Mustansiriyah Journal of Pure and Applied Sciences
https://mjpas.uomustansiriyah.edu.iq
Print ISSN: 2957-9910 Online ISSN: 2957-9929

Antibacterial Activity of Silver Nanoparticles and Lemon Peel Mixture on E-coli Bacteria

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A B S T R A C T

In this work, pulsed laser ablation in liquid (PLAL) technique is used to prepare the Ag nanoparticles in water, and prepare lemon peel extract by plasma discharge in a fabricated DC plasma jet in record time at two intervals (5,15) minutes, using Nd:YAG laser wavelength 532 nm at three energies (500, 700, and 900) mJ. Where mixed the colloidal Ag nanoparticles with lemon peel extract in different conditions and applied it on the antibacterial activity *Escherichia coli* (*E. coli.*). The antibacterial activity was performed by the agar disc diffusion method. The study confirmed that there was a significant improvement in bacterial inhibition in the case of mixing. Study the UV before and after mixing we note increases in intensity. the inhibition increasing with energy, the best inhibition take place at 900 mJ, at 15 minutes.

Keywords: *silver nanoparticles, a new method to prepare the extract, lemon peel extract, inhibition E. coli bacteria*

1. Introduction

Lemon is a great medicinal plant. It is primarily employed for its alkaloids, which have anticancer and antimicrobial activities in crude extracts of several parts of Lemon (leaves, stem, root, juice, peel, and flower) against clinically important bacterial strains.[1]. Lemon (Citrus lemon) peels simply disintegrate and pollute the environment. Lemon (Citrus lemon) peel is high in nutrients and may be used as a medicine as well as a nutritional supplement. These products have fewer side effects, are easy to find, and are fairly priced. Since the number of germs antibiotic resistant is increasing, there is constantly a hunt for an alternative medicine. [2]. In the present study the fruit peel of Citrus lemon was selected for assessing its antibacterial properties[3]. However, there are many fewer observations on the bactericidal properties of nanoparticles created by laser ablation in liquid (LAL). This approach, known

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as 'agnps,' although other forms of synthesis are also deemed green, allows for the manufacture of nanoparticles. [4]. Silver nanoparticles are employed for a variety of reasons, including their without chemical byproducts and stabilizing molecules high conductivity, chemical stability, catalytic activity, and antibacterial activity [5]. Silver nanoparticles offer antibacterial properties against bacteria, viruses, and other microbes [6]. Ag NPs have been shown to have antibacterial action against Escherichia coli [7]. Among the numerous types of non-thermal plasmas (NTP), air pressure plasma jet (APPJ) appears to be the most promising for a variety of applications, include hydrophilic and chemically active material modification [8, 9], surface etching of a material, Inactivation of bacteria, skin sterilization[10], treatment of wounds and caries[11], apoptosis of cancer cells and blood coagulation Pulsed direct current (DC), alternating current (AC), radio frequency (RF), and microwave power sources can all be used to create and power APPJ[12].

2. Materials and methods:

2.1. Lemon peel extract preparation:

Fig (1) shows The experimental setup of Dc plasma jet that consisted of a high voltage DC power supply (27 kV) and a stainless steel needle that acted as the cathode., 1 gram of lemon peel was taken and washed with distilled water and was immersed in a glass beaker containing 5 ml of deionized water (between the two electrodes). Argon gas with a purity of 99.99% was used as the gas discharge, and the gas flow was fixed at 2 (L/min) using a flowmeter to control the gas flow. Where the period of exposure to the plasma once was 5 minutes and the second time was 15 minutes as shown in fig.2.The way that used to prepare the extract is easy, clean and get the extract in a record time that has not been used before.

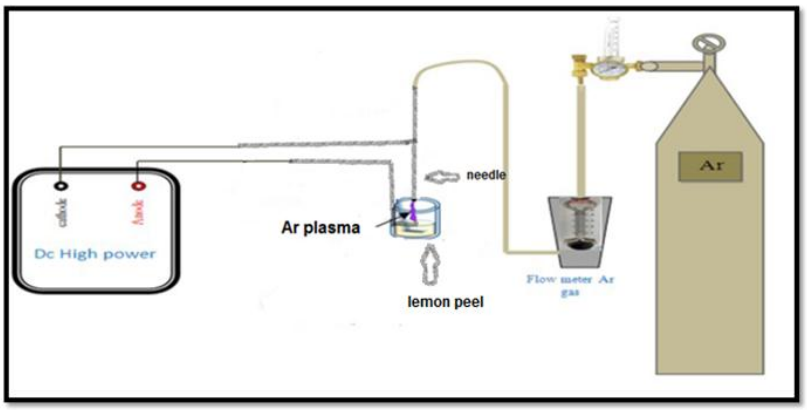

Figure 1. schematic of *DC* plasma Jet system

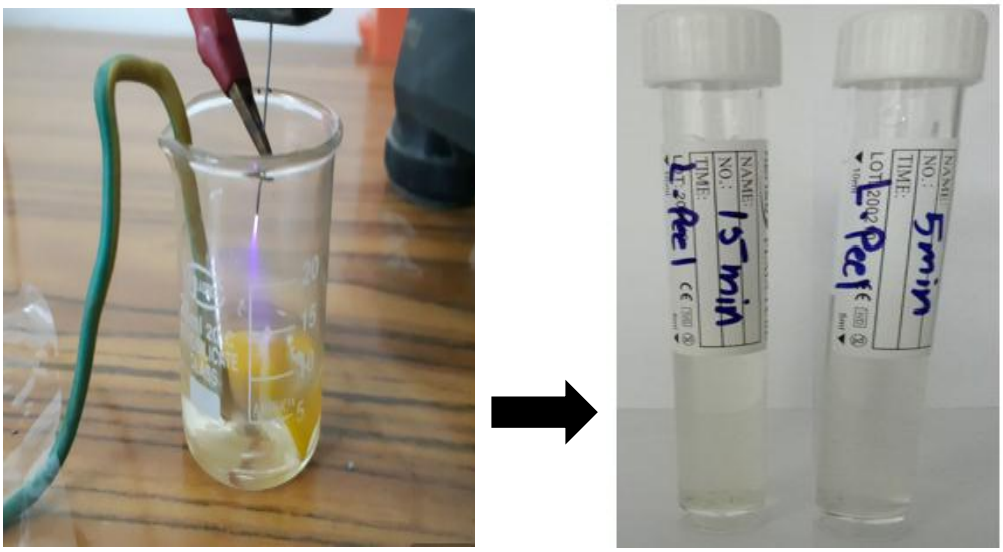

Figure 2. Stage of preparation the lemon peel extract by Dc Jet plasma

2.2 Ag Nanoparticles preparation

Silver powder with a purity of 99.99 percent are shown in fig. (3); was pressed into a circular geometric form. Compression was done at room temperature. Where there were placed inside a stainless steel cylinder, and pressure was applied for ten minutes using a hydraulic piston with a compressive strength of (6.5) tons to convert it into a disc with a thickness of 3 mm and a diameter of 10 mm. The samples were bombarded at a 45-degree angle with the target surface by a Nd:YAG pulse laser (9 nanosecond pulse duration time, 4Hz. frequency, and wavelength. 532nm).

Figure 3. Silver powder before and after pressing.

The experimental approach based on pulsed laser ablation in the liquid atmosphere. The nanoparticles were synthesized at the bottom of a glass container containing 5 mL of distilled water by pulsed laser ablation of the Ag target. The height of the distilled water above the target was 10 mm. The target was irradiated with Nd: YAG laser irradiation of (500, 700, 900) mJ per 1000 pulses as shown in fig. (4)

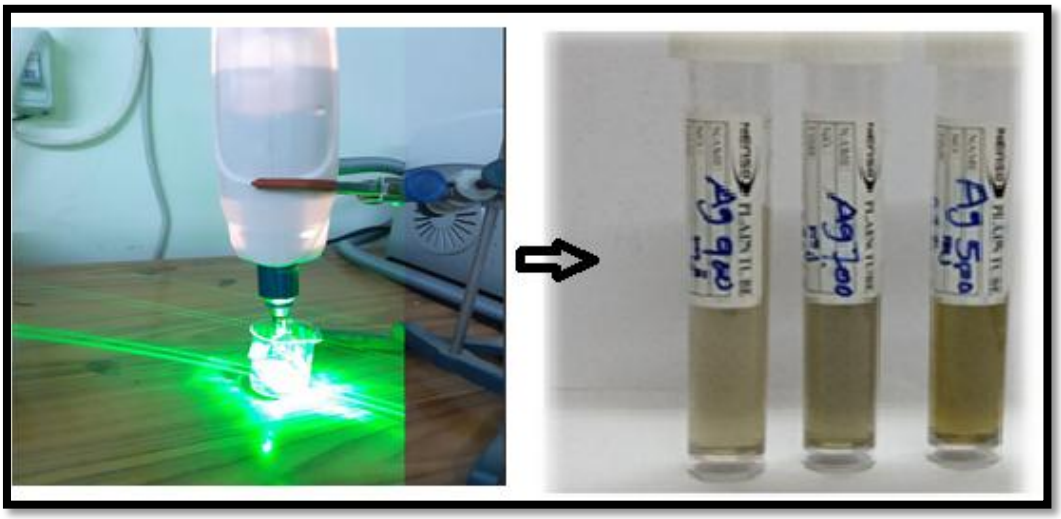

Figure 4. Stage of preparation Ag NPs at different laser energy (500-700- 900) mJ at 1000 pulse by (PLAL) method.

To prepare the mixture, take (1ml) of lemon peel extract and mix with (1ml) of a colloidal solution of silver nanoparticles to each energy (500,700 and 900) mJ at time 5min we get three tubes of (solution of silver + extract of cinnamon). Then **rework** and mix (solution of silver + lemon peel extract) for three energies (500,700 and 900) mJ at 15 min as shown in fig. (5).

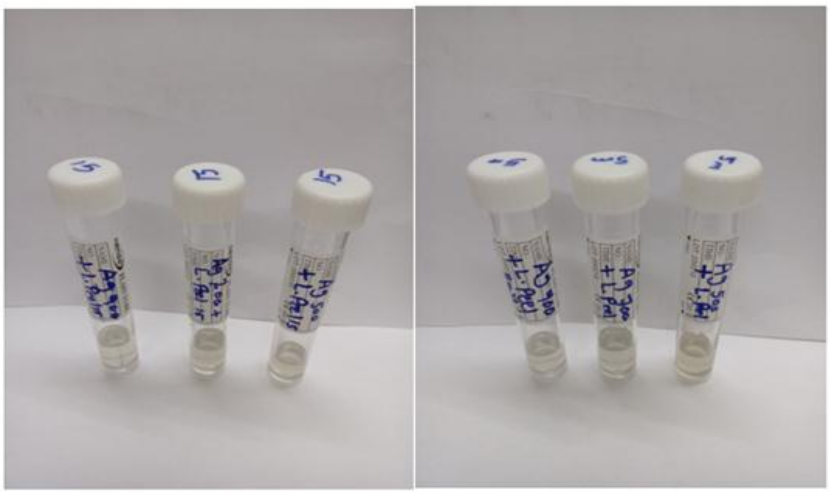

Figure 5. Nanoparticles liquid of Ag in different energy (500,700,900) +lemon peel extract prepared in 5min and 15 min.

2.3. Isolation and characterization of human pathogenic bacteria

A clinical Escherichia coli (*E. coli.)* isolate which isolated from urine sample. The bacterial isolate was identified by using selective culture media included MacConkey agar and Eosin Methylene Blue (EMB) agar media. The antibacterial activity of silver nanoparticles and

lemon peel mixture against E. coli has been investigated using agar well diffusion method on Muller-Hinton agar. Briefly, the bacterial isolate was cultured and incubated at 37 °C for 24 h then the bacteria were diluted (10^8 CFU/mL or 10^5 CFU/well) and spread on Muller-Hinton agar. Wells with size of 8 mm were cut on Muller-Hinton agar plates and separately filled with 100 µl from different energies (900, 700 and 500) for Ag-NPs, then incubated at 37°C for 24 h to measure the inhibition zone in mm.

3.Result and Discussion

X-Ray. Diffraction (XRD) was used to determine the lattice and the structure of Ag powder before and after laser ablation in distilled water. Checked for material where role the important technique to investigate the structural properties and crystalline nature. Fig. (5) Shows X-Ray Diffraction (XRD) analysis of the target pattern. Generated silver NPs by using laser ablation in distilled water. Fig. (5a) illustrated the x-ray form AG NPs before ablation and Fig. (5b) represents the x-ray of AgNPs after laser ablation. Four distinct peaks may be found in each of the two patterns for silver observed at 2θ = 38.2509, 44.4557, 64.6092, and 77.5520 that referred to (111), (200),(220) and (311) planes of Ag, respectively, demonstrate the presence of a pure cubic phase in the fcc structure according to (JCPDS. No. 004-0783). The prepared NPs by laser ablation have nearly the same peaks with a slight shift due to some strains in a lattice. We note that there is a decrease in intensity, which indicates less crystallization.

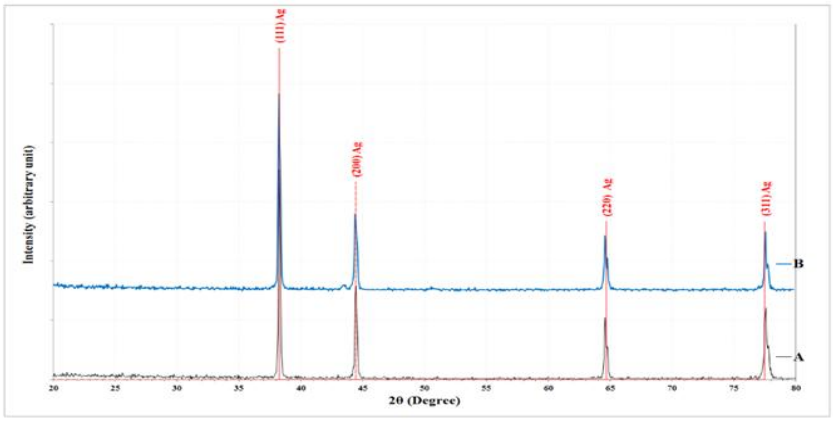

Figure 6. XRD pattern of a pure Ag Target A) before pulse laser ablation B) After pulse laser ablation.

3.1. Uv-vis absorbance of silver NPs

We obtained UV/Visible absorption spectra in the wavelength range of 300 to 900 nm to demonstrate the production process of Ag-NPs. Fig. 7 shows the absorption curves for colloidal contain silver nanoparticles prepared by various laser energies (500, 700, and 900) mJ.). It appears from the figure that the curves contain peaks that belong to the surface Plasmon resonance phenomenon and that the location of the peaks lies within limits (400-420) nm. It is also shown that the location of the peaks is red shifted from 400 nm to 420 nm

when the laser energy increased from 500 to 900 mJ, i.e., in the direction of increasing wavelength, and the reason for this is the increase in particle size

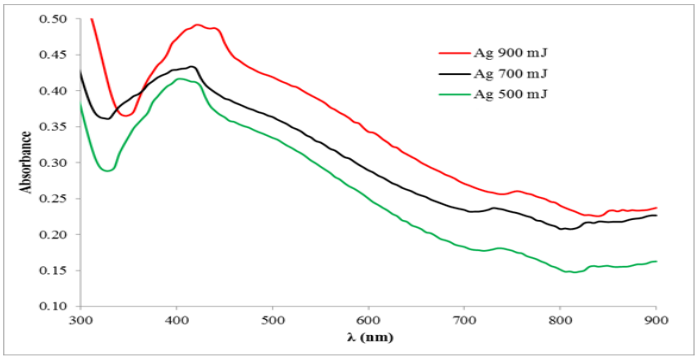

Figure 7: UV–vis spectra of Ag nanoparticle liquids in different energy (500,700,900) mJ

3.2. Uv-vis absorbance of silver NPs after adding lemon peel extract

From the fig.(8,9) it is observed that the surface Plasmon resonance(SPR) is broadened and shifted. The red shift in the SPR wavelength is consistent with the Mie theory for the optical properties of silver nanoparticles[13] This red shift in the SPR is around (400- 415) and (400-410) for Ag NPs after adding with lemon peel extract prepared at 5 and 15 min. respectively, may be explained in terms of the increased refractive index of the medium experienced by the silver particles in the organic solution when compared with silver particles in aqueous solution The increase in intensity of the surface Plasmon band for silver NPs after adding lemon peel extract prepared at 5,15 min., may be due to loss of surface-bound biomolecules from the lemon peel extract due to disturbance in the equilibrium between surface-bound and free biomolecules [14].

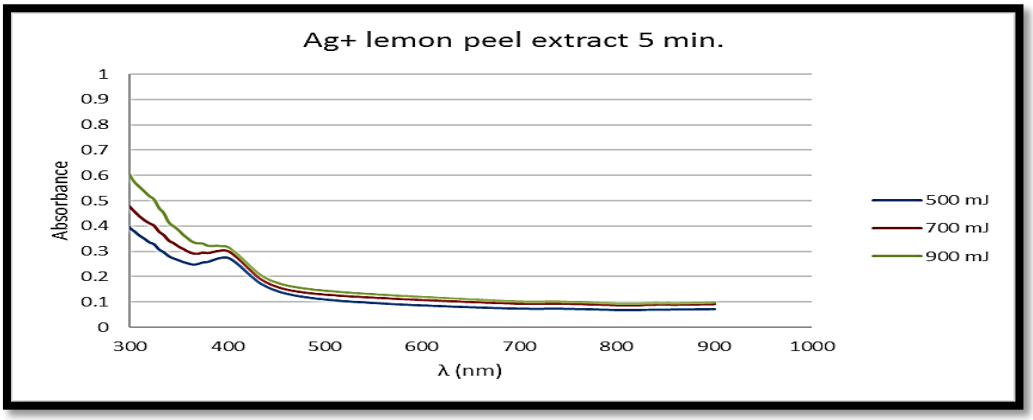

Figure 8. UV–vis spectra of Ag nanoparticle liquids in deferent energy (500,700,900) mJ +lemon peel extract prepared at 5 m

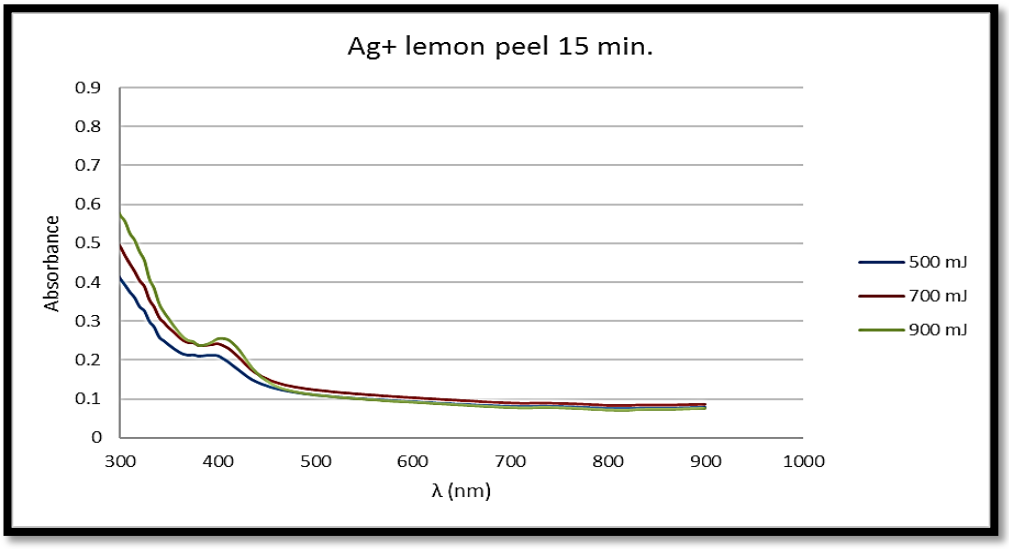

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Figure 9. UV–vis spectra of Ag nanoparticle liquids in deferent energy (500,700,900) mJ +lemon peel extract prepared at 15 min.

3.3. Antibacterial activity of silver NPs

The anti-bacterial activity was tested for silver nanoparticles prepared at three laser energies (500, 700 and 900) and compared with silver nanoparticles after mixing them with lemon peel extract prepared at two time intervals (5 minutes and 15 minutes) as seen in Table1. The results showed a significant improvement in the bacterial inhibition of silver nanoparticles after mixing them with lemon peel extract prepared at 15 minutes While silver nanoparticles mixed with lemon peel extract prepared for 5 minutes showed mild bacterial inhibition as shown in fig (10).

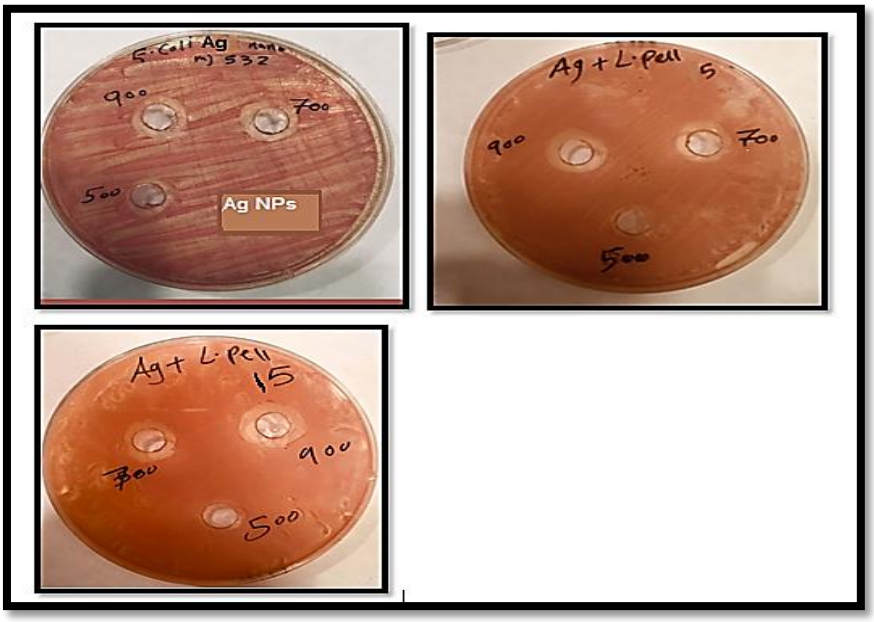

Figure 10. Image of the inhibition zone of silver nanoparticles at energies (500 - 700 - 900) mJ of *E. coli*. Bacteria after and before adding lemon peel extract prepared at 5min. and 15min.

Table 1. inhibition zone of Ag NPs before and after adding lemon peel extract against *E.coli.* Bacteria.

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No. of pulses	frequency (HZ)	λ (nm)	Energy (mJ)	inhibition zone (mm)Before adding lemon peel extract	inhibition zone (mm)after adding lemon peel extract at 5 min.	inhibition zone (mm)after adding lemon peel extract at 15 min.
1000	4	532	500	**8**	**8**	**8**
1000	4	532	700	**11**	**11**	**13**
1000	4	532	900	**13**	**15**	**18**

4. Conclusion

Lemon extracts are effective antibacterial agents against microbes. They are natural, inexpensive, and risk-free. The study succeeded in obtaining lemon peel extract in an easy and fast way through a plasma generation system (DC plasma jet system), where the extract was obtained in two time periods of 5 and 15 minutes, as well as silver nanoparticles was produced with three different energies (500-700-900) by pulsed laser ablation. Also, a comparison was made between the anti-bacterial effect of E. coli on each of the silver nanoparticles prepared at three energies after mixing them with lemon peel extract prepared

at two time intervals after mixing with lemon peel extract. It was concluded that the best bacterial inhibition was obtained by mixing the prepared silver nanoparticles (900mJ) with the lemon peel extract prepared with a time of 15 minutes.

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