JOTCS A
Journal of the Turkish Chemical Society
Section: A
Chemistry
TURKISH CHEMICAL SOCIETY

Eco-friendly Approach for Silver Nanoparticles Synthesis from Lemon Extract and their Anti-oxidant, Anti-bacterial, and Anti-cancer Activities

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Abstract: To create silver nanoparticles, researchers use bionanotechnology techniques because they are economical and environmentally friendly. The current study shows that lemon juice (*Citrus-limon*) can be used to biosynthesize silver nanoparticles (Ag NPs). The synthesized silver nanoparticles have been characterized by the surface plasmon resonance (SPR) measured at λ_{max} = 430 nm, confirming the formation of AgNPs. Moreover, Fourier Transform Infrared (FTIR) analysis was carried out to identify possible bio-molecules responsible for the bio-reduction of silver ions. The x-ray diffraction (XRD) peaks at (111, 200, 220, 222, and 311) confirm the found face-centered cubic (FCC) crystal structure of AgNPs in solution. Transmission-Electron-Microscopy (TEM) images showed that AgNPs have spherical morphology with sizes ranging from 10-50 nm. Furthermore, the Particles Size Analyzer (PSA) confirmed these sizes and ranges. Synthesized AgNPs have high anti-oxidant activity according to the (scavenging of DPPH radicals, total anti-oxidant, and reducing power) assays. Also, the anti-bacterial activity of AgNPs was evaluated by a well diffusion method, and the results suggest that they are more sensitive to gram-positive bacteria than gram-negative ones, with the average diameter of the inhibition zones for AgNPs ranging from 4.11 to 25.87 mm and 1.38 to 22.3 mm against *S. aureus* and *E. coli* bacteria, respectively. *In vitro* studies of AgNPs against MCF-7 breast cancer cell s lines showed a good cytotoxic effect $p<0.05$ with an IC_{50} value of 47 µg/mL; this study could be beneficial for nanotechnology-based pharmaceutical and biomedical applications.

Keywords: Silver nanoparticles, citrus limon juice, anti-oxidant activity, anti-bacterial activity, anti-cancer activity.

Submitted: August 09, 2022. **Accepted:** January 20, 2023.

Cite this: Alaallah NJ, AbdulKareem EA, Ghaidan AF, Imran NA. Eco-friendly Approach for Silver Nanoparticles Synthesis from Lemon Extract and their Anti-oxidant, Anti-bacterial, and Anti-cancer Activities. JOTCSA. 2023;10(1):205–16.

DOI: https://doi.org/10.18596/jotcsa.1159851.

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1. INTRODUCTION

Metal nanoparticles that appear in size ranging from 1 - 100 nm have received increasing interest due to their optical and optoelectronic properties that are unique compared to their bulk counterparts; this has made them enter into different fields such as catalysis, optics, environmental, electronics, and biotechnology, which is an area of constant interest (1,2). Among these, silver nanoparticles (AgNPs) are widely used in a variety of applications because they have a small size, large surface area, and high dispersive capacity and exhibit anti-microbial, anti-cancer, anti-diabetic, anti-oxidant, anti-inflammatory properties and also used in food preservation, water purifications ointment fabrication, and cosmetics (3, 4). Physical and chemical processes are still used today to create nanoparticles, but they result in many dangerous by-products that harm the environment. Therefore, there is a constant need for developing new methodologies and approaches for simple, rapid, consuming less energy, performing under moderate operation conditions, and eco-friendly nanoparticle synthesis methods. Many

scientists focus on the green synthesis of nanoparticles from plant extracts (5). It has been reported that nanoparticles synthesized by plant parts (such as seeds, roots, leaves, stems, flowers, and fruits) have more biological activities than nanoparticle synthesis by chemical methods (6-8). Figure 1 represents an illustrative scheme for forming silver nanoparticles from different plant parts.

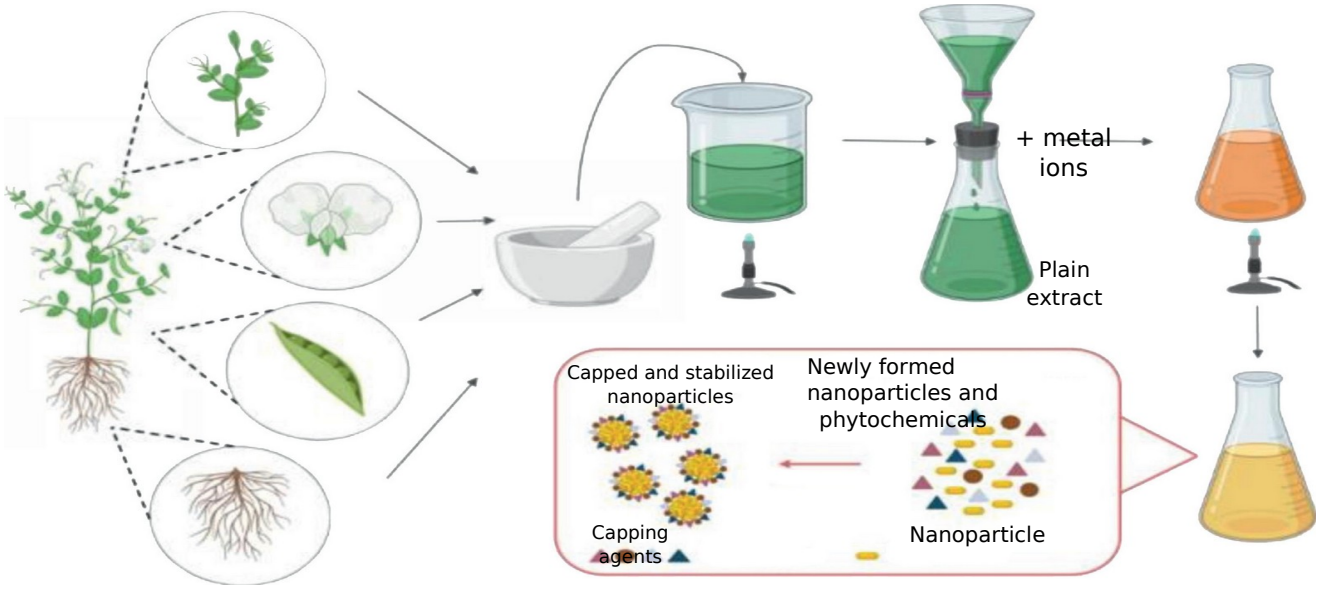

Figure 1: Green synthesis of metal nanoparticles by plant parts (9).

In recent times, nanoparticles using plant extracts have received attention as it is an economical and straightforward method (10). The main idea in the synthesis of AgNPs is by reducing agents that reduce silver ions (Ag^{+1}) to neutral silver (Ag^{0}) (22). Fruits, including lemon, are considered a rich source of flavanones, polys-methoxylated flavones, and carboxylic acids such as ascorbic acid and citric acid, which are very rare in other plants, and these materials could be used as reducing agents (5,11).

In this work, silver nanoparticles were synthesis through environmentally friendly synthesis techniques by using the extract from *Citrus limon* (lemon), the reason behind selecting this particular plant is that (i) lemon is a cheap, commercially available, and environmentally safe fruit and (ii) anti-microbial properties of the synthesized nanoparticles might be enhanced (12,13). In this regard, numerous studies on the synthesis of nanomaterials using plant parts such as *Citrus sinensis, Citrus tangerina*, and *Citrus limon* peel extract(5), *Citrus sinensis* peel extract (14), Lemons leaves extract (10,15), *Lemon Citrus Latifolia* extract (16), and *Citrus limon* (lemon) juice aqueous extract (13,17-19), Although there have been studies conducted with lemon, they have been limited to studying anti-bacterial activities. This study is the first to study the effectiveness of silver nanoparticles manufactured from lemon juice on cancer cell lines (MCF-7) and follow up on their anti-oxidant and anti-bacterial activities.

This work also provides more information on the chemical structure of silver nanoparticles synthesized from aqueous lemon juice by UV, FTIR, XRD, TEM, AFM, and PSA.

2. METHODOLOGY

2.1. Materials

Silver nitrate ($AgNO_3$) was purchased from BDH, Sigma Aldrich. Fresh lemon was taken from local markets in Baquba / Diyala. Other chemical compounds required for anti-oxidant, anti-bacterial, and anti-cancer studies were of the highest purity. In this study, all glass tool was washed using distilled water and dried in the oven till used in the experiments, and all solutions were prepared using distilled water.

2.2. Preparation of Lemon Juice

Fresh lemon (C. Limon) fruit samples were sliced and strained through a nylon mesh with fine holes to obtain the juice. Then, the juice obtained was put into a centrifuge at 10,000 rpm for 10 minutes to remove any unwanted impurities. This extract was collected in a dark volumetric flask (25 mL) and stored at 4 °C for further experiments (17).

2.3. Synthesis of Silver Nanoparticles (AgNPs) from Lemon Juice

For the green synthesis of AgNPs**,** 1 mL of the lemon juice was added to 4 mL of 1 mM silver nitrate ($AgNO_3$) solution in a suitable test tube. The solution was heated in a water bath at 100℃ for 30 minutes until AgNPs were formed, and reduction was confirmed by changing color from colorless to brownish-yellow (18).

2.4. Characterization of Synthesized Silver Nanoparticles

2.4.1. UV-vis spectroscopy

The optical properties of silver nanoparticles were calculated using ultraviolet-Visible spectrophotometer model Shimadzu UV-1700 (Shimadzu Corp, Kyoto, Japan). UV-Vis spectroscopic analysis was performed by continuous scanning in the range of 300-900 nm, an effective and widely applied technique for determining the formulation stability of metal nanoparticles (20) with some modification.

2.4.2. Fourier transform infrared (FTIR) analysis

FTIR is an analytical chemical method used to measure the intensity of infrared radiation against its wave number or wavelength light. It was performed to determine the functional groups involved in reducing Ag^{+} to AgNPs. The infrared spectra of the dried AgNPs and lemon powder were measured with the KBr disc in the range of 400-4000 cm^{-1} using (S-8400, Shimadzu, Japan) FTIR spectrometer (21).

2.4.3. X-Ray diffraction (XRD) analysis

To confirm and determine the crystal structure of AgNPs. The powder X-Ray diffraction (XRD) of the AgNPs was recorded using X-ray diffractometer model (Philips X'Pert-MPD diffractometer , the Netherlands) ranging from 10 to 80º, and monochromatic Cukα radiation having wavelength (λ = 1.5406°A) was used for XRD analysis (22).

2.5. Microscopy

2.5.1. Transmission electron microscopy (TEM) analysis

A thin film of the aqueous suspension of AgNPs was prepared by placing 10 *µL* of suspension on a small copper grid and letting the grid dry before starting the measurement. The morphology of the AgNPs was investigated by TEM using a (JEOL JEM-2100) with an acceleration voltage of 200kV which gives a high-resolution image (19).

2.5.2. Atomic force microscopy (AFM) analysis

AFM was used to assess the size and form of AgNPs produced through biological synthesis. The AFM usually measures the height of AgNPs. AFM is a measurement by taking a few drops of aqueous suspension and placing it on a glass slide, leaving 24 hours at room temperature to dry completely. Images obtained from the AFM were collected using Pico-Scan software (17).

2.5.3. Particle size analyzer (PSA) analysis

Particle size analyzer equipment (Horiba LA-3000Light Scattering Particle Size Distribution Analyzer, Horiba Ltd, Kyoto, Japan) was used to determine the particle size of the produced AgNPs. The normal log distribution of the nanoparticles based on intensity and size was studied comparatively. Measurements were taken between 5.0 and 500.0 nm(23).

2.6. Anti-Oxidant Assays

The synthesized AgNPs were estimated for anti-oxidant activity using three methods as follows: L-Ascorbic acid (AA) was used as a reference.

2.6.1. Scavenging of DPPH radical assay

The anti-oxidant activity of the lemon juices and AgNPs was measured by using (2,2-diphenyl-1-picryl-hydroxyl)(DPPH) assay, as described by Chang et al.(24). 1 mL of serial dilutions of lemon juice and synthesized AgNPs were prepared in different concentrations (100, 200, 300, and 400 µg/mL). To each dilution, 1 mL of DPPH (0.135 mM in ethanol) was added, incubated, and left in the dark at room temperature for (30 minutes). The absorbance of the control and samples were determined at λ_{max} = 517 nm, and ascorbic acid was used as a positive control.

The capability of AgNPs to scavenge the DPPH radical was recorded as a percentage inhibition in Equation 1:

$$\%Inhibition = \left(\frac{OD_{Control} - OD_{Sample}}{OD_{Control}} \times 100 \right) \quad \text{(Eq. 1)}$$

where $OD_{control}$ is the optical density of the control samples (DPPH solution without samples) and $OD_{samples}$ is the optical density of the sample (DPPH solution and samples).

2.6.2. Total anti-oxidant assay

The total anti-oxidant activity of lemon juice and AgNPs was estimated according to the method conducted by Prieto et al. (25). An aliquot of 0.3 mL of each diluted juice prepared as described above was mixed with (3 mL) of reagent solution (4 mM ammonium-molybdate ($(NH_4)_2MoO_4$), 28 mM sodium phosphate (Na_3PO_4), and 0.6 M sulfuric acid (H_2SO_4), incubated at 95 °C for 90 minutes. After cooling the tubes, the samples' absorbance was measured at λ_{max} = 695 nm, and the anti-oxidant activity was determined as (%) using this formula:

$$Total\ Anti-Oxidant = \left(\frac{OD_{Control} - OD_{Sample}}{OD_{Control}} \times 100 \right)$$

(Eq. 2)

2.6.3. Reducing power assay

The ferric (Fe^{3+}) reducing the power of lemon juice and AgNPs was evaluated according to methods described by Oyaizu (26). Various concentrations of 1ml lemon juice and AgNPs were mixed with equal volumes of sodium phosphate buffers (0.2 M, pH=6.6) and 1 mL of potassium hexacyanoferrate ($K_3Fe(CN)_6$) (1%, w/v), and the resultant mixtures were incubated at 50 ^{0}C for 20 minutes. The reaction was stopped by adding 1 mL of trichloroacetic acid (TCA) (10%, w/v) and then centrifuging at 10,000 rpm for 10 minutes. A resultant supernatant solution (1.5 mL) was mixed for 10 minutes with distilled water (1.5 mL) and 0.1 mL of ferric chloride ($FeCl_3$)

solution (0.1%, w/v). Then, the absorbance of the mixture was measured at λ_{max} = 700 nm against a blank solution. Higher absorbance values of the reaction mixture determined increased reducing power. Three replicates of each sample were made, and the average was recorded.

2.7. Antimicrobial Activity

A standard well diffusion procedure depends on investigating the inhibition effect of lemon juice and AgNPs of bacterial growth for two human pathogenic bacteria, *Staphylococcus aureus* as Gram-positive and *Escherichia coli* as Gram-negative (27). About 25 mL of nutrient agars were poured on Petri dishes and left to solidify. Inoculation of bacteria was performed by streaking on the agar surface of each plate hole 5 mm wide was on agar by punched with a sterile cork borer (10, 25, 50, and 100) µL of lemon juice, and a solution containing AgNPs was transported and placed in these holes. The plates were permissible to stand by for (30 minutes) to (1 hour). The incubation of inoculated agar was done for 24 hours at an optimum temperature for bacteria growth equal to 37 °C. Then, the inhibition zone of bacteria growth was determined in millimeters. All experiments were conducted in triplicate.

2.8. Cytotoxicity Assay

The cytotoxicity assay of lemon juice and AgNPs was estimated by the MTT assay using a human breast cancer cell line MCF-7. In this method, MCF-7 cells were seeded onto 96-well plates ~1×10^5 cells/wells at (37 ^{0}C) for 24 hours, and then cells were treated with different concentrations ranging from 10 to 100 µg/mL of AgNPs along with cell control and lemon juice. Cells that had been treated with MTT (0.5 mg/L) were added, and they were then incubated at 37°C in a CO_2 incubator for 4 hours. Cells were washed with 100 µl of phosphate-buffered saline (PBS) after the MTT-containing medium was discarded. After incubation, the MTT formazan crystals were dissolved in (100 µl) of dimethyl sulfoxide (DMSO, 10%), and purple-blue formazone dye was measured at 620 nm in a multiwell ELISA plate reader (Thermo-Multiskan EX)(28). The optical density (OD) value was used to compute the percentage of cell viability by using the following formula.

$$Percentage\,of\,cell\,viability = \frac{OD_{cells\ \ with\,AgNPs}}{OD_{cells\ \ without\,AgNPs}} \times 100$$

(Eq. 3)

Also, the cytotoxicity assay was estimated by finding the IC_{50} value, which was the concentration showing 50% inhibition activity.

2.9. Statistical Analysis

A statistical comparison was carried out using a one-way analysis of variance (ANOVA), followed by Tukey's test. Each experiment was performed in triplicate, and the results were expressed as the mean± standard deviation; statistical significance was accepted at a level of ($p< 0.05$).

3. RESULTS AND DISCUSSION

3.1. Biosynthesis of AgNPs

In this study, the biosynthesis of AgNPs was carried out by reducing the aqueous silver solution of $AgNO_3$ with lemon juice. The results indicated an apparent change in the color of the solution from colorless to yellowish-brown; this occurred in a period of (30 minutes), and this change indicates the formation of AgNPs. Figure 2 shows the color change obtained, which agrees with a number of other studies (16,17,19).

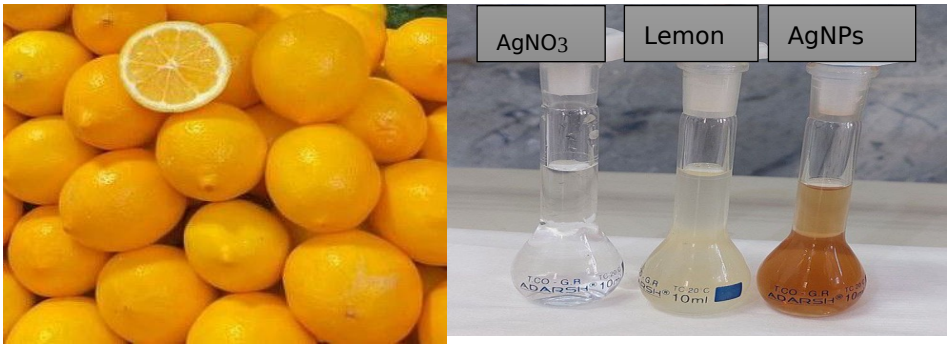

Figure 2: The change in color indicating the synthesis of AgNPs.

3.2. Characterization of AgNPs

3.2.1. UV-Visible analysis

Ultraviolet-Visible (UV-Vis) spectroscopy was used to characterize the synthesis of AgNPs. Figure 3 shows UV-Visible spectra of the synthesis of AgNPs. Lemon juice and $AgNO_3$ were involved as controls. The peak at λ_{max} = 430 nm in Uv absorption refers to the conformation of AgNPs; the peaks' broadness exhibits the particle size variability. The result of this study was similar to the results observed by Patil et al. (29).

3.2.2. FTIR analysis

FT-IR absorption spectra of lemon juice before and after bio-reduction, as shown in Figure 4, shows various bending and stretching bands viz, 3429, 1730, 1630, 1400, 1225, 1106, 899, 596, and 520 cm^{-1}. The broad peak at 3429 cm^{-1} corresponded to (–N-H) stretching of amides (II). The peak at 1730 cm^{-1} is sharp and related to (-C=O) stretching in (-

COOH) carboxylic acid. The peak at 1630 cm^{-1} (weaker), related to amide I, arisen due to carbonyl stretch in the protein. The peak at 1400 cm^{-1} corresponded to -O-H and -C-H bending. The peak at 1200 cm^{-1} corresponds to -C-O stretching in the carboxylic acid group (COOH). The peak at 1021 cm^{-1} is related to C-N stretching vibrations of amine. The peak at 1106 cm^{-1} appeared as a significant peak, which might be attributed to the C-O groups of the polyols such as polysaccharides, flavones, and terpenoids excessively found in plant extract used as reducing agents during the synthesis of AgNPs (30). The peak at 899 cm^{-1} is related to C-H aromatic in benzene groups. The peaks between 596 and 520 cm^{-1} represent to silver nanoparticle's connection with oxygen from the hydroxyl group. From the measurement of FT-IR, it can be concluded that some of the bio-organic compounds present in lemon juice, such as flavonoids, alkaloids, and phenols, can play an essential role in reducing silver salt to metallic silver (Ag^0). Moreover, these composites act as capping and stabilizing agents, which helps prevent the silver nanoparticles from accumulating. This suggests that the biological components included in lemon juice perform dual functions of conformation and stabilization of AgNPs in an aqueous medium. Additionally, this study's results agree with those of other studies (10).

3.2.3. XRD analysis

The XRD spectra of the synthesized AgNPs are shown in (Figure 5). Bragg's diffraction peaks for AgNPs are observed at 38.4°, 44.53°, 64.45°, 79.77°, and 82.96°, equivalent to 111, 200, 220, 311, and 222, representative face centered cubic (FCC) structure of silver, respectively. The average crystallite size of AgNPs were calculated to be about 17.8 nm, using Scherer formula $D=0.94\ \lambda/\beta \cos\theta$, D represents the size of particles (nm), λ is the wavelength of the X-ray (Cu $K\alpha$ = 1.5406˚ A), β is the full width of the (XRD) peak at half height, and θ is the Bragg angle, that is (111), and θ is the position of that line in the pattern. The X-ray diffraction pattern demonstrated the lack of contaminants and the effective synthesis of pure Ag-NPs (31). The results of XRD analysis agree with the previously published research demonstrating the cubic structure of silver (32).

3.2.4. TEM analysis

TEM analysis of silver nanoparticles shows that AgNPs were mainly spherical with average sizes of 19 nm (Figure 6). AgNPs are usually present and readily aggregate during the manufacturing process. However, the fact that they were present and homogenous in our approach suggests that the AgNPs were stabilized using a capping agent, possibly the bio-organic chemicals found in lemon juice (33). Compared with the previous studies, the silver nanoparticles in this work were similar in shape and size to those obtained in studies (16,34). In comparison, it was smaller than AgNPs obtained in studies (35-37).

3.2.5. AFM analysis

Atomic force microscopy (AFM) is used to evaluate the surface topography and size of AgNPs. Figures (7 - A) and (7 - B) showed the three-dimensional (3D) and two-dimensional (2D) images of AgNPs showing spherical or mostly spherical shapes of nanoparticles with an average size was <100 nm. AgNPs have been shown as a topographic image.

3.2.6. PSA analysis

The AgNPs size and distribution profile are depicted in Figure 8 using particle sizes analysis (PSA). The result showed that most particle distributions (90%) have modest nanoscale sizes, with a mean particle size of 35 nm.

3.3. Antioxidant Activity

Oxidation is an important biological process that contributes to energy production in many living organisms; however, the out-of-control production of oxygen-derived frees radicals. Reactive oxygen species (ROS) cause damage to complex life molecules such as proteins, carbohydrates, nucleic acid, and lipids (38). This causes the emergence of several health problems, such as cancer, hepatic diseases, cardiovascular diseases, and renal failure (39). Anti-oxidants are agents that restrict the harmful effects of these oxidant reactions. These restrictions can include preventing the formation of free radicals or permanently removing free radicals, thus enhancing the immune defense and reducing the incidence of diseases (40). In this work, the anti-oxidant activity of lemon juice and synthesized AgNPs was examined using three different methods because a single, universal technique cannot accurately assess anti-oxidant capacity. The (DPPH) free radical scavenging activity is the better test for measuring anti-oxidant activity due to its simplicity, stability, and testing speed. In DPPH assay of lemon juice and AgNPs compared with ascorbic acid as standard. The results in (Figure 9) indicate that AgNPs have higher DPPH activity by 86% compared with lemon juice at 400 µg/mL concentration, and their activity increased with increased concentration. Therefore, we can say that AgNPs could be used as treatment agents for several diseases caused by oxidative stress. The result obtained in this study is in agreement with previous studies (41-43).

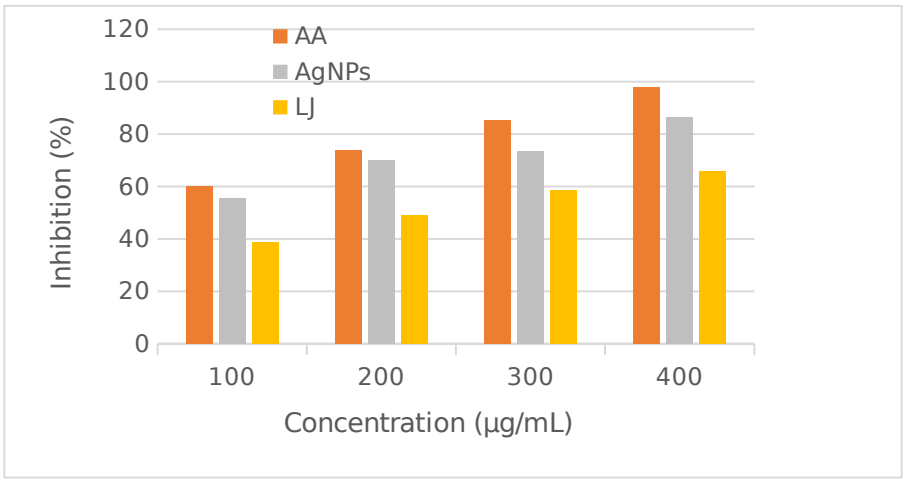

Figure 9: %Inhibition of DPPH free radical scavenging activity of Lemon juice (LJ), bio-synthesized AgNPs, and Ascorbic Acid (AA) at different concentrations.

In the total anti-oxidant method (phosphomolybdate), the basis of this method is the reduction of Mo(VI) to Mo(V) by the anti-oxidant compound and conformation of green phosphate, Mo(V) complex. The results presented in Figure 10 showed that AgNPs have more anti-oxidant activity than ascorbic acid or lemon juice.

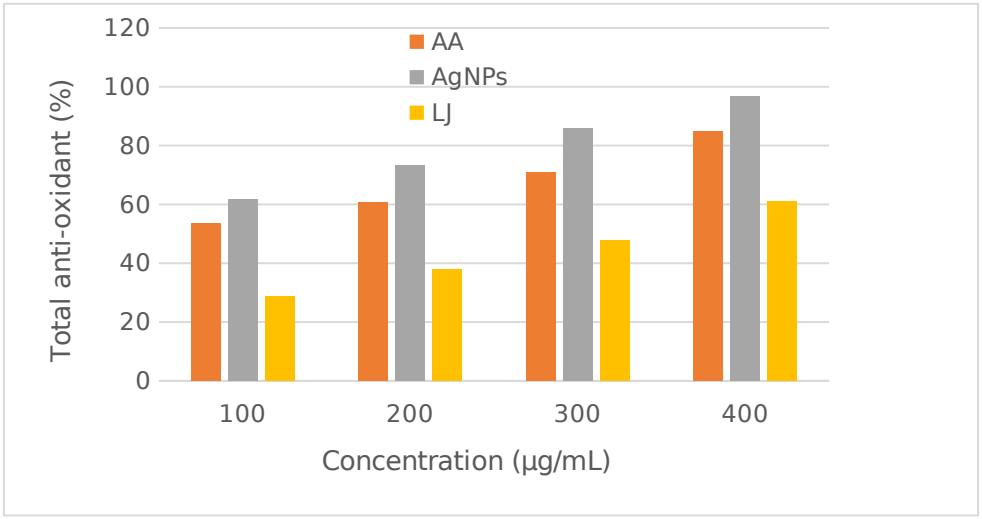

Figure 10: Total anti-oxidant capacity of Lemon Juice (LJ), biosynthesized AgNPs, and Ascorbic Acid (AA), at different concentrations.

In the reducing power assay**,** the ability of an anti-oxidant to donate an electron is measured when a reaction occurs between samples and potassium ferricyanide (Fe^{3+}) to generate potassium ferro-cyanide (Fe^{2+}), followed by a reaction with ferric chloride ($FeCl_3$) to form a ferric-ferrous complex (44). As shown in Figure 11, AgNPs have more anti-oxidant activity than lemon juice. The maximum and minimum optical densities were detected for AgNPs and lemon juice at 2.11 and 0.99 at 400 µg/mL.

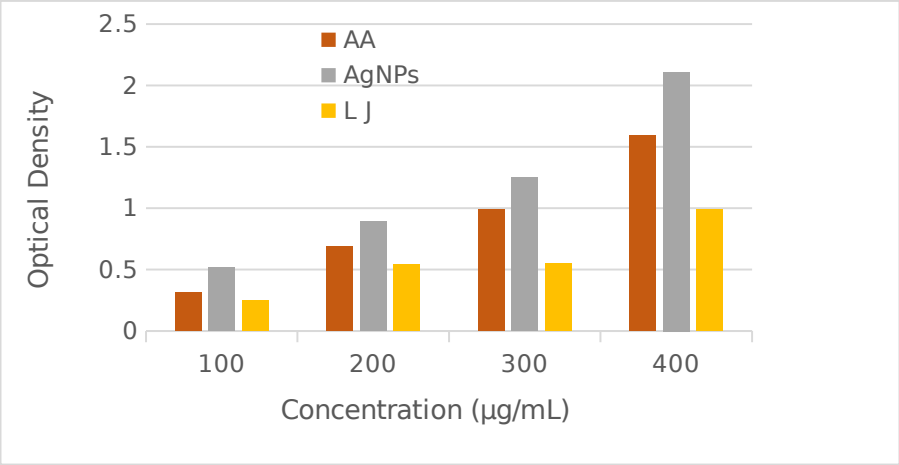

Figure 11: Reducing power activity of Lemon Juice (LJ), biosynthesized AgNPs, and Ascorbic Acid (AA) at different concentrations.

The analysis of variance (ANOVA) test findings showed statistically significant differences between lemon juice and the AgNPs when compared to ascorbic acid as a reference.

We conclude from this study that AgNPs synthesized from lemon juice have a stronger anti-oxidant capacity than the juice. This work highlights the importance of the therapeutic value of AgNPs synthesized from lemon juice as a source for developing anti-oxidant drugs.

3.4. Anti-Bacterial Effectiveness of Silver Nanoparticles

The anti-bacterial activity of lemon juice and synthesized AgNPs is shown in Figure 12 and Figure 13 against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus (S. aureus*), respectively. Also, the values of zone of inhibition observed round the wells of synthesized AgNPs are given in Table 1 as (Mean±SD). and lemon juice and silver nitrate (control) are given in Table 2. The results in Table 2 showed that the effect of $AgNO_3$ for the dose of 60 µL against *E. coli and S. aureus* was low, and the reason for this may be due to its very low concentration (1 mM). Similarly, when comparing the effect of lemon juice and AgNPs as anti-bacterial, the results indicated a slight effect of lemon juice compared with the activity of AgNPs as an anti-bacterial for all doses (Tables 1 and 2). Although silver has anti-bacterial properties, lemon juice is rich in biologically active compounds such as flavonoids, carotenoids, and phenolic compounds, which act as natural antibiotics against pathogens in the body, such as bacteria (10,43). AgNPs differed significantly at $p < 0.05$ from lemon juice and $AgNO_3$ (45). AgNPs have activity of silver NP derived from lemon juice showed enhancement in activity due to the synergistic effect of silver and biologically active compounds of lemon juice. As shown in the data in Tables 1 and 2. This is in agreement with many studies; Samreen, Muzaffar, et al. (2018) conducted a study on different bacterial strains, and the results indicated a significant inhibition of AgNPs compared to the absence of any inhibition for lemon extract (45). Furthermore, Mosae Selvakumar, Antonyraj, et al. (2016), and Niluxsshun, Masilamani, et al. (2021) discovered that lemon extract has slight activity while the zone of inhibition around AgNPs was significantly higher than the extract and controls and nearer to the inhibition of standard antibiotics (5,19). Also, it has been observed that AgNPs were more sensitive to, Gram-positive bacteria (*S. aureus*) than Gram-negative bacteria (*E. coli*). However, the mean inhibition zone diameter against *E. coli* for this study at a dose of 100 µL was higher compared to other studies such as *lemon* extract 3.0 mm (46), *Nicotiana tobaccum* leaf 4.0 mm (47), and *Neem* leaves 6.0 mm (48). Moreover, the anti-bacterial activity of AgNPs was found to increase with increasing doses (Table 1). This may be because of the different sizes and shapes of nanoparticles, especially AgNPs, which are spherical and have a high surface to volume ratio to interact with the cell walls of pathogens giving the best anti-microbial activity (49). This result agrees with several works of literature revealing that nanoparticles are more active against Gram-positive bacteria than Gram-negative bacteria (50, 51). And that the bactericidal property of nanoparticles depends entirely on the dose and particle size (52). The mechanism underlying the anti-bacterial activity of AgNPs is very complex. This can be explained as silver ions (Ag^+) acting as an anti-bacterial by interacting with the peptidoglycan cell wall and that the thickness of the peptidoglycan layer differs in species of bacteria (Gram-negative bacteria and Gram-positive bacteria). This is the main reason why bacteria are differently affected by the attack by AgNPs (29). Other investigations have reported that the positive charge of AgNPs interacts with the negative charge on the cell wall of bacteria, which leads to changes in the morphology of the cell wall and increases the permeability of the membrane by making pores and thereby causing the death of bacteria (53,54). Also, it is said that Ag^+ interaction with amino ($-NH_2$) and thiol (-SH) groups of protein on cell membrane, which results may be responsible for the induction of ROS, which leads to the inhibition of respiratory enzymes and, consequently death (5). Ag^+ affects

bacterial cells through several mechanisms, including cell wall leakage, interaction with bacterial enzymes and DNA, ribosomal destabilization, and interruption in the electron transport chain, resulting in cell death (55).

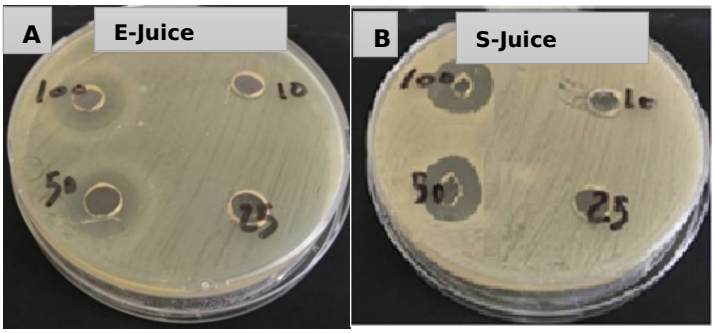

Figure 12: Anti-bacterial activity of lemon juice against (A) E= *Escherichia coli*, (B) S= *Staphylococcus aureus*.

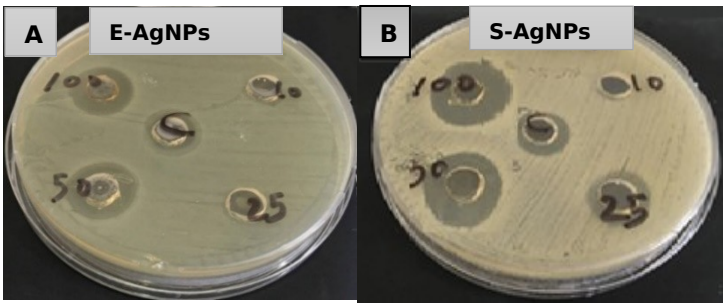

Figure 13: Anti-bacterial activity of AgNPs against (A) E= *Escherichia coli*, (B) S= *Staphylococcus aureus*.

Table 1: Effect of AgNPs on the growth of bacterial species.

Dose of lemon juice (*µL*)	*E. coli* (Mean±SD)	*S. aureus* (Mean±SD)	Dose of AgNPs (*µL*)	*E. coli* (Mean±SD)	*S. aureus* .(Mean±SD)
10	0.00±0.00	0.00±0.00	10	1.38±0.41	4.11±0.39
25	0.00±0.00	5.17±0.29	25	3.93±0.83	13.13±0.32
50	10.2±0.62	11.4±0.40	50	17.5±0.5	20.47±0.45
100	13.03±0.35	14.5±0.45	100	22.3±0.57	25.87±0.96

Table 2: Effect of AgNPs, lemon juice (control), and $AgNO_3$ on the growth of bacterial species.

Dose of solution (µL)	Zone of inhibition (mm) AgNPs		Lemon juice		$AgNO_3$	
60	***E. coli* (Mean±SD)**	***S. aureus* (Mean±SD)**	***E. coli* (Mean±SD)**	***S. aureus* (Mean±SD)**	***E. coli* (Mean±SD)**	***S. aureus* (Mean±SD)**
	19.0±0.26	22.67±0.32	11.4±0.62	13.2±0.40	5.53±0.50	6.06±0.60

3.5. Anti-cancer activity

In our study, the anti-cancer activity of the green synthesis AgNPs at different concentrations levels 10, 25, 50, 75, and 100 µg mL^{-1} was studied against a human breast cancer cell line MCF-7. The result showed that AgNPs had positive anti-cancer effects on MCF-7 cancer cells. In figure 14, the nanoparticles showed dose-dependent anti-cancer activity. The required concentrations for 50% inhibition of the cell viability (IC_{50}) were calculated graphically; it was about 47 µg/mL for AgNPs, while IC_{50} was recorded at 215 µg/mL for lemon juice. AgNPs showed significant inhibition of cell growth of about 80%, while the lemon juice showed very low cytotoxicity of 29%. According to the optical microscopic analysis of cancer cells, exposure to an aqueous suspension of AgNPs caused significant morphological changes in the cells, including aggregation, cellular shrinkage, and rounding, compared to untreated cells. The result obtained in this study was in agreement with another study (56).

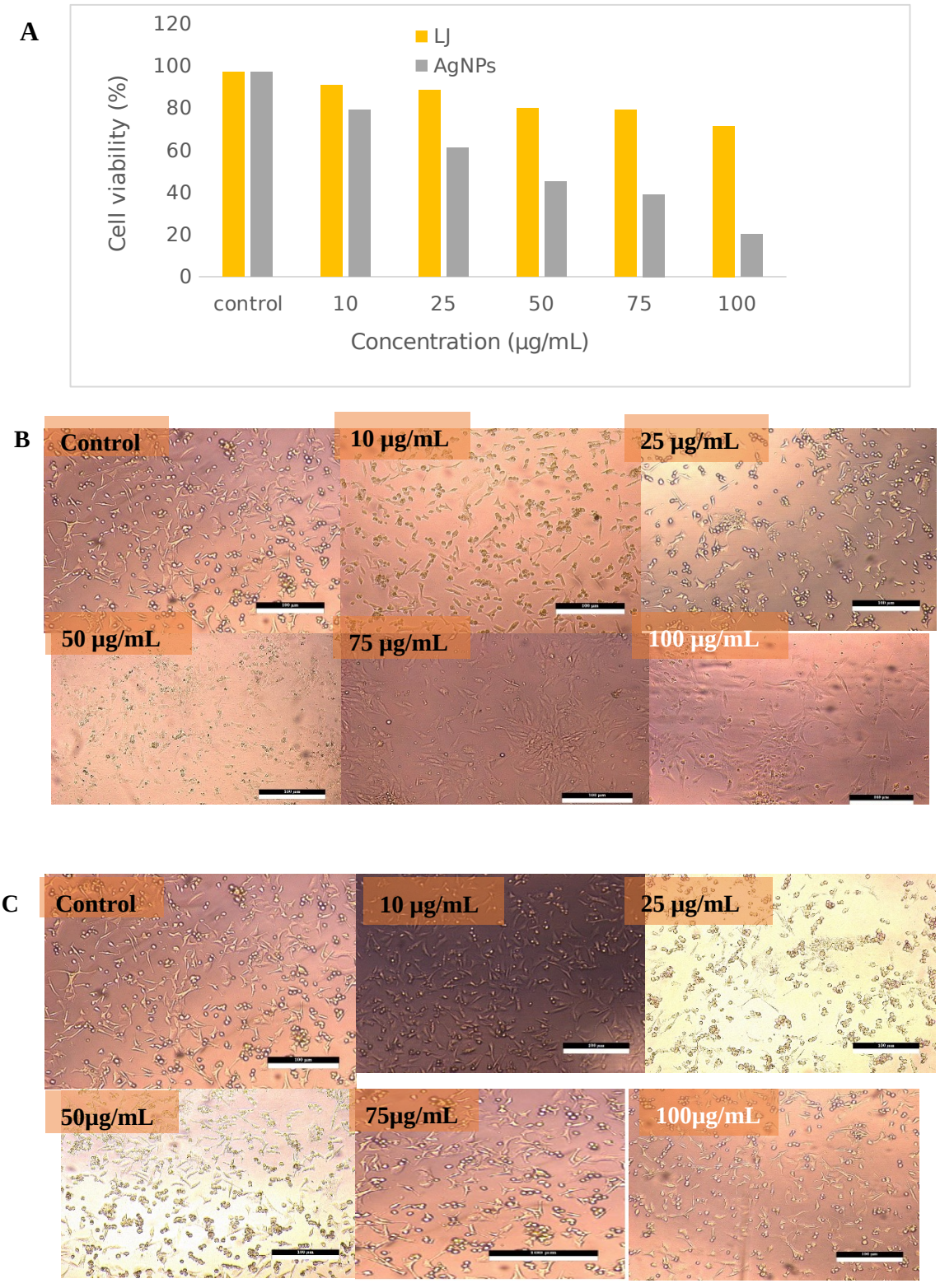

Figure 14:- Cytotoxic activity of AgNPs and lemon juice (LJ) MCF-7 cell line **(A)** Histogram demonstrating the cell viability percentage of MCF-7 cells at different concentrations 10, 25, 50, 75, and 100 µg/mL of AgNPs and lemon juice**. (B)** Photomicrographs showing changes in MCF-7 cell line after being treated with different concentrations 10, 25, 50, 75, and 100 µg/mL of AgNPs, and **(C)** Photomicrographs showing MCF-7 cell line after being treated with different concentrations 10, 25, 50, 75 and 100 µg/mL of lemon juice.

One of the potential mechanisms for inducing apoptosis in cancer cells involves the synthesis of AgNPs, which trigger reactive oxygen species (ROS) and, in turn, the production of toxic free radicals and the disruption of mitochondria, both of which result in cellular apoptosis (57). Many researchers have

reported the cellular internalization of AgNPs, according to the surface properties of AgNPs. AgNPs carry a positive charge, while normal-cancer cell membranes contain substances such as lipids (especially PO_4^{3-} groups) with a negative charge; Having an opposite charge is responsible for cellular internalization and thus causes cancer cell death (55). In another study conducted by one of the researchers on the breast cancer cell line, the study reported that silver nanoparticles (GSNPs) manufactured from Mentha-arvensis induce cytotoxicity by mediating caspase 9-dependent apoptosis in breast cancer cell lines (58). In a study conducted by one of the researchers on silver nanoparticles, it was found that these AgNPs nanoparticles can contribute to cellular degradation by autophagy and thus cause cancer cell death (59). Three methods have been proposed. To take advantage of the biosynthesis of nanoparticles that cause apoptosis in cancer cells, that is mitochondrial damage, activation of death transmembrane receptors, and injury of the endoplasmic reticulum (60-62).

4. CONCLUSION

In this study, the Ag-NPs were successfully synthesized by green synthesis using lemon (*Citrus limon*) juice with different groups of phytochemicals such as phenols, terpenes, and flavonoids. The characteristics of the biosynthesized AgNPs were measured by different equipments (UV-visible spectrophotometer, FT-IR spectrometer, TEM, AFM, and PSA). Moreover, several medicinal aspects of these NPs, including anti-oxidant, anti-bacterial, and anti-cancer activity, were evaluated, and the AgNPs showed potential anti-microbial activity against Gram-positive and Gram-negative bacteria. The anti-oxidant activity of AgNPs showed the highest effect. In addition, the results indicate that AgNPs had anti-cancer activities against human breast (MCF-7) cancer cell lines in a dose-dependent manner. These green methods of AgNP formation open a new window for treating many infectious diseases and cancers.

5. CONFLICT OF INTEREST

Regarding the current manuscript, the researchers affirm that there are no conflicts of interest

6. ACKNOWLEDGMENTS

The researchers are grateful to the University of Diyala and the Faculty of Sciences for supplying the resources necessary for this study.

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