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Synthesis of Silver Nanoparticles by Using Aloe Vera and Bio Application

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ABSTRACT

The present study aimed at the biosynthesis of silver nanoparticles by using aloe Vera leaves extract. Silver nanoparticles (Ag NPs) synthesized by the green method have shown several applications such as biomedical, anticancer, etc. Measurement of the size of silver nanoparticles by AFM (showing an average diameter of 54 nm), XRD and FE-SEM analyses. Four different concentrations (25, 50, 100, and 200 µg/ml) were prepared from the synthesized Ag NPs and examined for their antibacterial efficacy against both *E.coli* Furthermore, *pseudomonas aeruginosa*. Cytotoxicity was evaluated using MTT assays on (TCP -1013) cancer cell lines. As shown by the results, Ag NPs inhibit bacterial growth over the concentration range evaluated. High cytotoxicity against the investigated cancer cell line was also seen with all doses of Ag NPs tested, corroborating the antibacterial activity findings. There was a dosage relationship between Ag NPs antibacterial and anticancer properties.

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INTRODUCTION

Nanotechnology involves making nanoparticles (NPs) with different shapes, sizes, and controlled distributions so that they can be used in a variety of ways [1-3]. It could affect society if it is used a lot in different areas of science and technology. Metal NPs can be used as an antimicrobial, anticancer, wound healer, surgery function, catalyst, and in biomedical devices. green synthesis provides a viable alternative [4-8]). The word "nanoparticle" (NP) refers to the very small particles that are made in a wide range of industrial physicochemical processes. Two such processes are photochemical and electrochemical reduction, respectively. Other methods include catalytic routes, hydrolysis precipitation, wet chemical methods, hydrolysis precipitation, and laser desorption [9-12]. In place of harmful physical and chemical processes, green synthesis provides a viable alternative [13-15]. that pose no danger to humans or the environment Furthermore, the importance of sustainable resources cannot be overstated in an eco-friendly plan the shift toward greener methods from the more traditional ones has several causes. Contrary to popular belief, "green" does not relate to the colour green, but rather to the process of making nanoparticles out of metal salts by using the reducing property of biologically active compounds [16,17]. These biologically active compounds may be isolated from a wide variety of plant, animal and microbial sources Plants (leaves, roots, whole

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plants, flowers, fruits, bark, latex, etc.), microbes, and animals all contribute to this category. "Green nanoparticle synthesis" refers to the process of creating nanoparticles using renewable resources The purpose of this research is to evaluate green nanoparticles' (AgNPs) biological activity as a potential broadly available, cost-effective antibacterial and anticancer treatment.

MATERIALS AND METHODS

BioSynthesis of Ag NPs by aloe vera leaf extract

to preparation of aloe vera extract, we used plant extract from the aloe vera plant. 50 grams of aloe vera leaves were cleansed with deionized water and then coarsely chopped to make the aloe vera extract solution. After twenty minutes of cooking in fifty millilitres of deionized water, the aloe vera leaves were allowed to cool. We cooled the leaf broth, strained it, and put it in the fridge at 4 degrees Celsius to keep the nutrients in the leaves. To get to the aloe vera, this extract was used as a solvent [15].

Synthesis and characterization of silver

preparation of Ag NPs were performed according to the procedure described by [9]. with minor modification All of the chemicals and reagents used were acquired from the American company Sigma, Ltd. After dissolving 0.3 mol of $AgNO_3$ in 20 ml of deionized water, 20 ml of an aloe vera extract solution was added and the mixture was vigorously agitated for 30 minutes at room temperature. The mixtures were put into Teflon-lined (Parr, USA) 100 mL containers, heated for varying amounts of time, and then gradually cooled to room temperature. Synthesized Ag NPs were concentrated to concentrations of 25, 25,100, and 200 g/ml by filtering a grey precipitate, washing it several times with deionized water, and drying it in the air at 60 degrees Celsius for six hours.

Nanoparticles Characterization Techniques

To see their 2D and 3D topologies, atomic force microscopy (AFM) was used to quantify the average crystalline size of the generated Ag NPs, as shown in Fig. 1A. We know the crystal structure because of the X-ray diffraction patterns seen in Fig. 2. Additional characterization using FE-SEM is shown in Fig. 3. [9]. Synthesis of Ag NPs was performed at the biochemistry laboratory of the Biomedical Engineering Department at the University of Technology in Iraq.

Evaluation of antibacterial activity Preparation of bacterial isolates

These E. *coli* and *pseudomonas aurginosa* strains are from the Biotechnology Department of the University of Applied Science and Technology. Before the trials began, all of the necessary confirmatory tests for this investigation were performed in the lab. After being cultured in nutrient broth at 37 degrees Celsius for 24 hours, the bacteria were ready to be put to the test for antibiotic efficacy.

Antibacterial activity

The effectiveness of Ag NPs as an antibacterial agent was tested against *Escherichia coli* and *pseudomonas aurginosa*. The antimicrobial efficacy was tested using the agar well diffusion technique. Using sterile Petri dishes, 25 ml aliquots of (Muller-Hinton) agar medium were poured in and allowed to set at room temperature. An agar plate with an overnight culture of active testing bacteria was swabbed with sterile cotton to ensure even distribution. The antimicrobial performance of the synthesized Ag NPs was assessed after wells were drilled into each plate. Subsequently, silver nanoparticles of varying concentrations (25, 50, 100, and 200 g/ml) were added to the wells. After incubation for 24 hours at 37°C [8]. The inhibitory zones around each well were analyzed.

Evaluation of anticancer activity

To determine anticancer efficacy, CAC Baghdad/ Iraq donated MCF-7 cancer cell lines, which were plated in 96-well culture plates with RPMI 1640 medium supplemented with 10,000 IU penicillin, 10% fetal bovine serum, and 100 g/ ml streptomycin. Plates were kept at 37 degrees Celsius in a humidified incubator with 5 % CO_2.

MTT Cytotoxicity Assay

As per the Manufacturer's Instructions (MTT Kit/Intron Biotech, Germany), both the planning of the solution and experimental tests were carried out. 1 x 104 cells/ml were cultured in 96-well plates, with 200 microliters of RPMI medium added to each well. The panels were gently moved, sterile-topped, and incubated at 37°C for 24 hours with 5% Carbon dioxide. The medium and 200µl Ag NPs were then separated. The medium is isolated from the wells and 200µL (25, 50, 100, and 200 (µg/ml of Ag NPs are used. In addition to the others, three replicates of every regulation

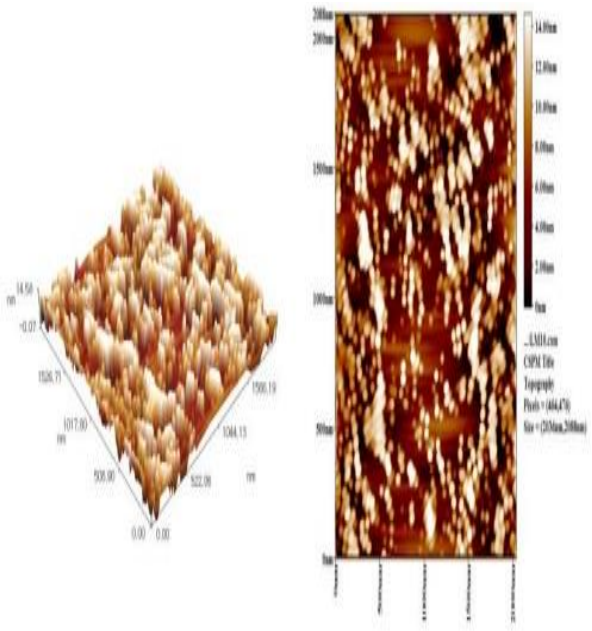

Fig. 1. A. Atomic force microscopy of Ag NPs synthesized using aloe vera 2D and 3D topological. B. The average size of Ag nanoparticles by AFMM

and concentration process have been performed, with each experimental replicate containing a positive control (doxorubicin 50 mg/mL) and an adverse control (DMSO). Over 48 hours, the flat was replenished with 5% CO_2 at 37°C. After the Ag NPs treatment, a solution of 10ml of MTT was added to each well and for four hours it was reincubated at 37° C, 5% CO_2. 100 µg/ml of DMSO solution was added to each well and incubated for five minutes after the removal of the medium. The optical intensity at a wavelength of 575 nm was used to figure out how alive the cells were [16].

Statistical analysis

The statistical method utilized here was one-way analysis of variance (ANOVA). The p-value

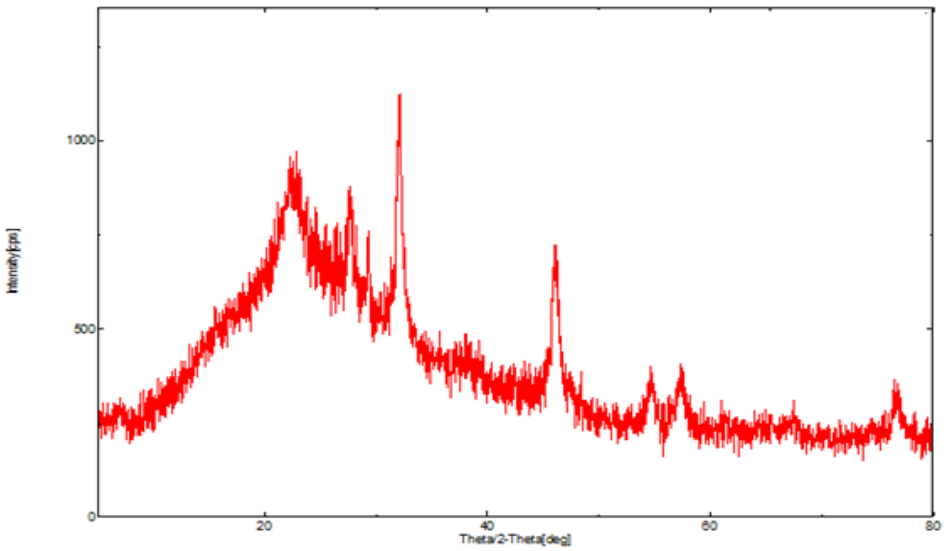

Fig. 2. XRD pattern of Ag NPs

chosen to indicate statistical significance was 0.05. SPSS version 23 was used to calculate significance levels for differences and correlations between findings. The information was presented as a mean and standard deviation.

RESULTS AND DISCUSSION

The topography and surface morphology were assessed with the use of an atomic force microscope (AFM). The AFM is shown in Fig. 1-B. Provides two- and three-dimensional reconstructions of the nanoparticle's atomic-level surface. Average particle sizes were measured to be on the nanoscale scale. AFM-SPM (atomic force microscopy and scanning probe microscopy) measurements (Fig. 1B). As shown in Fig. 1, silver nanoparticles were created using topological 2D and 3D aloe vera structures, as shown in Fig. 1-A using atomic force microscopy.

The XRD pattern of Ag nanoparticles was obtained from green synthesis as shown in Fig . 2. XRD patterns show that all Ag NPs in the anatase phase and these findings were in good agreement with the JCPDS number of card 21-1272. Peaks were absorbed in 31°, 73°, 45°, 72°, and 76 along with miller indices values (110), (200), (311), respectively. This results in agreement with [4]. Fig. 3 reveals that the silver nanoparticles formed were spherical in shape, with sizes ranging around 100 nm and grain sizes of 10 to 40nm in diameter some of the particles were slightly agglomerated because of the heat produced in the annealing process, However, the small-sized particles were very reactive because of their sharp edges.

In Table 1, we see that Ag NPs had significant antibacterial activity at all concentrations (25, 50, 100, and 200 g/ml) against the two species used in this study (*E.coli* and *pseudomonas aurginosa*). Different bacteria used in this study range in sensitivity, which may account for the observed differences in diameters of inhibition caused by Ag NPs. Probably the most significant toxicological mechanism is the adhesion of Ag NPs to negatively charged bacterial cell walls [11]. where they may change the shape and permeability of the plasma membrane. Each investigation also confirmed that nanoparticle toxicity is exacerbated by the release of silver ions from their surfaces [5]. Ag NPs get into bacterial cells by binding to macromolecules that contain sulfur. Once inside, they stop proteins from working and kill cells by stopping enzymes in the respiratory chain [14]. The MTT assay was applied to determine the cytotoxic activity of the Ag NPs on tumour (TCP -1013) cell lines. This assay

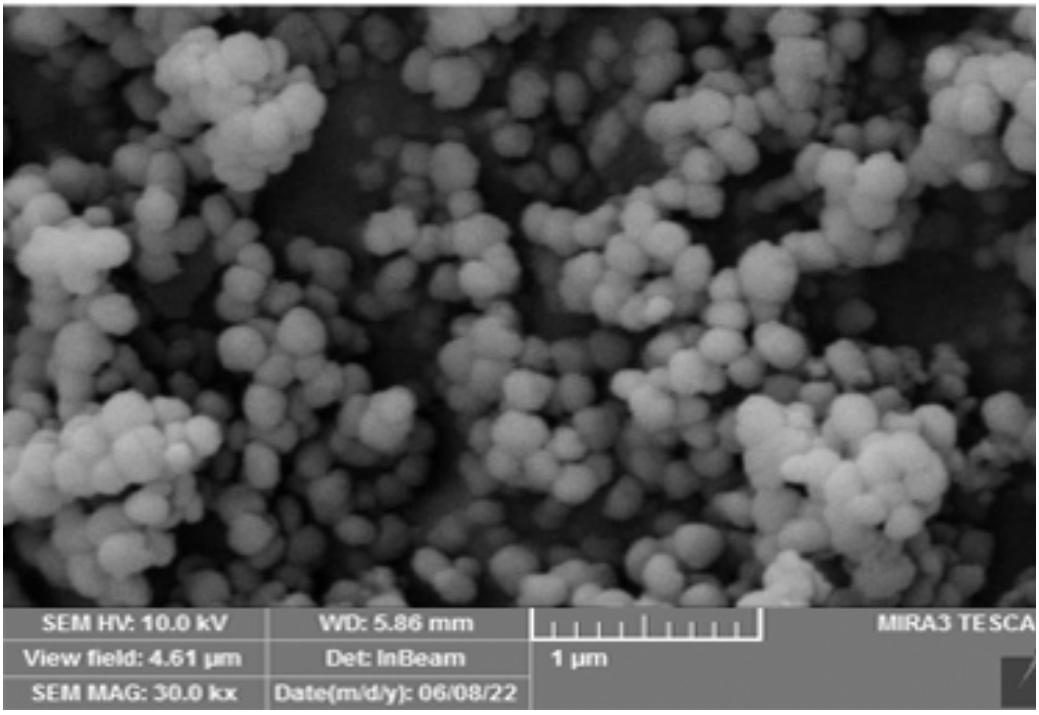

Fig. 3. (FE-SEM) of Ag NPs

Table 1. Antibacterial susceptibility test

No.	Ag NPs concentration µg/ml	Inhibition Zone (mm)	
		E.coli	*pseudomonas aurginosa*
1	25	2±0.16	1±0.10
2	50	5±1.16	3±1.22
3	100	11±2.16	12±3.73
4	200	22±4.2	18±1.16

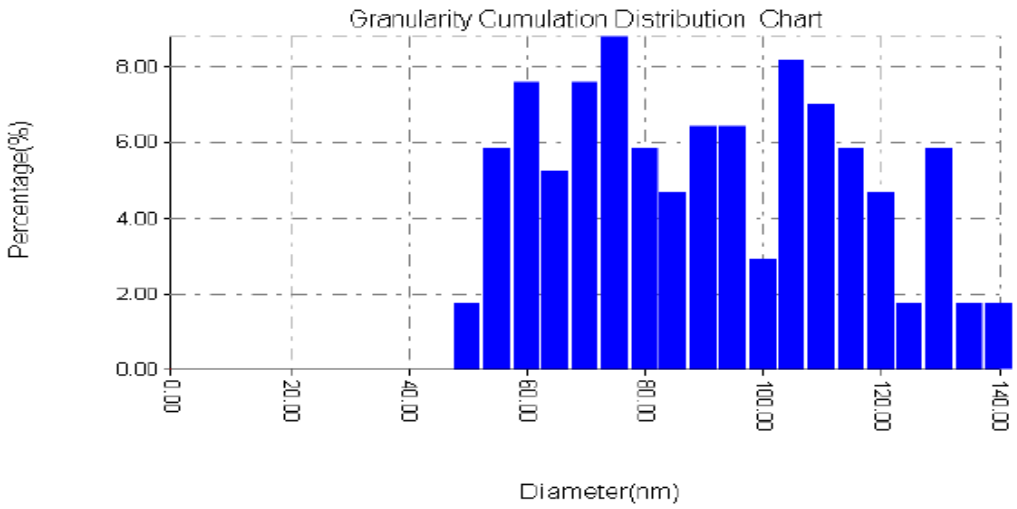

Fig. 4. The average size of Ag nanoparticles by AFM.

Table 2. Statistical analysis of Cytotoxicity effect of Ag NPs on TCP -1013 cell line

No.	Concentration µg/ml	TCP-1013)	
		Mean Diff	SD
1	200	51.10	5.01
2	100	60.64	4.45
3	50	79.56	6.83
4	25	92.84	1.26

used a range of Ag NPs concentrations on tumour cell lines (1x10 ^{4}–1x10 ^{6} cells / mL) to detect the cell viability. Results in Fig. 4. showed that the dose-dependent manner of Ag NPs caused a reduction in the cell viability of TCP 1013 cell lines, in addition to measuring the IC50 of 71.55µg/ml. Ag NPs displayed a dose-dependent sequence of progressive cytotoxicity beginning at a lower concentration to its maximum inhibition at 200µg/mL, (55) % inhibition of TCP-1013cells. Evidence

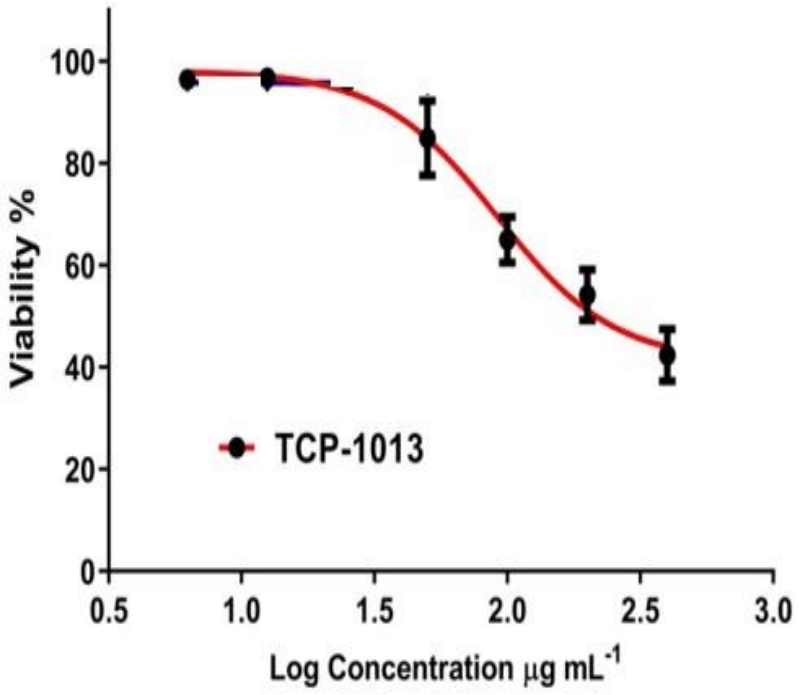

Fig. 5. Cytotoxicity effect of Ag NPs on TCP -1013 cell line

of the cytotoxic effect of Ag NPs revealed that treatment of TCP- 1013 cells at concentrations between (25) and (200) µg / mL for 24 hours showed substantial cell viability mortality by rising dose-dependent concentrations, reaching a death rate of up to 55% at (200) µg / mL with IC50 of (71.55) µg / mL (Fig. 5) and (Table 2). When exposed to nanomaterials, cells are substantially more likely to experience DNA damage, membrane collapse, and eventual cell death because of oxidative stress and lipid peroxidation. By collecting electrons and interacting with water molecules in the cell medium, this nanoparticle makes ROS free radicals [1].

CONCLUSION

We conclude that the fabrication of AgNPs using aloe vera extract is a very simple, rapid, and efficient method.at size 54 nm Additionally, the use of plant extract makes it even more sustainable and less toxic to humans and their surrounding environment. demonstrated that Ag NPs was a promising effect on gram-negative and positive bacteria. in addition to cytotoxicity evaluation of Ag nanoparticles against TCP -1013 cancer cells therefore this can be considered a promising candidate in anticancer studies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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