate polymers*, **178**, 18-26(2017).
 39. Forootanfar, H., Adeli-Sardou, M., Nikkhoo, M., Mehrabani, M., Amir-Heidari, B., Shahverdi, A.R. and Shakibaie, M., Antioxidant and cytotoxic effect of biologically synthesized selenium nanoparticles in comparison to selenium dioxide. *Journal of Trace Elements in Medicine and Biology*, **28**(1), 75-79(2014).
- Egypt. J. Chem.* Vol. **63**, No. 4 (2020)

40. Huang, B., Zhang, J., Hou, J. and Chen, C., Free radical scavenging efficiency of Nano-Se *in vitro*. *Free Radical Biology and Medicine*, **35**(7), 805-813(2003).
41. Huang, Z., Guo, B.J., Wong, R.N.S. and Jiang, Y., Characterization and antioxidant activity of selenium-containing phycocyanin isolated from *Spirulina platensis*. *Food Chemistry*, **100**(3), 1137-1143(2007).
42. Chen, W., Li, Y., Yang, S., Yue, L., Jiang, Q. and Xia, W., Synthesis and antioxidant properties of chitosan and carboxymethyl chitosan-stabilized selenium nanoparticles. *Carbohydrate polymers*, **132**, 574-581(2015).
43. Morry, J., Ngamcherdtrakul, W., Gu, S., Goodyear, S.M., Castro, D.J., Reda, M.M., Sangvanich, T. and Yantasee, W., Dermal delivery of HSP47 siRNA with NOX4-modulating mesoporous silica-based nanoparticles for treating fibrosis. *Biomaterials*, **66**, 41-52(2015).
44. Li, Y., Lin, Z., Zhao, M., Xu, T., Wang, C., Xia, H., Wang, H. and Zhu, B., Multifunctional selenium nanoparticles as carriers of HSP70 siRNA to induce apoptosis of HepG2 cells. *International journal of nanomedicine*, **11**, 3065(2016).
45. Wang, C., Zhang, Y., Guo, K., Wang, N., Jin, H., Liu, Y. and Qin, W., Heat shock proteins in hepatocellular carcinoma: Molecular mechanism and therapeutic potential. *International journal of cancer*, **138**(8), 1824-1834(2016).
46. Ahmad, M.S., Yasser, M.M., Sholkamy, E.N., Ali, A.M. and Mehanni, M.M., Anticancer activity of biostabilized selenium nanorods synthesized by *Streptomyces bikiniensis* strain Ess_amA-1. *International journal of nanomedicine*, **10**, 3389(2015).