

RESEARCH ARTICLE

Eman Abdullah M. Ali

Synthesis, characterization and biological activities of copper nanoparticles

ABSTRACT:

Nanotechnology is the most advanced research area in the present era. Copper nanoparticles displayed a significant role in many biological fields. The aim of the present study was to synthesize, characterize and study the biological activities of copper nanoparticles. Copper nanoparticles were produced from copper nitrate by chemical reduction method using isopropyl alcohol and cetyl trimethylammonium bromide (CTAB). Synthesized particles were characterized by fourier transform infrared (FTIR) spectrophotometer and transmission electron microscopy (TEM). Spherical particles were produced with size range 5-13 nm. Antimicrobial activities of both copper nanoparticles and copper nitrate precursor were assayed against *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans*, *Penicillium expansum*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Streptococcus mutans*, *Escherichia coli*, *Neisseria gonorrhoeae* and *Pseudomonas aeruginosa*. Both copper nanoparticles and copper nitrate precursor exhibited higher antibacterial activities than antifungal activities. Copper nanoparticles and copper nitrate precursor showed antioxidant activity relatively close to each other, with efficient antioxidant activity reached to 95.42% at 500 µg/ml of copper nanoparticles. Copper nanoparticles revealed higher significant cytotoxic effects than copper nitrate precursor. In conclusion, synthesis of copper nanoparticles by using chemical reduction method was simple, inexpensive and fast. Copper nanoparticles exhibited significant biological activities.

KEY WORDS:

Copper nanoparticles, FTIR, TEM, Antimicrobial activity, Antioxidant activity, Cytotoxicity.

CORRESPONDENCE:

Eman Abdullah M. Ali

Botany and Microbiology Department, Faculty of Science, Cairo University, 12613, Giza, Egypt.

E-mail: emanali@sci.cu.edu.eg

ORCID: <https://orcid.org/0000-0001-9595-8323>

ARTICLE CODE: 37.02.19

ISSN: 1687-7497

Online ISSN: 2090 - 0503

<https://www.ejmanager.com/my/ejeb>

INTRODUCTION:

Nanotechnology is one of the most important technologies in the current century. It includes the ability to modify the molecule structure at atomic level at nanometer scale within range of 1 to 100 nm. Changing to the nanodimensional level results in changing in the fundamental properties of a substance because of the display of the so-called "quantum dimensional effects". Metallic nanoparticles have high thermal conductivity and high surface to volume ratio. Metallic nanoparticles are applied in many science areas as agriculture, biotechnology, chemistry, pharmaceuticals and medicine. Copper is one of the most important noble metals. Advantages of using copper nanoparticles (CuNPs) are their high electrical and thermal conductivity, low cost, easy availability and their catalytic properties (Gubin *et al.*, 2005; Saterlie *et al.*, 2011; Jain *et al.*, 2014; Kanhed *et al.*, 2014; Sorbiun *et al.*, 2018).

Numerous techniques have been used for copper nanoparticles production. Methods included chemical reduction (Zhang *et al.*, 2010), electrochemical reduction (Han *et al.*, 2006), thermal reduction (Salavati-Niasari *et al.*, 2009), mechano-chemical process (Sheibani *et al.*, 2008) and thermal decomposition (Salavati-Niasari and Davar, 2009). For synthesis of metallic nanoparticles, chemical reduction method is very suitable method because it is economic, simple and fast (Jain *et al.*, 2014; Kanhed *et al.*, 2014).

Microbial resistance to antibiotics makes it is necessary to find new alternative antimicrobial drugs (Baker-Austin *et al.*, 2006; Hajipour *et al.*, 2013). Metallic nanoparticles and their oxides are making a new class of antibacterial agents (Leid *et al.*, 2012).

Antioxidants are defined as the molecules that are able to prevent oxidation of other molecules. These molecules have very important role in treatment of various diseases. The role of these molecules is preventing the oxidative stresses and protecting cells by scavenging free radicals (Imran *et al.*, 2011). Copper nanoparticles showed Antioxidant activity against DPPH (Ghosh *et al.*, 2015).

Nanoparticles introduce a new view for discovery, protection and treatment of tumors. They are expected to improve cancer diagnosis and therapy (Zhu *et al.*, 2016; Yesilot and Aydin, 2019).

The aim of the present research was to synthesize, characterize and study the biological activities of copper nanoparticles.

MATERIAL AND METHODS:

Copper nanoparticles synthesis:

Copper(II) nitrate solution was prepared at a concentration of 0.003 M in isopropyl alcohol. Cetyl trimethylammonium bromide (CTAB) solution was also prepared in isopropyl alcohol at a concentration of 0.09 M. In Erlenmeyer flasks (250 ml), copper nitrate solution was added drop by drop to the CTAB solution with strong stirring by using a magnetic stir plate (Athawale *et al.*, 2005).

Characterization of the synthesized copper nanoparticles:

Both solutions of copper nitrate (metal precursor) and copper nanoparticles were analyzed using SHIMADZU fourier transform infrared (FTIR) spectrophotometer (Japan) in the range of 400 – 4000 cm^{-1} . Size and shape of synthesized copper nanoparticles were determined using transmission electron microscopic (TEM) analysis (JEOL-JEM-2100).

Assay of antimicrobial activity:

Antimicrobial activities of both copper nitrate and synthesized copper nanoparticles were assayed against some pathogenic microorganisms using well diffusion method (Hindler *et al.*, 1994). The well diameter was 6 mm contained 100 μl of the tested sample at concentration of 10 mg/ml. The tested fungal species were *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans* ATCC 10231 and *Penicillium expansum*. The tested Gram positive bacterial species were *Staphylococcus aureus* ATCC 25923, *Streptococcus faecalis* ATCC 19433 and *Streptococcus mutans* ATCC 25175. Gram negative bacterial species were *Escherichia coli* ATCC 25922, *Neisseria gonorrhoeae* ATCC 19424 and *Pseudomonas aeruginosa* ATCC 10145. Malt extract agar media was used for fungal tests, while Mueller-Hinton agar medium was used for bacterial tests. Fungal plates were incubated at 25°C for 3 days, while bacterial plates were incubated at 37°C for 24 hours. Ketoconazole was used as a standard for fungi, while gentamycin was used as bacterial standard. Standards were used as positive controls at concentration of 10 mg/ml, while dimethyl sulfoxide (DMSO) was used as a negative control. After incubation periods, the inhibition zones diameters were determined in millimeters.

Determination of Minimum Inhibitory Concentration (MIC):

The MIC values of synthesized copper nanoparticles were determined by well diffusion method (Hindler *et al.*, 1994) according to NCCLS recommendations (NCCLS, 1993). Bifold serial dilutions of copper nanoparticles were prepared using DMSO. Mueller-Hinton agar media were seeded by bacterial suspensions, while Malt extract agar media were seeded by fungal suspensions. Media were poured in plates and left to solidify. Wells of diameter 6 mm were made in plates using sterile cork borer. Each well contained 100 μl of each concentration of copper nanoparticles. Plates were incubated at 25°C for 3 days for fungal species, while at 37°C for 24 hours for bacterial species. MIC value was determined as the lowest concentration of copper nanoparticles that prevented the visible microbial growth after incubation period.

Antioxidant Assay:

Antioxidant activities of synthesized copper nanoparticles and its precursor copper nitrate were determined by using DPPH free radical scavenging assay (Yen and Chen, 1995). Freshly prepared solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was prepared in methanol (0.16 mM) and stored in the dark. Methanol solutions of the tested compounds and the standard ascorbic acid were prepared at different concentrations. Equal amounts of DPPH solution and each concentration of the tested compound were added to test tubes. Mixtures were vortexed for 1 min and incubated in the dark for 30 min at room temperature. DPPH solution without antioxidant was used as control. Absorbance measurements were determined by using a UV-visible spectrophotometer. The reduction in absorbance was determined at 517 nm. The percentage (%) of scavenging activity was determined as follow:

$$\text{DPPH scavenging \%} = \left[\frac{(A_c - A_t)}{A_c} \times 100 \right]$$

Where, A_c = Absorbance of the control and A_t = absorbance of the sample.

The IC_{50} (50% inhibitory concentration) values were determined from the curve plotted between the concentration and DPPH scavenging %. The IC_{50} was defined as the concentration required to inhibit DPPH radical by 50%.

In vitro cytotoxicity assay:

• Human cell lines:

Human breast cancer cell line (MCF-7) and human hepatocellular carcinoma cell line (HepG-2) were gotten from the American Type Culture Collection (ATCC, Rockville, MD).

• Propagation of cell lines:

The cells were propagated on RPMI-1640 medium (Lonza, Belgium) with 50 $\mu\text{g}/\text{ml}$

gentamycin and 10% inactivated fetal bovine serum (Lonza, Belgium). The cells were kept at 37°C in a humidified atmosphere with 5% CO₂. They were subcultured from 2 to 3 times per week.

- **Cytotoxicity assay:**

In Corning® 96-well tissue culture plates, the tumor cell lines were suspended in the medium at concentration of 5x10⁴ cell/well. Cells were incubated for 24 hr. Copper nanoparticles and copper nitrate solutions were then added into 96-well plates (3 replicates) at different concentrations. Media or DMSO (0.5%) was used as control. The numbers of viable cells were estimated after 24 hours incubation by the MTT test. Media were removed from plates and fresh culture RPMI 1640 medium (100 µl) was added without phenol red. Ten µl of MTT (Sigma, USA) stock solution (12 mM) was added to each well including controls. Plates were then incubated for 4 hours at 37°C and 5% CO₂. An aliquot of media (85 µl) was removed from wells, and replaced with DMSO (50 µl) and mixed carefully with pipette then incubated for 10 min at 37°C. Optical densities were determined with the microplate reader (SunRise, TECAN, Inc, USA) at 590 nm. Percentage of cell viability was determined as follow:

$$\text{Cell viability \%} = \left[\frac{A_T}{A_C} \right] \times 100\%.$$

Where, A_T = absorbance of the sample and A_C = Absorbance of the control.

A curve was plotted between sample concentrations versus percentages of cell

viability. IC₅₀ values were determined from the plotted curve which defined as the concentration needed to make toxic effects in intact cells by 50% (Mosmann, 1983).

- **Statistical analysis:**

All results were displayed as mean ± standard deviations (mean ± SD). Data were analyzed by one-way analysis of variance (ANOVAs) using SPSS 20 statistical software. The differences between mean values were analyzed at $p \leq 0.05$ with Duncan's multiple range test (Duncan, 1955).

RESULTS:

Synthesis of copper nanoparticles:

Synthesis of copper nanoparticles from copper nitrate solution was done by using chemical reduction method. Visual observation of the produced copper nanoparticles was primarily shown by formation of violet color solution.

Characterization of synthesized copper nanoparticles:

- **FTIR analysis:**

The FTIR spectrum of synthesized copper nanoparticles displayed 14 distinct peaks at 447.49, 640.37, 725.23, 910.40, 964.41, 1242.16, 1342.46, 1481.33, 1697.36, 2330.01, 2846.93, 2916.37, 3016.67, and 3394.72 cm⁻¹ (Fig. 1A). FTIR analysis of copper nitrate showed ten peaks at 594.08, 717.52, 825.53, 1049.28, 1342.46, 1411.89, 1620.21, 1766.80, 2075.41, and 2414.88 cm⁻¹ (Fig. 1B).

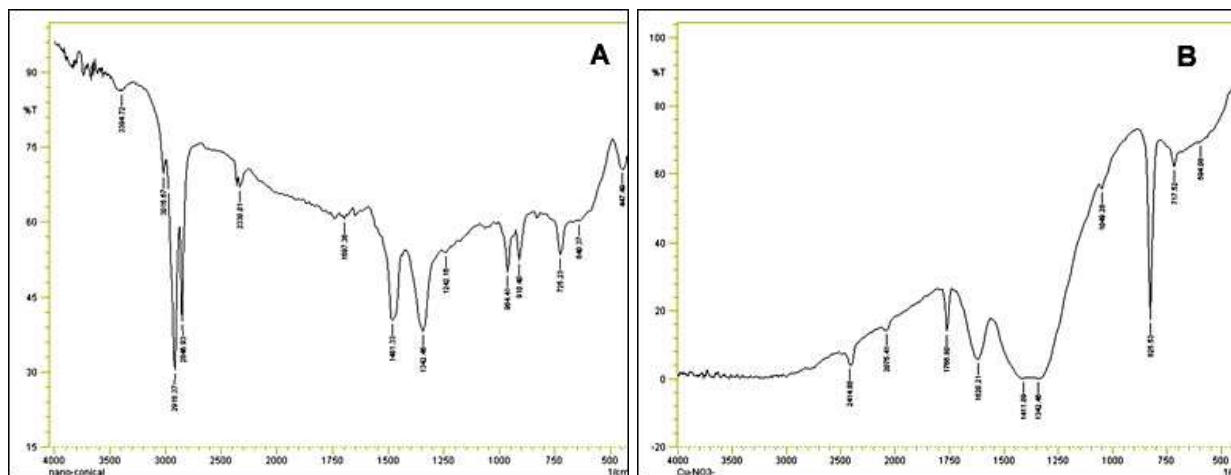


Fig. 1. FTIR spectra. (A) CuNPs, (B) Cu(NO₃)₂.

- **TEM analysis:**

Characterization using TEM analysis displayed spherical copper nanoparticles with size in the range of 5 – 13 nm (Fig. 2).

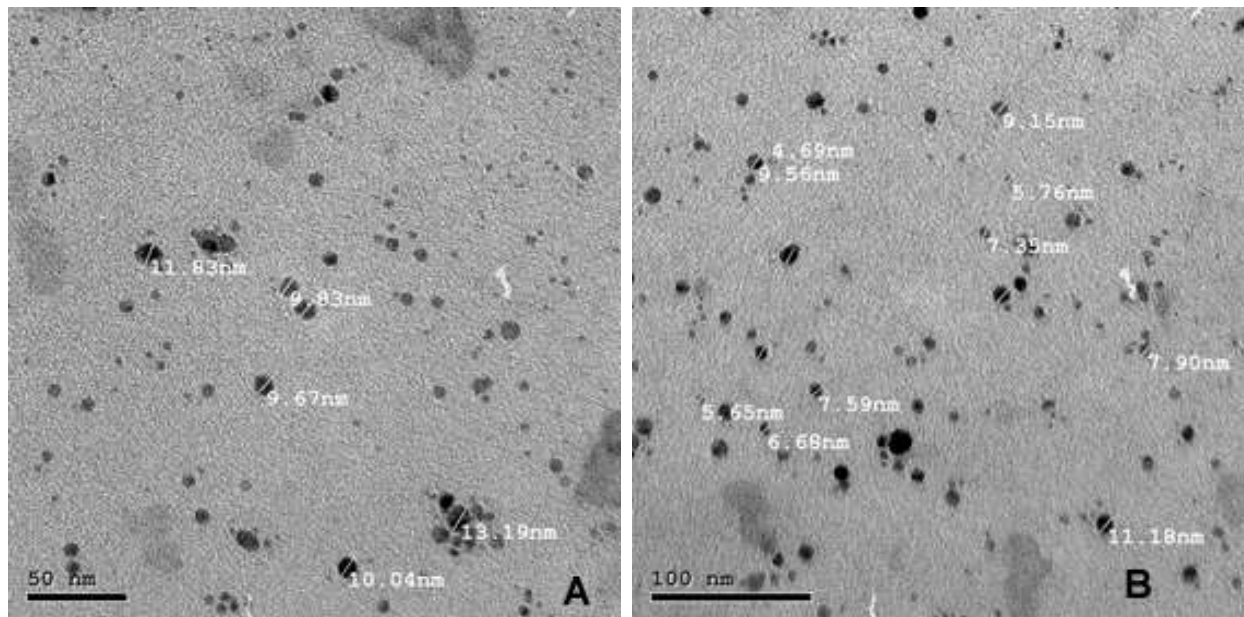


Fig. 2. TEM micrographs of copper nanoparticles. (A) 50 nm scale, (B) 100 nm scale.

Antimicrobial activity Assay and MIC determination:

Antimicrobial activities of both synthesized copper nanoparticles and copper nitrate precursor were assayed against some pathogenic microorganisms using well diffusion method. All tested bacterial species, either Gram positive or Gram negative, were sensitive to both CuNPs and $\text{Cu}(\text{NO}_3)_2$. *Aspergillus niger* was resistant to both tested materials, while *Aspergillus fumigatus* was resistant only to $\text{Cu}(\text{NO}_3)_2$ (Table 1). Copper nanoparticles and copper nitrate displayed higher antibacterial activities than antifungal properties. The synthesis of CuNPs from

copper nitrate precursor significantly enhanced the antimicrobial activity toward *Aspergillus flavus* and *Aspergillus fumigatus*, while no significant difference was observed against *Aspergillus niger*, *Candida albicans*, *Streptococcus faecalis* and *Pseudomonas aeruginosa*. Decreased antimicrobial activities of CuNPs compared to copper nitrate precursor was observed in other tested microorganism. Table 2 indicated that the highest MIC values of CuNPs were attained against *A. fumigatus* and *P. expansum* (78 $\mu\text{g}/\text{ml}$), while the least values were achieved toward *S. faecalis*, *S. mutans*, *N. gonorrhoeae* and *P. aeruginosa* (19.5 $\mu\text{g}/\text{ml}$).

Table 1. Antimicrobial activities of CuNPs, $\text{Cu}(\text{NO}_3)_2$ and antimicrobial standards.

Tested microorganisms	Inhibition zone diameter (mm)		
	CuNPs	$\text{Cu}(\text{NO}_3)_2$	Control
<u>Fungi</u>			<u>Ketoconazole</u>
<i>Aspergillus flavus</i>	15 ± 1 ^{de}	10 ± 0 ^b	20 ± 2 ⁱ
<i>Aspergillus fumigatus</i>	13 ± 0 ^c	0 ± 0 ^a	17 ± 1 ^{fg}
<i>Aspergillus niger</i>	0 ± 0 ^a	0 ± 0 ^a	15 ± 0 ^{de}
<i>Candida albicans</i>	14 ± 2 ^{cd}	14 ± 1 ^{cd}	20 ± 1 ⁱ
<i>Penicillium expansum</i>	13 ± 1 ^c	16 ± 1 ^{ef}	17 ± 1 ^{fg}
<u>Gram positive bacteria</u>			<u>Gentamycin</u>
<i>Staphylococcus aureus</i>	14 ± 1 ^{cd}	16 ± 0 ^{ef}	24 ± 1 ^j
<i>Streptococcus faecalis</i>	19 ± 1 ^{hi}	19 ± 2 ^{hi}	30 ± 2 ^l
<i>Streptococcus mutans</i>	16 ± 0 ^{ef}	20 ± 1 ⁱ	27 ± 1 ^k
<u>Gram negative bacteria</u>			<u>Gentamycin</u>
<i>Escherichia coli</i>	13 ± 0 ^c	14 ± 1 ^{cd}	30 ± 2 ^l
<i>Neisseria gonorrhoeae</i>	18 ± 2 ^{gh}	19 ± 2 ^{hi}	28 ± 2 ^k
<i>Pseudomonas aeruginosa</i>	17 ± 1 ^{fg}	17 ± 1 ^{fg}	31 ± 1 ^l

Values are mean of triplicate readings (mean ± SD).

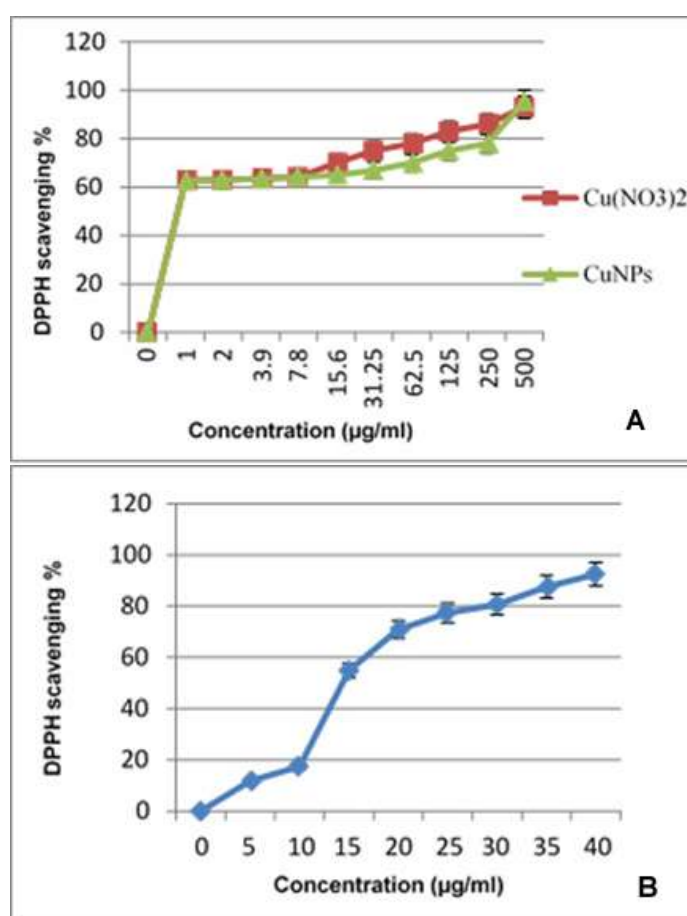
Mean values with different letters are significantly different at 5% level according to Duncan's multiple range test.

Table 2. Determination of MIC values of CuNPs

Microbial species	MIC value ($\mu\text{g/ml}$)
<i>Aspergillus flavus</i>	39
<i>Aspergillus fumigatus</i>	78
<i>Candida albicans</i>	39
<i>Penicillium expansum</i>	78
<i>Staphylococcus aureus</i>	39
<i>Streptococcus faecalis</i>	19.5
<i>Streptococcus mutans</i>	19.5
<i>Escherichia coli</i>	39
<i>Neisseria gonorrhoeae</i>	19.5
<i>Pseudomonas aeruginosa</i>	19.5

Antioxidant Assay:

The antioxidant activities of both CuNPs and $\text{Cu}(\text{NO}_3)_2$ were determined by bleaching of the purple colour of DPPH methanol solution (Fig. 3A). The DPPH scavenging percent of the standard ascorbic acid was also investigated (Fig. 3B). It was observed that no significant differences between antioxidant activities of both CuNPs and $\text{Cu}(\text{NO}_3)_2$ at lower concentrations ranged between 0.0 to 7.8 $\mu\text{g/ml}$. However, at higher concentrations from 15.6 to 250 $\mu\text{g/ml}$, the antioxidant activities of $\text{Cu}(\text{NO}_3)_2$ increased significantly than CuNPs. At concentration of 500 $\mu\text{g/ml}$, equal antioxidant activities were achieved. IC_{50} value was 3.40 $\mu\text{g/ml}$ for CuNPs, 3.13 $\mu\text{g/ml}$ for $\text{Cu}(\text{NO}_3)_2$ and 14.2 $\mu\text{g/ml}$ for the standard ascorbic acid.

Fig. 3. Antioxidant activities. (A) CuNPs and $\text{Cu}(\text{NO}_3)_2$, (B) Standard ascorbic acid.**In vitro cytotoxicity assay:**

The *in vitro* cytotoxicity of both CuNPs and $\text{Cu}(\text{NO}_3)_2$ was assayed against human breast cancer cell line (MCF-7) and human hepatocellular carcinoma cell line (HepG-2) by using MTT method. IC_{50} values were also determined. As shown in figure 4, CuNPs had higher significant cytotoxicity than $\text{Cu}(\text{NO}_3)_2$ against breast carcinoma cells with IC_{50} values of 5.88 and 27.9 $\mu\text{g/ml}$, respectively (Table 3). Moreover, higher significant cytotoxicity of CuNPs than $\text{Cu}(\text{NO}_3)_2$ was observed against hepatocellular carcinoma

cells (Fig. 5) with IC_{50} values of 3.94 and 9.63 $\mu\text{g/ml}$, respectively. In case of breast carcinoma cell line, the cytotoxicity of CuNPs was significantly higher than the medically used standard vinblastine at concentrations of 7.8-500 $\mu\text{g/ml}$. No significant difference between IC_{50} values of CuNPs and the standard vinblastine indicating the high efficacy of CuNPs against breast carcinoma cells. In hepatocellular carcinoma cell line, the same cytotoxicity of CuNPs and vinblastine was observed at concentration 31.25 $\mu\text{g/ml}$, while at higher concentrations

(62.5-500 $\mu\text{g/ml}$) CuNPs had higher significant cytotoxic effect than vinblastine. Briefly,

CuNPs exhibited efficient cytotoxicity against breast and liver cancers.

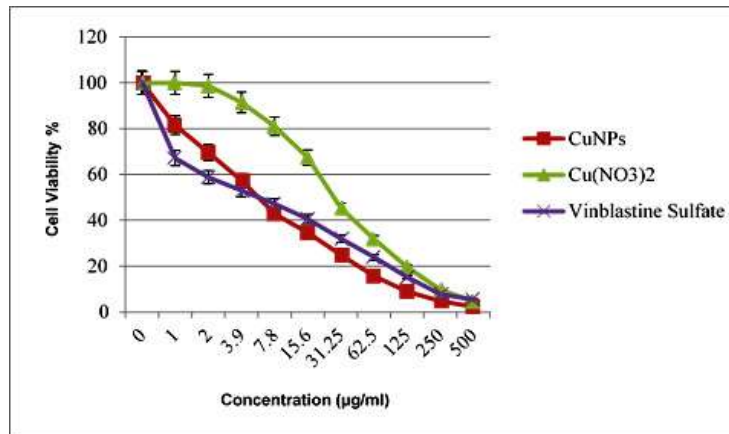


Fig. 4. *In vitro* cytotoxicity of CuNPs, $\text{Cu}(\text{NO}_3)_2$ and vinblastine against breast carcinoma cells (MCF-7) using MTT assay.

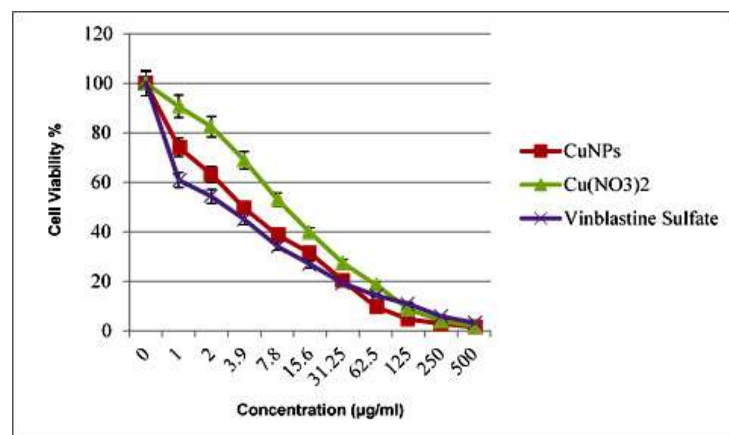


Fig. 5. *In vitro* cytotoxicity of CuNPs, $\text{Cu}(\text{NO}_3)_2$ and vinblastine against hepatocellular carcinoma cells (HepG-2) using MTT assay.

DISCUSSION:

Copper nanoparticles were synthesized from $\text{Cu}(\text{NO}_3)_2$ solution by reduction of Cu^{2+} ions to Cu^0 . Isopropyl alcohol was the reducing agent. CTAB catalyzes the reduction reaction with isopropyl alcohol. CTAB also surrounded the surface of copper nanoparticles as a capping agent. The long chain cetyl groups present in CTAB stabilize copper nanoparticles and prevent their aggregation (Kanhed *et al.*, 2014).

Liquid phase chemical reduction is the most common method for copper nanopowders synthesis due to its simple operation, low cost and good control of morphology and size (Tamilvanan *et al.*, 2014). It includes the precipitation of metallic copper nanoparticles in solution by chemical reduction of copper hydroxide, oxide or salt (Tan and Cheong, 2013). Reducing agents used for reduction of copper salt as CTAB (Wu and Chen, 2004), hydrazine (Saikova *et al.*, 2010) and ascorbic acid (Liu *et al.*, 2012).

Copper nanoparticles preparation suffers from some problems when compared with noble metals. They are highly sensitive to oxygen and be oxidized when exposed to air. Also, aggregation of colloidal particles takes place due to surface oxidation (Jain *et al.*, 2014; Tamilvanan *et al.*, 2014). To prevent this problem, surfactants are used to prevent oxidation (Pham *et al.*, 2012). Using of reducing and capping agents decreases particle-particle interaction and accumulation of colloidal particles (Kogiso *et al.*, 2002; Khanna *et al.*, 2007; Khanna *et al.*, 2008).

FTIR spectroscopy is used to estimate functional atoms on nanoparticles surface and chemical bonds in surface of atoms. It can be used also for characterization of physical properties and functions of nanoparticles (Morais *et al.*, 2006). FTIR analyses were done for both synthesized copper nanoparticles and $\text{Cu}(\text{NO}_3)_2$ solutions. Among the produced peaks, CuNPs displayed two peaks at 1342.46 and 1481.33 cm^{-1} . In

relation to the present work, Kanhed *et al.* (2014) found that FTIR analysis of CuNPs displayed two peaks at 1370 and 1473 cm^{-1} . These peaks also match with functional groups present in the capped CTAB. They reported that the peak position lies within range of ~ 1500 and 1300 cm^{-1} displayed C–H, CH₂ and CH₃ bending vibrations. Athawale *et al.* (2005) detected a general broadening without significant move in peak positions of CTAB and IPA when they were mixed. The peak may result from interaction of hydroxyl groups and ammonium with oxygen, which is an electron rich center having two lone electron pairs. Cu^{2+} ions may be reduced by the electron rich oxygen center and cetyl chain stabilizes the reduced copper nanoparticles.

FTIR analysis of biocapped synthesized copper nanoparticles displayed main peaks at 1382 cm^{-1} corresponding to C–N stretching vibration of the aromatic amine and at 2362 cm^{-1} corresponding to aldehydic C–H stretching (Yallappa *et al.*, 2013). Subbaiya and Selvam (2015) studied the FTIR spectra of copper nanoparticles. They found characteristic peaks at 1103 cm^{-1} (C–O stretch), 1381 cm^{-1} (N–O stretch), 1627 cm^{-1} (C=C stretch) and 3391 cm^{-1} (O–H stretch, H bonded). The wide absorption bands between 2800 cm^{-1} and 4000 cm^{-1} chiefly relayed to O–H and C–O groups on copper crystals nanostructure surface. The peak at 591 cm^{-1} indicated copper nanostructure formation.

Characterization using TEM analysis gives the real shape and size of the synthesized nanoparticles. In the current work, the synthesized copper nanoparticles were spherical with size ranged between 5 to 13 nm by TEM analysis. Kanhed *et al.* (2014) revealed that synthesized nanoparticles had spherical shape with size in the range of 3 – 10 nm. Tamilvanan *et al.* (2014) reported that the synthesized colloidal copper nanoparticles in ethylene glycol and water were spherical in shape using TEM. The average size of copper nanoparticles was in range of 15 ± 2 nm. They also explained copper nanoparticles synthesis by copper nitrate reduction in aqueous solution using hydrazine monohydrate reducer with silver nanoparticles as catalysts. They determined particle size using Scherrer equation which was found in 7 – 12 nm range.

In the current work, copper nanoparticles showed higher, equal or decreased antimicrobial activities related to $\text{Cu}(\text{NO}_3)_2$ with higher antibacterial activities than antifungal activities. Copper compounds were used as antimicrobial agents several years ago. The aim was to improve their activities, especially towards microbial resistance, by production of nanoparticles. Microbial sensitivity was found to vary depending on the microbial species.

Malandrakis *et al.* (2019) reported that CuNPs had higher antifungal activity than CuSO_4 toward tested species except for *Botrytis cinerea*, *Alternaria alternata* and *Monilinia fructicola* indicating a potential difference in the mode of action of these metals.

Nanoparticles displayed powerful antimicrobial activities and expected to be an alternative to antimicrobial agents (Martinez-Gutierrez *et al.*, 2010; Oves *et al.*, 2013). Copper nanoparticles have small dimensions and high surface-to-volume ratio, so they were employed as antimicrobial agents. They can also interact with other particles easily improving their antimicrobial efficiency. Copper nanoparticles are more reactive than other metallic nanoparticles. Metallic copper nanoparticles were found to be anti-infective agents instead of silver and other noble metal (Karthik and Geetha, 2013; Tamilvanan *et al.*, 2014). Ramyadevi *et al.* (2012) studied the antimicrobial activity of copper nanoparticles against some microbial species and found that the antibacterial activity was more than the antifungal activity.

Significant antifungal activity of copper nanoparticles was displayed against *Alternaria alternate*, *Curvularia lunata* and *Fusarium oxysporum* (Kanhed *et al.*, 2014). Copper nanoparticles displayed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Lee *et al.*, 2011; Tamilvanan *et al.*, 2014). Regarding to the antibacterial mechanism, electron microscope revealed the contact of copper nanoparticles on bacterial cell surface. This interaction leads to an increase in osmotic pressure, resulting in extraction of cytoplasmic components and cell wall fragments (Deryabin *et al.*, 2013).

The biological activity of nanoparticles is due to their small size (< 100 nm). This size is the same of cells, viruses, proteins, and DNA. So, nanoparticles can come near to a biological object, become compatible with it, then bind to it (Pershina *et al.*, 2008). Nanoparticles attached to cell membrane then penetrate into bacteria. Metallic nanoparticles, like CuNPs, interact with sulfur-containing proteins (present in bacterial membrane) and DNA. Nanoparticles attack the respiratory chain and cell division resulting in cell death (Priester *et al.*, 2009; Zain *et al.*, 2014; Sorbiun *et al.*, 2018).

CuNPs showed high antioxidant activity at concentrations of 1-500 $\mu\text{g/ml}$ with DPPH scavenging percent ranging from 62.54% to 95.42%. Ghosh *et al.* (2015) displayed that copper nanoparticles synthesized from *Dioscorea bulbifera* (a medicinal plant) showed 40.81% DPPH scavenging activity. Niramathi *et al.* (2013) reported that as copper nanoparticles concentration increased, the DPPH scavenging activity increased.

CuNPs exerted higher significant cytotoxic effects than Cu(NO₃)₂ precursor. It also exhibited higher significant cytotoxicity than the standard vinblastine against breast and liver cancers at the high concentrations. Fan *et al.* (2013) reported that metallic nanoparticles displayed a potential anticancer activity. Jose *et al.* (2011) found that copper nanoparticles had cytotoxic effect, by inducing apoptosis, towards U937 of human histiocytic lymphoma and HeLa cells of human cervical

cancer origins. Shobha *et al.* (2019) displayed that the morphology of cells of cancer lines MCF-7 altered significantly when treated with copper nanoparticles using MTT assay with IC₅₀ value of 1.71 µg ml⁻¹

In conclusion, copper nanoparticles can be synthesized easily from copper nitrate by using chemical reduction method. Copper nanoparticles exhibited efficient antimicrobial, antioxidant and cytotoxic effects.

REFERENCES:

- Athawale A, Katre PP, Kumar M, Majumdar MB. 2005. Synthesis of CTAB-IPA reduced copper nanoparticles. *Mater. Chem. Phys.*, 91(2-3): 507-512.
- Baker-Austin C, Wright MS, Stepanauskas R, Arthur JV. 2006. Co-selection of antibiotic and metal resistance. *Trends Microbiol.*, 14(4): 176-182.
- Deryabin DG, Aleshina ES, Vasil'chenko TD, Deryabina TD, Efremova LV, Korimov IF, Korolevskaya LB. 2013. Investigation of copper nanoparticles antibacterial mechanisms tested by luminescent *Escherichia coli* strains. *Nanotechnologies Russia*, 8(5-6): 402.
- Duncan DB. 1955. Multiple range and multiple F tests. *Biometrics*, 11(1): 1-42.
- Fan Y, Ma L, Fan B, Leng S. 2013. Cytotoxicity of gold, silver and copper nanoparticles and their applications. *Hans J. Nanotechnol.*, 3: 24-34.
- Ghosh S, More P, Nitnavare R, Jagtap S, Chippalkatti R, Derle A, Kitture R, Asok A, Kale S, Singh S, Shaikh ML, Ramanamurthy B, Bellare J, Chopade BA. 2015. Antidiabetic and antioxidant properties of copper nanoparticles synthesized by medicinal plant *Dioscorea bulbifera*. *J. Nanomed. Nanotechnol.*, S6: 007.
- Gubin SP, Koksharov YA, Khomutov GB, Yurkov GY. 2005. Magnetic nanoparticles: preparation, structure and properties. *Russ. Chem. Rev.*, 74(6): 489-520.
- Hajipour MJ, Fromm KM, Ashkarran AA, Aberasturi DJ, Larramend IR, Rojo T, Serpooshan V, Parak WJ, Mahmoudi M. 2013. Antibacterial properties of nanoparticles. *Trends Biotechnol.*, 31(1): 61-62.
- Han WK, Choi JW, Hwang GH, Hong SJ, Lee JS, Kang SG. 2006. Fabrication of Cu nanoparticles by direct electrochemical reduction from CuO nanoparticles. *Appl. Surf. Sci.*, 252(8): 2832-2838.
- Hindler JA, Howard BJ, Keiser JF. 1994. Antimicrobial agents and susceptibility testing. In: "Clinical and pathogenic Microbiology, (Howard BJ. Ed)". Mosby-Year Book Inc., St. Louis, MO, USA.
- Imran MM, Raja MMM, Abdul Basith J, Asarudeen A. 2011. Determination of total phenol, flavonoid and antioxidant activity of edible mushrooms *Pleurotus florida* and *Pleurotus eous*. *International Food Res. J.*, 18(2): 579-582.
- Jain S, Jain A, Devra V. 2014. Experimental investigation on the synthesis of copper nanoparticles by chemical reduction method. *Int. J. Sci. Eng. Res.*, 5(11): 973-978.
- Jose GP, Santra S, Mandal SK, Sengupta TK. 2011. Singlet oxygen-mediated DNA degradation by copper nanoparticles: potential towards cytotoxic effect on cancer cells. *J. Nanobiotechnol.*, 9: 9.
- Kanhed P, Birla S, Gaikwad S, Gade A, Seabra AB, Rubilar O, Duran N, Rai M. 2014. *In vitro* antifungal efficacy of copper nanoparticles against selected crop pathogenic fungi. *Mater. Lett.*, 115: 13-17.
- Karthik AD, Geetha K. 2013. Synthesis of copper precursor, copper and its oxide nanoparticles by green chemical reduction method and its antimicrobial activity. *J. Appl. Pharm. Sci.*, 3(5): 16-21.
- Khanna PK, Gaikwad S, Adhyapak PV, Singh N, Marimuthu R. 2007. Synthesis and characterization of copper nanoparticles. *Mater. Lett.*, 61(25): 4711-4.
- Khanna PK, Kale TS, Shaikh M, Rao NK, Satyanarayana CVV. 2008. Synthesis of oleic acid capped copper nanoparticles via reduction of copper salt by SFS. *Mater. Chem. Phys.*, 110(1): 21-25.
- Kogiso M, Yoshida K, Yase K, Shimizu T. 2002. One-dimensional organization of copper nanoparticles by chemical reduction of lipid-copper hybrid nanofibers. *Chem. Commun.*, 8(21): 2492-2493.
- Lee HJ, Lee G, Jang NR, Yun JH, Song JY, Kim BS. 2011. Biological synthesis of copper nanoparticles using plant extract. *Nanotechnology*, 1: 371-374.
- Leid JG, Ditto AJ, Knapp A, Shah PN, Wright BD, Blust R, Christensen L, Clemons CB, Wilber JP, Young GW, Kang AG, Panzner MJ, Cannon CL, Yun YH, Youngs WJ, Seckinger NM, Cope EK. 2012. *In vitro* antimicrobial studies of silver carbene complexes: activity of free and nanoparticle carbene formulations against clinical isolates of pathogenic bacteria. *J. Antimicrob. Chemother.*, 67(1): 138-148.
- Liu Q-m, Yasunami T, Kuruda K, Okido M. 2012. Preparation of Cu nanoparticles with ascorbic acid by aqueous solution reduction method. *T. Nonferr. Metals Soc.*, 22(9): 2198-2203.
- Malandrakis A, Kavroulakis N, Chrysikopoulos C. 2019. Nano-fungicides against plant pathogens: Copper, silver and zinc NPs.

- Geophys. Res. Abst., 21, EGU2019-3081-1, 2019
- Martinez-Gutierrez F, Olive PL, Banuelos A, Orrantia E, Nino N, Sanchez EM, Ruiz F, Bach H, Av-Gay Y. 2010. Synthesis, characterization, and evaluation of antimicrobial and cytotoxic effect of silver and titanium nanoparticles. *Nanomedicine*, 5(6): 681-688.
- Morais PC, Santos RL, Pimenta ACM, Azevedo RB and Lima ECD. 2006. Preparation and characterization of ultra-stable biocompatible magnetic fluids using citrate-coated cobalt ferrite nanoparticles. *Thin Solid Films*, 515(1): 266-70.
- Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, 65(1-2): 55-63.
- NCCLS. 1993. Performance standard for antimicrobial disc susceptibility tests. Approved Standard. National Committee for Clinical Laboratory Standards, Villanova, P.A. Publication M2-A5. USA.
- Niramathi KL, Sudha V, Lavanya R, Brindha P. 2013. Biosynthesis of silver nanoparticles using *Alternanthera sessilis* (Linn.) extract and their antimicrobial, antioxidant activities. *Colloids surf. B: Biointerfaces*, 102: 288-291.
- Oves M, Khan MS, Zaidi A, Ahmed AS, Ahmed F, Ahmad E, Sherwani A, Owais M, Azam A. 2013. Antibacterial and cytotoxic efficacy of extracellular silver nanoparticles biofabricated from chromium reducing novel OS4 strain of *Stenotrophomonas maltophilia*. *PLoS ONE*, 8(3): e59140.
- Pershina AG, Sazonov AE, Milto IV. 2008. Application of magnetic nanoparticles in biomedicine. *B. of Siberian Med.*, 2: 70-78. [in Russian]
- Pham LQ, Sohn JH, Kim CW, Park JH, Kang HS, Lee BC, Kang YS. 2012. Copper nanoparticles incorporated with conducting polymer: effects of copper concentration and surfactants on the stability and conductivity. *J. Colloid Interf. Sci.*, 365(1): 103-109.
- Priester JH, Stoimenov PK, Mielke RE, Webb SM, Ehrhardt C, Zhang JP, Stucky GD, Holden PA. 2009. Effects of soluble cadmium salts versus CdSe quantum dots on the growth of Planktonic *Pseudomonas aeruginosa*. *Environ. Sci. Technol.*, 43(7): 2589-2594.
- Ramyadevi J, Jeyasubramanian K, Marikani A, Rajakumar G, Rahuman A. 2012. Synthesis and antimicrobial activity of copper nanopart. *Mater. Lett.*, 71: 114-116.
- Saikova S, Vorobev S, Nikolaeva R, Mikhlin YL. 2010. Conditions for the formation of copper nanoparticles by reduction of copper(II) ions with hydrazine hydrate solutions. *Russ. J. Gen. Chem.*, 80(6): 1122-1127.
- Salavati-Niasari, Davar F. 2009. Synthesis of copper and copper (I) oxide nanoparticles by thermal decomposition of a new precursor. *Mater. Lett.*, 63(3-4): 441-443.
- Salavati-Niasari, Fereshteh Z, Davar F. 2009. Synthesis of oleylamine capped copper nanocrystal via thermal reduction of a new precursor. *Polyhedron*, 28(1): 126-130.
- Saterlie M, Sahin H, Kavlicoglu B, Liu Y, Graeve O. 2011. Particle size effects in the thermal conductivity enhancement of copper-based nanofluids. *Nanoscale Res. Lett.*, 6(1): 217.
- Sheibani S, Ataie A, Heshmati-Manesh S. 2008. Role of process control agent on synthesis and consolidation behavior of nanocrystalline copper produced by mechanochemical route. *J. Alloy. Compd.*, 465(1-2): 78-82.
- Shobha G, Sagar S, Shashidhara KS, Mahadimane V, Ananda S. 2019. *In vitro* cytotoxicity study of green synthesized copper nanoparticles. *Res. J. Biotechnol.*, 14(8): 105-111.
- Sorbiun M, Mehr, ES, Ramazani A, Malekzadeh AM. 2018. Biosynthesis of metallic nanoparticles using plant extracts and evaluation of their antibacterial properties. *Nanochem. Res.*, 3(1): 1-16.
- Subbaiya R, Selvam MM. 2015. Synthesis and characterisation of copper nanoparticles using *Eupatorium glandulosum* extract and their antimicrobial, antioxidant activities. *RJPBCS*, 6(2): 1117- 1127.
- Tamilvanan A, Balamurugan K, Ponappa K, Kumar BM. 2014 Copper nanoparticles: Synthetic strategies, properties and multifunctional application. *Int. J. Nanosci.*, 13(2): 1430001 (22 pages).
- Tan KS, Cheong KY. 2013 Advances of Ag, Cu, and Ag-Cu alloy nanoparticles synthesized via chemical reduction route. *J. Nanopart. Res.*, 15(4): 1537 (29 pages).
- Wu SH, Chen DH. 2004 Synthesis of high-concentration Cu nanoparticles in aqueous CTAB solutions. *J. Colloid Interface Sci.*, 273(1): 165-169.
- Yallappa S, Manjanna J, Sindhe MA, Satyanarayan ND, Pramod SN, Nagaraja K. 2013. Microwave assisted rapid synthesis and biological evaluation of stable copper nanoparticles using *T. arjuna* bark extract. *Spectrochim. Acta A: Mol. Biomol. Spectrosc.*, 110: 108-115.
- Yen GC, Chen HY. 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J. Agric. Food Chem.*, 43(1): 27-37.
- Yesilot S, Aydin C. 2019. Silver Nanoparticles; A New Hope in Cancer Therapy? *East. J. Med.*, 24(1): 111-116.
- Zain NM, Stapley AGF, Shama G. 2014. Green synthesis of silver and copper nanoparticles using ascorbic acid and chitosan for antimicrobial applications. *Carbohydr. Polym.*, 112: 195-202.
- Zhang Q, Yang Z, Ding B, Lan X, Guo Y. 2010. Preparation of copper nanoparticles by chemical reduction method using potassium borohydride. *T. Nonferr. Metal. Soc.*, 20(Suppl 1): S240-S24.
- Zhu B, Li Y, Lin Z, Zhao M, Xu T, Wang C, Deng N. 2016. Silver nanoparticles induce HePG-2 cells apoptosis through ROS-mediated signaling pathways. *Nanoscale Res. Lett.*, 11(1): 198.

تصنيع وتوصيف ودراسة الأنشطة البيولوجية لجسيمات النحاس النانوية

إيمان عبد الله محمد علي

قسم النبات والميكروبيولوجي، كلية العلوم، جامعة القاهرة، الجيزة، مصر

بينيسيليوم إكسانسوم، ستافيلوكوكس ايربوس، ستريبتوكوكاس فيكالييس، ستريبتوكوكاس ميوتانس، اشيريشيا كولاي، نيسيريا جونوريا وسودوموناس ايريجينوزا. أظهرت نتائج كلا من جسيمات النحاس النانوية ونواتر النحاس المصنعة لها أنشطة مضادة للبكتيريا أكثر من الأنشطة المضادة للفطريات. كشفت النتائج ان النشاط المضاد للأكسدة لكلا من جسيمات النحاس النانوية ونواتر النحاس متقارب الى حد ما مع نسبة وصلت الى 95.42% مع تركيز 500 ميكروجرام/مل لجسيمات النحاس النانوية. كشفت جسيمات النحاس النانوية آثار السمية الخلوية أعلى بكثير من نواتر النحاس. كان إنتاج جسيمات النحاس النانوية باستخدام طريقة الاختزال الكيميائي بسيطاً وغير مكلف وسريع. أظهرت جسيمات النحاس النانوية أنشطة بيولوجية بكفاءة عالية.

تقنية النانو هي أكثر مجالات البحث تقدماً في العصر الحالي. أظهرت جسيمات النحاس النانوية دوراً مهماً في العديد من المجالات البيولوجية. الهدف من هذه الدراسة هو تصنيع وتوصيف ودراسة الأنشطة البيولوجية لجسيمات النحاس النانوية. تم تصنيع جسيمات النحاس النانوية بواسطة طريقة الاختزال الكيميائي باستخدام كحول الأيزوبروبيل وبروميد الثلاثي ميثيل أمونيوم سيتيل (CTAB). تم توصيف الجسيمات المصنعة باستخدام مقياس الطيف الضوئي للأشعة تحت الحمراء (FTIR) وتحليل المجهر الإلكتروني (TEM). تم إنتاج جسيمات كروية بنطاق مقاس 5-13 نانومتر. تم اختبار الأنشطة المضادة للميكروبات لكل من جسيمات النحاس النانوية ونواتر النحاس ضد ميكروبات أسبرجيلس فلافس، أسبرجيلس فوميغاتوس، أسبرجيلوس نيجر، كانديكا أليكانز،