RESEARCH PAPER

Antibacterial Activity of Silver Nanoparticles and Cinnamon Bark Extract Prepared by Pulse Laser Ablation and Plasma Jet Methods

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ABSTRACT

This study explores a new facile method of obtaining plant extract using a atmospheric pressure and a way of producing Ag nanoparticles (Ag NPs) using the pulsed laser ablation(PLA) in distilled water. A 532 nm Nd:YAG laser at varying energies (500, 700, and 900 mJ) was used to prepare Ag NPs. Cinnamon bark extract was prepared by using an atmospheric pressure plasma jet in cinnamon bark and distilled water mixture with exposure times, (5 and 10 min). The physical characterization of these silver nanoparticles and Cinnamon bark extract were verified using X-ray diffraction patterns (XRD) , Field Emission Scanning Electron Microscopy(FESEM) and Ultraviolet-visible (UV-Vis) spectroscopy. The antibacterial activity of Ag NPs and a mixture (Ag NPs with Cinnamon extract) were evaluated for NPs obtained by PLA using different laser energy in distilled water, on the Gram-negative isolate (Escherichia coli). The results and images of inhibition zone diameter showed that the NPs have synergistic effects on the studied bacteria it increase with the increases of laser energy. A significant improvement in the bacterial inhibition of silver nanoparticles after mixing them with cinnamon bark extract.

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INTRODUCTION

Nanoparticles NPs are suitable for a variety of applications due to their small size, large surface area, and unique chemical and physical properties. [1-5]. Due to their improved properties, metal and metal oxide nanoparticles have recently attracted a lot of attention among essential materials, leading to a variety of applications in various fields [6, 7]. Medicinally, cinnamon is used in the treatment of diarrhea, flatulent dyspepsia, poor appetite, low vitality, kidney weakness and rheumatism, influenza, cough, bronchitis, fever, arthritic angina, palpitations, hypertension, and nervous disorders, stimulating the circulatory system and capillary circulation, spasms, vomiting and controlling infections, reducing blood sugar levels in diabetics and as a skin antiseptic[8]. Inhibitory effect restored to the nature of the material contained in cinnamon[9]. As the presence of compounds alkaloids, tannins, volatile oils, saponins, tenpins flavones, and coumarins which is one of the antibacterial was inhibiting the growth of bacteria, alkaloids characterized by their ability to break into the bacterial cell and interfere with DNA, while working tannins on the inhibition of enzymes and transport proteins in the cell membrane saponins are working to reduce the proportion of sugar within the bacteria that lead to bacterial cell death

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as well as for to glycosides which have a similar effect but has a lesser effect [10-12]. Various methods of preparation of nanoparticles have been developed and they are suitable for synthesis of nanoparticles in different sizes and shapes. Pulsed laser ablation is a method for fabricating nanoparticles with advantages over chemical methods. For example, the method reduces chemical contamination in the final nanoparticles. The generation of NPs from almost any metal or semiconductor is made simple and reliable by laser ablation of a solid target in liquids [13-14]. In general, non- thermal plasmas have gained a broad interest due to advantages such as low cost, low operating temperature, smaller volume, and lack of solvents. Nonthermal plasma is used extensively for surface activation or modification, since the ions, atoms, and molecules are comparatively cold (near room temperature) and do not cause any thermal damage to the surfaces of heat-sensitive materials, such as polymers and biological tissues . This gives it the potential to efficiently amend the surface properties without distressing the bulk properties of the material. Various mechanisms occur in parallel when a polymer surface is exposed to plasma environment: surface etching, cross-linking, and chemical modifications [15-19]. Among the numerous types of NTP, air pressure plasma jet (APPJ) appears to be the most promising for a variety of applications, including hydrophilic and chemically active material modification [20, 21], surface etching of a material, Inactivation of bacteria, skin sterilization [22], treatment of wounds and caries [23] 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 [24]. In our study, we discuss the synthesis of silver nanoparticles and Cinnamon bark extract using PLA and plasma jet techniques technique respectively. Then, the synthesized specimens were characterized by using different techniques. The antibacterial activities of silver nanoparticles and mixture with Cinnamon bark extract were study against human pathogenic bacteria of Escherichia coli.

MATERIALS AND METHODS

Cinnamon bark extract preparation

Aqueous cinnamon bark extract (Fig. 1a) was prepared by washing 1g of cinnamon bark with distilled water and adding 5 mL distilled water. This solution was exposure to atmospheric pressure plasma jet to obtain the extract, as shown in Fig. 1b. The atmospheric plasma jet allows the gas to flow into a small tube with argon gas flow rate of 2 l/min. and the higher voltage (27 kV) under atmospheric pressure. The sample was exposed to the plasma jet once for 5 min and another for 15 min to obtain the extract, as shown in Fig. 1c. Our extract preparation method is simple and, to the best of our knowledge, the fastest compared to other methods.

Ag nanoparticles preparation

The silver powder (99.99% purity) used in the work and pressed into a disc target with a thickness of 3 mm and a diameter of 10 mm using a stainless steel cylinder. A pressure of 6.5 tons was applied for 10 min using a hydraulic piston in air and at room temperature. The surface of the Ag target was bombarded at a 45° angle by an Nd:YAG pulses

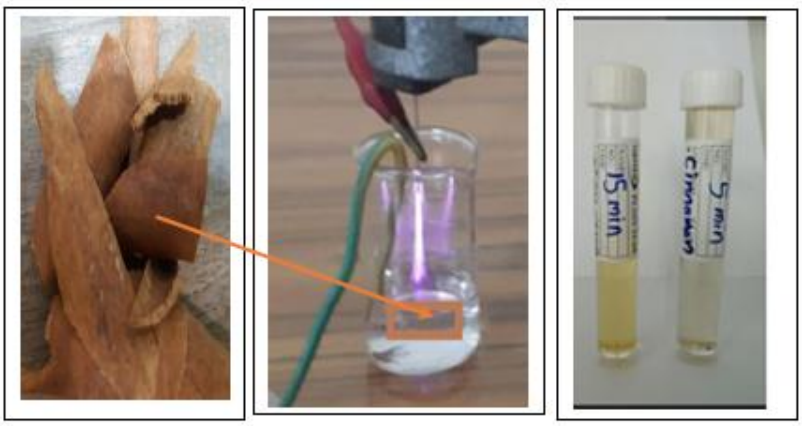

Fig. 1. a) bark cinnamon, b) cinnamon under plasma jet c) cinnamon extract in 5 min and 15 min.

laser (9 nanosecond pulse duration, 4 Hz frequency, and 532 nm wavelength). The experimental approach was based on PLA in distilled water. The nanoparticles were synthesized at the bottom of a glass container containing 5 mL of distilled water. The height of the distilled water above the target was 10 mm. The target was irradiated with Nd:YAG laser at 500, 700, 900 mJ and applied 1000 pulses.

Preparation of AgNps with cinnamon extract mixture

To prepare the mixture, take (1ml) of extract cinnamon and mixed 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) as shown in Fig. 2a. Then rework and mixed (solution of silver + extract of cinnamon) for three energies (500,700 and 900) mJ at 15 min as shown in Fig. 2b.

Isolation and characterizations of human pathogenic bacteria

A clinical *Escherichia coli* was isolated from a urine sample. The bacteria were identified using selective culture media, including MacConkey and Eosin Methylene Blue (EMB) agar media. The antibacterial activity of Ag NPs against *E. coli* was investigated using the agar well diffusion method on Muller-Hinton agar. Briefly, the bacterial isolate was cultured and incubated at 37 °C for 24 h. The bacteria were then diluted (10^8 CFU/mL or 10^5 CFU/well) and spread on Muller-Hinton agar. Wells with a size of 8 mm were cut on Muller-Hinton agar plates and separately filled with 100 µL of Ag-NPs from different energies 900, 700, and 500 mJ. The wells were then incubated at 37 °C for 24 h to measure the inhibition zone (expressed in mm).

RESULTS AND DISCUSSIONS

X-ray diffraction analysis

X-Ray Diffraction (XRD) was used to determine the lattice and the structure of Ag powder before and after laser ablation in distilled water. Fig. 3a illustrated the X-ray patterns form Ag NPs before ablation and Fig. 3b represents the X-ray patterns 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. Line broadening increased after ablation, indicating reduced crystalline size according to Scherer's equation [1,2].

UV-Vis absorbance spectroscopy

Ultraviolet-visible (UV-Vis) absorbance spectroscopy.is a powerful tool for analyzing and studying Plasmon resonance in metallic nanoparticles, including peak positions and forms. We obtained UV-Vis absorption spectra in the wavelength range of 300 to 900 nm to demonstrate the production process of Ag-NPs. Fig. 4 shows the absorption curves of the colloidal silver nanoparticles prepared under various laser energies (500, 700, and 900 mJ). The surface Plasmon resonance (SPR) influenced the absorption spectra of metal nanoparticles. It appears from the figure that the curves contain peaks that belong to the SPR phenomenon and that the location of the peaks lies within limits

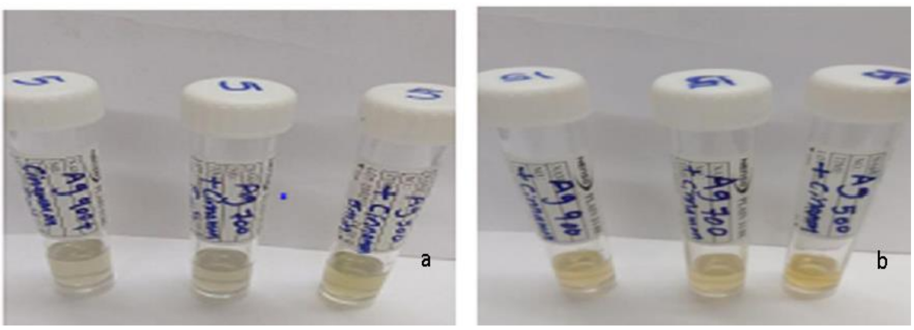

Fig. 2. Colloidal solution of silver nanoparticles with cinnamon extracts at energies (500,700,900) a) 5min. and b) 15 min prepared at (a) 5 min and (b) 15 min by jet plasma.

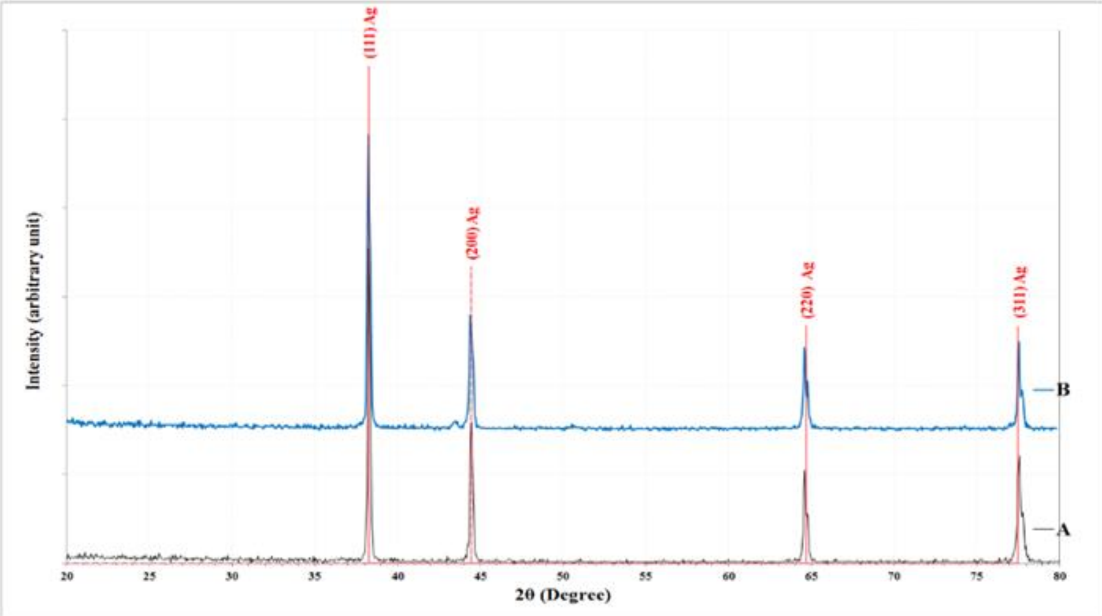

Fig. 3. XRD pattern of Ag NPs via a) before, and, b) after pulsed laser ablation

400–420 nm). The location of the peaks was also red-shifted from 400 to 420 nm when the laser energy increased from 500 to 900 mJ, i.e., in the direction of increasing wavelength. The red shift is due to the increase in particle size. Hence a growth attributed to particle coagulation [1, 2].

The SPR was broadened and shifted, as shown in Figs. 5,6. This red shift in the SPR wavelength is similar with the Mie theory for the optical characteristics of copper nanoparticles and may be understood by the higher refractive index of the medium experienced by silver nanoparticles in organic solution compared to silver particles in aqueous solution. The increase in the intensity

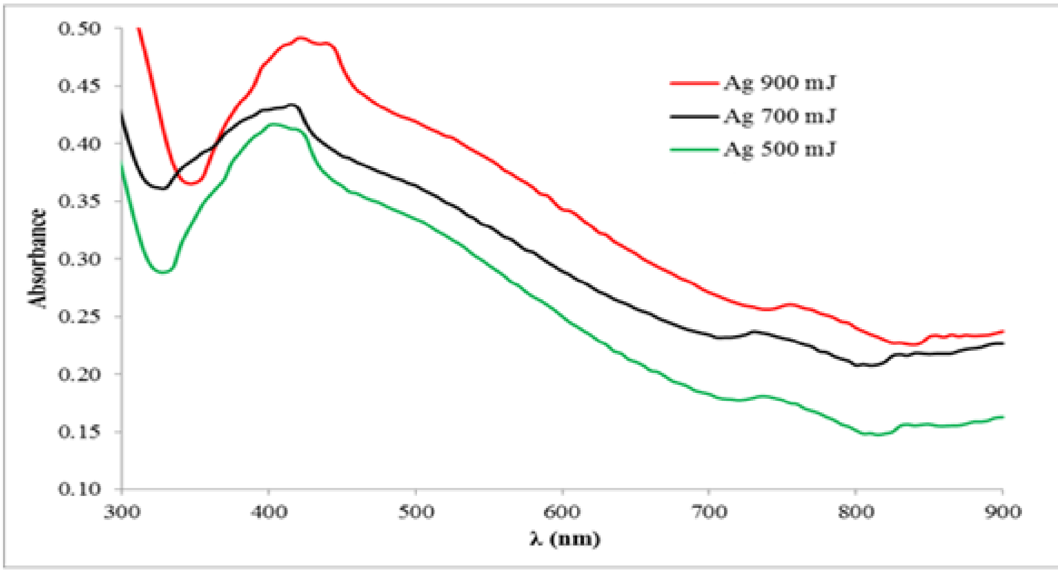

Fig. 4. UV–vis spectra of Ag nanoparticle liquids in different energy (500,700,900mJ)

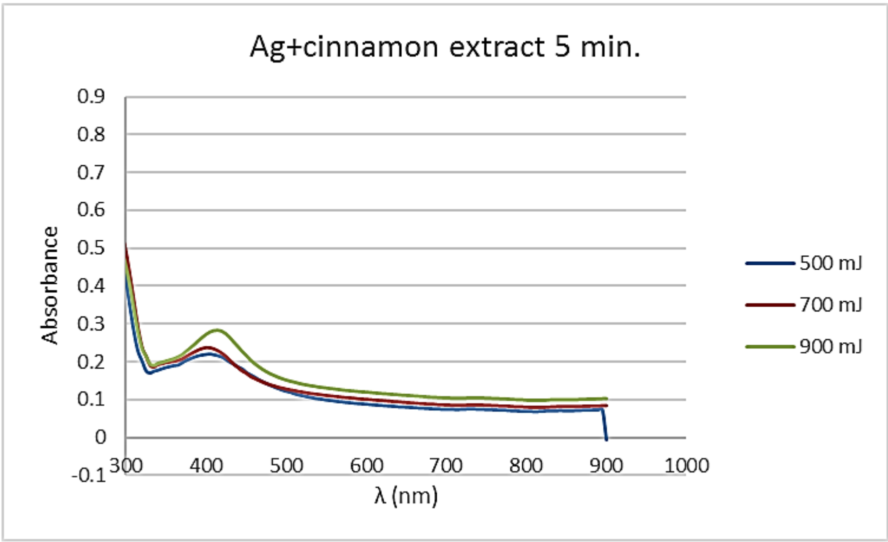

Fig. 5. UV–Vis spectra of Ag nanoparticle liquids in different energy (500,700,900mJ) mixed cinnamon bark extract prepared at 5 min.

of the surface Plasmon band may be due to the loss of surface-bound biomolecules from the cinnamon bark extract due to the disturbance in the equilibrium between surface-bound and free biomolecules [13, 14].

Antibacterial activity

The antibacterial activity of Ag NPs prepared at three different laser energies (500, 700, and 900 mJ) was compared with that of Ag NPs after mixing with cinnamon bark extract prepared at two time intervals (5 and 15 min) as seen in Figs.

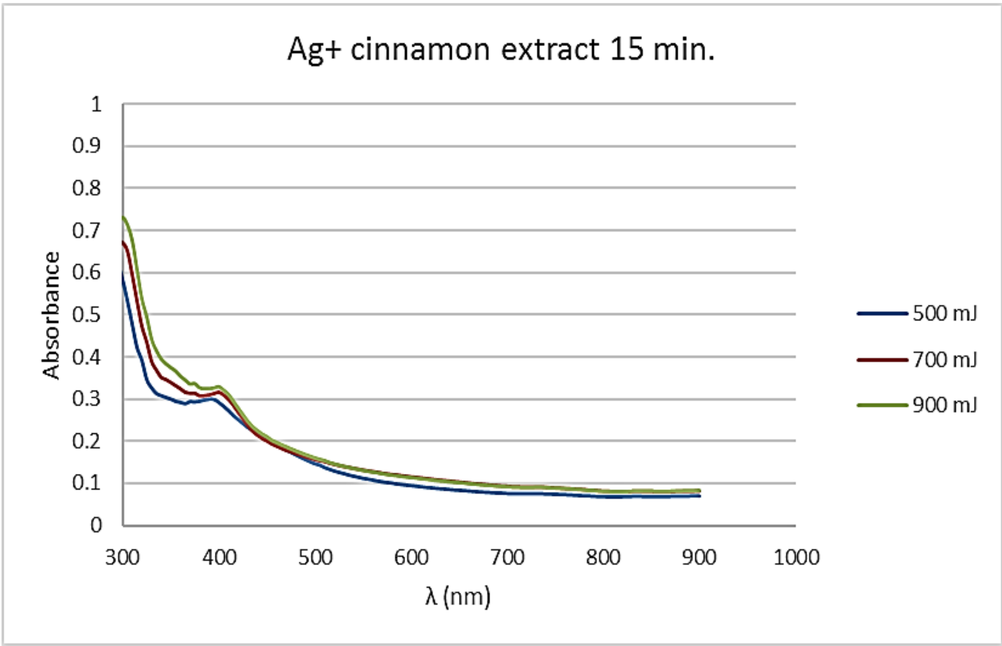

Fig. 6. UV–vis spectra of Ag nanoparticle liquids in deferent energy (500,700,900) mixed cinnamon bark extract prepared at 15 min.

Table 1. Inhibition zone of Ag NPs before and after adding cinnamon bark extract against *E.coli.* Bacteria

Laser energy nergy (mJ)	Inhibition zone (mm) of Ag NPs	Inhibition zone (mm) after adding cinnamon extract at 5 min.	Inhibition zone (mm) after adding cinnamon extract at 15 min.
500	8	12	15
700	11	14	17
900	13	18	20

7, 8 and Table 1. The results showed a significant improvement in the bacterial inhibition of Ag NPs after mixing with cinnamon bark extract prepared at 15 min. The Ag NPs mixed with cinnamon bark extract prepared at 5 min showed mild bacterial inhibition. The action of antimicrobial activity is not clearly known in previous researches, the reason may be because the Silver nanoparticles have stick on bacterial cell wall and then enter it causing death of cell, or by forming free silver nanoparticles radicals when contact with bacteria, these free radicals have the ability to make porous and then damage the cell membrane [25]. In addition, cell membranes have pores of nanoscale so they are easy to penetrate and disrupt the permeability process, thus affecting DNA replication and gene expression [26].

Field Emission Scanning Electron Microscopy

Field Emission Scanning Electron Microscopy (FESEM) characterizing of Ag NPs was obtained for different laser energies (500, 700, and 900 mJ) in

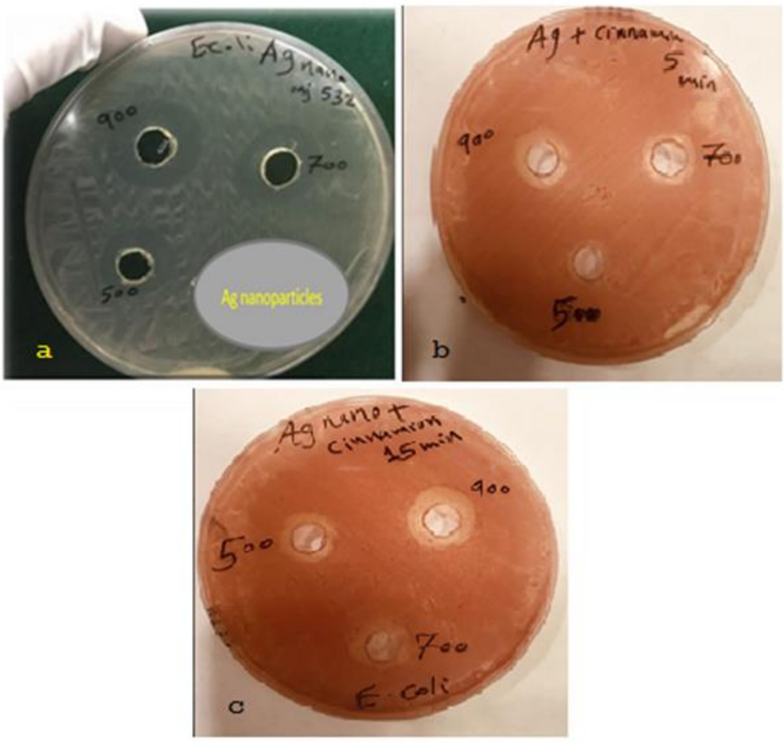

Fig. 7. Image of the inhibition zone of silver nanoparticles at energies (500 – 700- 900) against of E. coli bacteria a) before adding cinnamon bark extract; b) after adding cinnamon bark extract prepared at 5 min.; c) after adding cinnamon bark extract prepared at 15 min

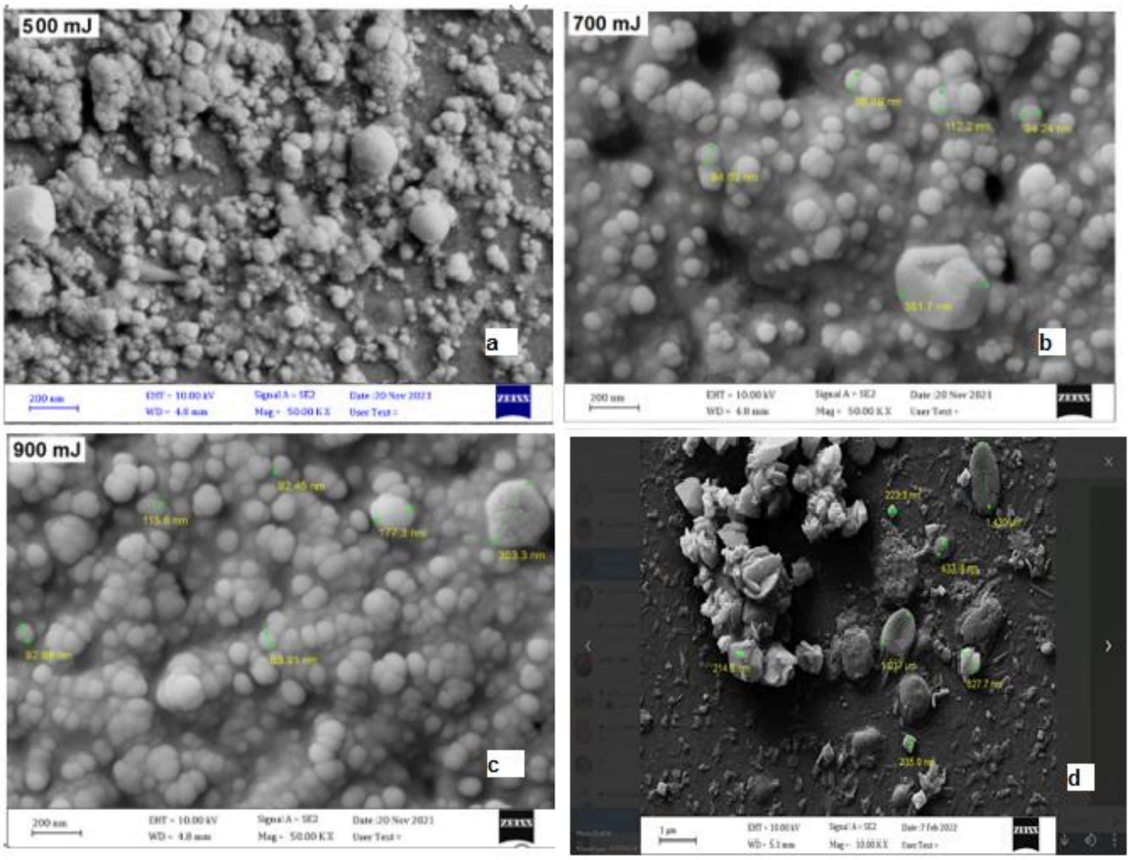

Fig. 8. FESEM images for synthesized silver NPs at different energies, a) 500mJ b) 700mJ c) 900mJ d) Ag NPs prepared at 900 mJ with cinnamon bark extract.

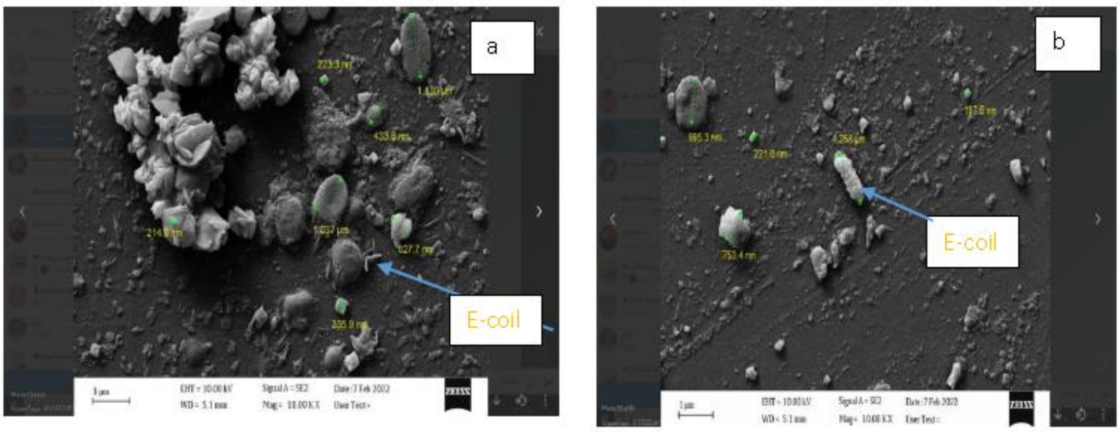

Fig. 9. FESEM images for E. coli treated with Ag NPs prepared at 900 mJ with cinnamon bark extract.

water and depositing it on glass slides. The results showed that, the nanoparticles varied in size and distribution patterns depending on the energy of the laser employed as shown in Fig. 8. The average diameters of the particles were 58.98, 87.41, and 116.13 nm for ablation energy 500, 700, and 900 mJ, respectively. The Ag NPs had a spherical shape and were aggregated in a few regions due to the drying processes during sample preparation. The high surface energy of the produced NPs may have caused particle aggregation.

The FESEM image of E. coli treated with Ag NPs prepared at 900 mJ with cinnamon bark extract is shown seen in Fig. 9. As seen in Fig. 9a, b, Ag NPs adhered to the surface of the bacterial cell wall. The image demonstrates that the Ag NPs not only adhered to a surface of cellular membrane, but also entered within the bacterial cells. The decrease of bacteria and morphological changes, such as the formation of pores and cell debris, also occurred in some bacteria.

CONCLUSION

In this study, we employed a plasma jet for the first time to obtain cinnamon bark extract that are effective antibacterial agents. Ag NPs were also prepared by pulsed laser ablation. The study analysed the laser energy induced changes for optical, structural, morphological and antibacterial properties of AgNPs and Ag with cinnamon bark extract mixture. The study showed the effects of a mixture of AgNPs and Ag with cinnamon bark extract on E. coli bacteria. The inhibition rate increased with increased with laser energy and concentration of cinnamon bark in a distilled water after 15 min exposure to atmospheric plasma.

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CONFLICT OF INTEREST

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

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