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Effect of Colloidal Cu NPs/Cinnamon Extract on the Antibacterial Activity

Maryam Mohammed K.*, Baida M. Ahmed

Department of Physics, College of Science, Mustansiriyah University, Baghdad, IRAQ.
*Correspondent contact: meryammeme2015@gmail.com

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ABSTRACT

This study explores a new facile method of obtaining plant extract using a plasma jet and a way of producing Cu nanoparticles (Cu NPs) using pulsed laser ablation in liquid. A 532 nm Nd:YAG laser at varying energies (500, 700, and 900 mJ) was used to prepare Cu NPs. Cinnamon bark extract was prepared quickly by DC plasma discharge (plasma jet) exposure at 5 and 15 min. The study showed the effects of a mixture (Cu NPs with cinnamon extract) on Escherichia coli bacteria. The colloids inhibited E. coli; the inhibition increased with duration and laser energy. The characterization of the Cu NPs was performed using ultraviolet-visible spectrophotometry, and field emission-scanning electron microscopy (FE-SEM).

KEYWORDS: Copper nanoparticles, using DC plasma discharge to prepare the extract, cinnamon extract, inhibition *E. coli* bacteria

الخلاصة

تستكشف هذه الدراسة طريقة سهلة للحصول على مستخلص نباتي باستخدام نفث البلازما وطريقة لإنتاج جزيئات النحاس النانوية (Cu NPs) باستخدام الاستئصال بالليزر النبضي في السائل. تم استخدام ليزر 532 نانومتر Nd:YAG عند طاقات مختلفة (500 و 700 و 900 مللي جول) لتحضير جسيمات النحاس النانوية NPs. تم تحضير مستخلص لحاء االقرفة بوقت قياسي عن طريق التعرض لتفريغ البلازما DC في 5 و 15 دقيقة. أظهرت الدراسة تأثير خليط (Cu NPs مع مستخلص لحاء القرفة) على بكتيريا Escherichia coli. الغرويات تعمل على تثبيط الإشريكية القولونية. يزداد التثبيط مع مرور الوقت وطاقة الليزر. تم إجراء توصيف Cu NPs باستخدام القياس الطيفي المرئي فوق البنفسجي، الفحص المجهري الإلكتروني FE-SEM.

INTRODUCTION

Since the birth of civilization, medicinal plants have been an integral component of human life. They are the basis of numerous indigenous traditional systems of medicine in Asia. Pharmacological research has recognized the importance of medicinal plants as a possible source of biologically active chemicals [1]. Cinnamon is being used to flavor meals, drinks, boiling meat, pickles, chutneys, and ketchup in India, Southeast Asia, the United States, and Europe. Cinnamon is used for medicinal purposes to treat 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 infection control, reducing blood sugar levels in diabetics, and as a skin antiseptic [2]. Cinnamon's inhibitive action has been returned to its natural state [3, 4]. The presence of chemicals such as alkaloids, tannins, volatile oils, saponins, terpins, flavones, and coumarins, which are antibacterial, inhibited the growth of bacteria. Alkaloids are distinguished by their ability to enter the bacterial cell and interfere with DNA [5]. While tannins function to block enzymes and transport proteins in the cell membrane, saponins work to decrease the quantity of sugar within bacteria that results to bacterial cell death, as well as glycosides, which have a similar but less powerful effect. We proposed a technique for obtaining) colloidal copper nanoparticles (Cu NPs) at various laser energies in a single process using laser ablation by nanosecond (Nd:YAG) laser ablation of the copper target soaked in distilled water, and we used UV-Visible spectrophotometer to characterize the structure, morphology, particle size, and absorption spectroscopy of the obtained colloids and nanoparticles [6, 7]. However, there

are far fewer publications on the bactericidal properties of nanoparticles created by (PLAL). This approach, known as 'green synthesis,' although other forms of synthesis are also regarded green, allows for the synthesis of nanoparticles [8]. Peisheng Liu *et al.* (2015) [9] reported on the facile and rapid fabrication of colloidal copper nanoparticles by laser ablation in liquid (LAL) operated in ambient conditions, and their SPR properties. A formation mechanism was put forward to reveal the optical properties of colloidal copper nanoparticles. J. M. J. Santillán *et al.* (2013) [10] reported on the analysis of the structure, configuration, and sizing of Cu and Cu oxide nanoparticles (NPs) produced by laser ablation of the solid copper target in liquids. Hadeel K. Nasif *et al.* (2021) [11] produced Cu NPs by Q-switched Nd: YAG liquid laser ablation technique (PLAL) using distilled water at room temperature at different energies (300-600 800) mJ. With a constant wavelength (1064nm). At a constant frequency (6Hz), 300 laser pulses were used to ablate the target placed in distilled water to study the effect of these materials in inhibiting bacteria. Laser pulse energy Cu NPs have antibacterial capabilities that are effective against bacteria, viruses, and another organism [12, 13]. Moreover, Cu NPs have been also shown to have antibacterial action against Escherichia coli [14, 15].

MATERIALS AND METHOD

Cinnamon bark extract preparation

Figure 1 shows the experimental setup of Dc plasma jet that consisted of a high voltage DC power supply (26 kV) and a stainless-steel needle that acted as the cathode., 1 gram of cinnamon bark was taken and washed with distilled water and was immersed in a glass beaker containing 5 ml of deionized water (between the two electrodes). Ar 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 Figure 2.

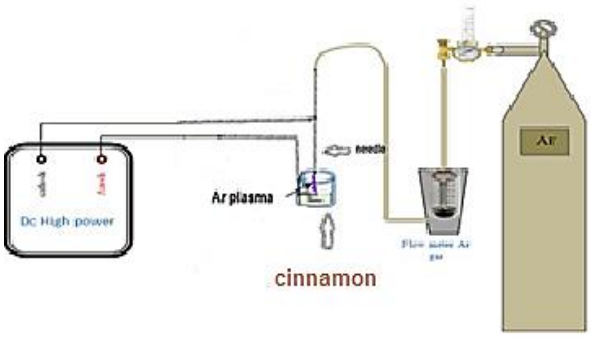


Figure 1. Schematic of DC plasma Jet system.

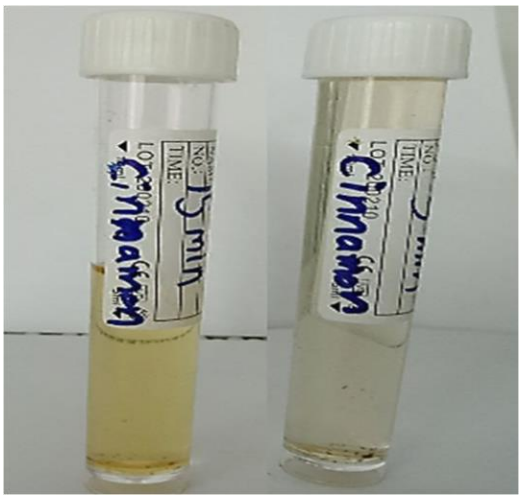


Figure 2. cinnamon extract prepared at two-time interval (5 min. ,15 min.) by Dc plasma jet.

Cu Nanoparticles preparation

Copper powder with a purity of 99.99 percent is shown in Figure 3 was pressed into a circular geometric form. In most cases, compression was done at room temperature. Where it was 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 an Nd:YAG pulses laser (9 nanosecond pulse duration time, 4 Hz. frequency, and wavelength. 532 nm).

Figure 3. Copper powder before and after pressing.

The experimental approach based on pulsed laser ablation is 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 Cu 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 Figure 4.

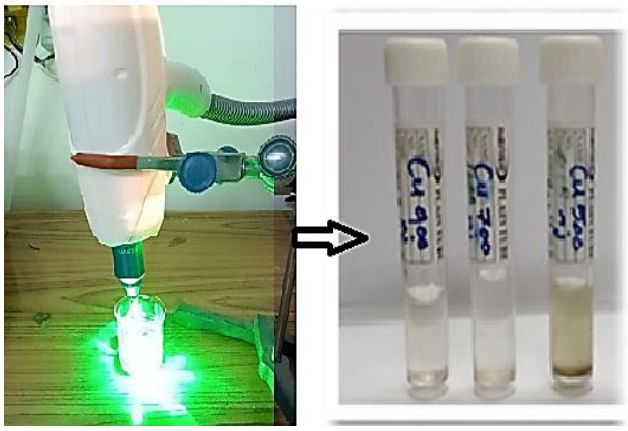


Figure 4. Stage of preparation Cu NPs at different laser energy (500-700- 900) mJ at 1000 pulse by (PLAL) method.

After that 1 ml of the extract at (5min. and 15 min.) was mixed with 1 ml of a colloidal solution of copper nanoparticles which prepared in different energies (500,700,900) shown in Figure 5.

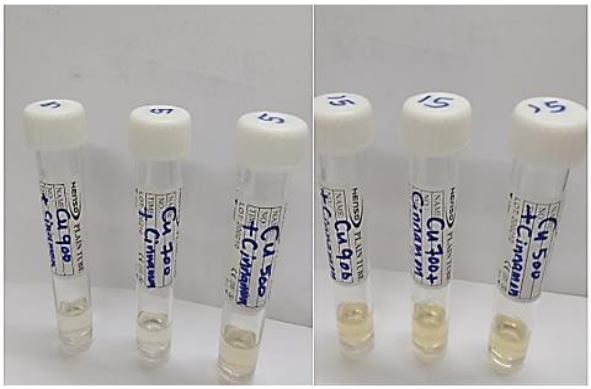


Figure 5. Nanoparticles liquid of Cu in different energy (500,700,900) +cinnamon bark extract prepared in 5min.and 15 min.

Isolation and characterization of human pathogenic bacteria

A clinical Escherichia coli (*E. coli*) 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 copper nanoparticles 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 Cu-NPs, then incubated at 37°C for 24 h to measure the inhibition zone in mm.

RESULTS AND DISCUSSION

Uv-vis absorbance of copper NPs before and after adding lemon peel extract

UV-Vis absorbance spectroscopy is a powerful tool for analyzing and studying Plasmon resonance in metallic nanoparticles, including peak positions and forms. it was obtained UV/Visible absorption spectra in the wavelength range of 300 to 900 nm to demonstrate the production process of Cu-NPs. Figure 6 shows the absorption curves for colloidal containing copper nanoparticles prepared by various laser energies (500, 700, and 900) mJ. Surface Plasmon resonances (SPRs) influence the absorption spectra of metal nanoparticles. 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 (550- 575) nm. It is also shown that the location of the peaks is red shifted from 550 nm to 575 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 [16].

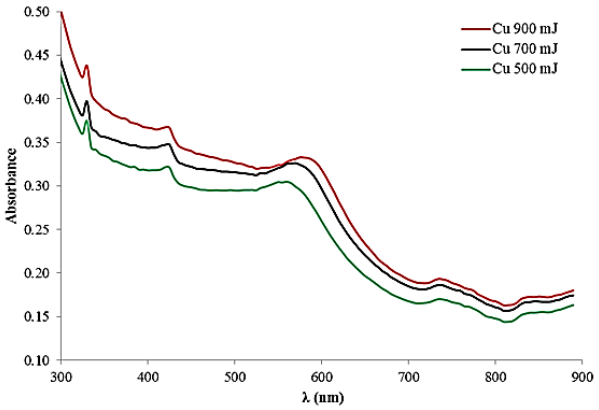


Figure 6. UV–vis spectra of Cu nanoparticle liquids in different energy (500,700,900).

From Figures 7 and 8, it is observed that the surface Plasmon resonance is broadened and shifted. This red shift in the SPR wavelength is consistent with the Mie theory for the optical properties of copper nanoparticle [17] and may be explained in terms of

the increased refractive index of the medium experienced by the copper particles in the organic solution when compared with copper particles in aqueous solution. The increase in intensity of the surface plasmon band may be due to loss of surface-bound biomolecules from the cinnamon extract due to disturbance in the equilibrium between surface-bound and free biomolecules [18].

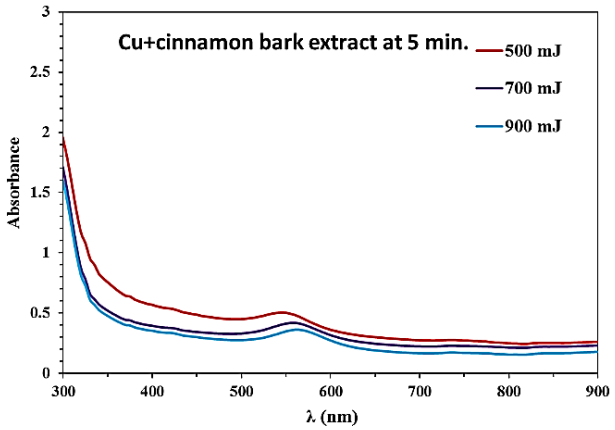


Figure 7. UV–vis spectra of Cu nanoparticle liquids in deferent energy (500,700,900) + cinnamon bark extract prepared at 5 min.

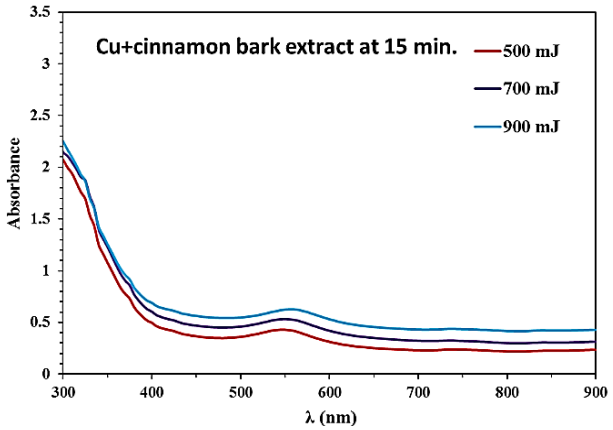


Figure 8. UV–vis spectra of Cu nanoparticle liquids in deferent energy (500,700,900) + cinnamon bark extract prepared at 15 min.

Antibacterial activity of copper NPs

The anti-bacterial activity was tested by Cu NPs prepared at three laser energies (500, 700 and 900) and compared with copper nanoparticles after mixing them with cinnamon bark extract prepared at two-time intervals (5 minutes and 15 minutes) as seen in Table 1. The results showed a significant improvement in the bacterial inhibition of copper nanoparticles after mixing them with cinnamon bark extract prepared at 15 minutes. While copper nanoparticles mixed with cinnamon bark extract prepared at 5 minutes showed mild bacterial inhibition as shown in Figures 10 and 11.

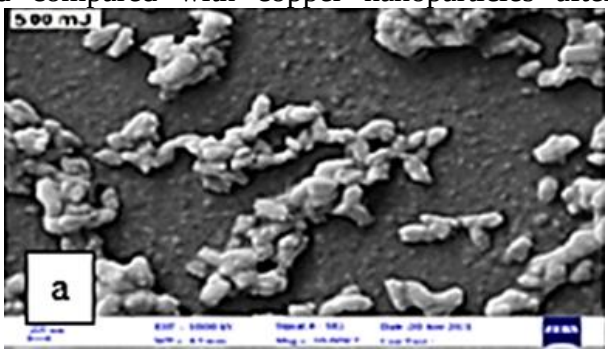
Table 1. Inhibition zone of Cu NPs before and after adding the cinnamon extract against E-coli bacteria.

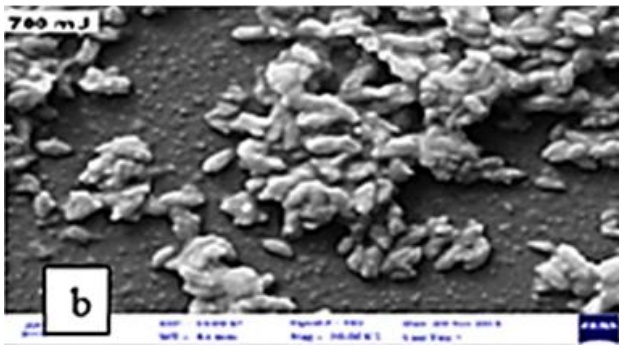
No. of pulses	Frequency (Hz)	λ (nm)	Energy (mJ)	E. coli sample No. (mm)*	E. coli sample No. (mm)**	E. coli sample No. (mm)***
1000	4	532	500	8	12	15
1000	4	532	700	11	14	17
1000	4	532	900	13	15	18

* Before adding cinnamon extract.
** After adding cinnamon extract at 5 min.
*** After adding cinnamon extract at 15 min.

Scanning electron microscopy of Cu NPs

Scanning electron microscopy (SEM) is one of the important methods of characterizing the morphology of materials. The Cu NPs for SEM imaging were prepared by exposing the samples to 1000 pulses at different laser energies (500, 700, and 900 mJ) in water and depositing the films on glass slides. The nanoparticles varied in size and distribution patterns depending on the energy of the laser employed. The average diameters of the particles were 193.71, 200.24, and 247.71 at 500, 700, and 900 mJ, respectively. The SEM image of *E. coli* treated with Cu NPs prepared at 900 mJ with lemon peel extract is shown seen in Figure 9. The Cu NPs had an ovoid shape and were aggregated in a few regions due to the drying processes during sample preparation, as shown in the FE-SEM pictures. The high surface energy of the produced NPs may have caused particle aggregation. As seen in Figure 9d, Cu NPs adhered to the surface of the bacterial cell wall. The image demonstrates that the Cu NPs not only adhered to a surface of the cellular membrane, but also entered the bacterial cells.





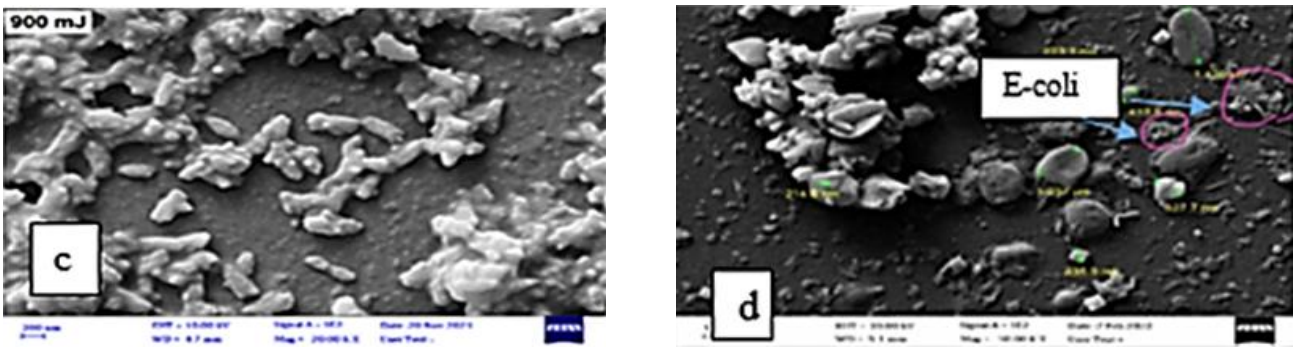


Figure 9. SEM images for synthesized copper NPs at different energies, a) 500mJ b) 700mJ c) 900mJ d) *E. coli* treated with Cu NPs prepared at 900 mJ with cinnamon bark extract.

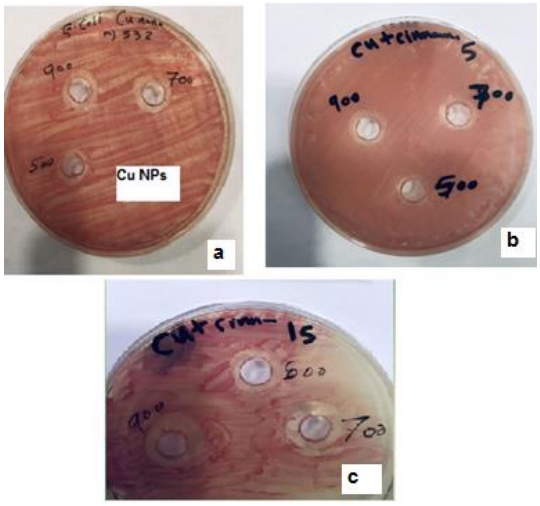


Figure 10. Image of the inhibition zone of copper nanoparticles at energies (500 - 700 - 900) mJ of *E. coli*. Bacteria after and before adding cinnamon extract prepared at 5min. and 15min.

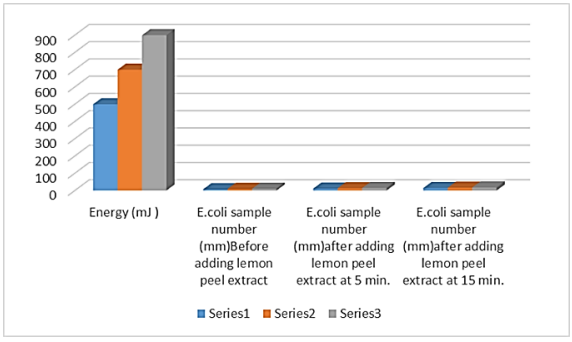


Figure 11. Antibacterial activity of copper nanoparticles before and after adding cinnamon extract prepared at 5min. and 15min. against E. coli bacteria.

CONCLUSIONS

Cinnamon extracts are effective antibacterial agents against microbes. They are natural, inexpensive, and risk-free. The study showed the feasibility of obtaining cinnamon bark extract in an easy and fast way through a by (DC plasma system) in two time periods of 5 and 15 minutes, as well as Cu NPs prepare by PLAL technique. This study showed the effects of a mixture the colloids on the *Escherichia coli* (*E. coli*) Bacteria. Note that these effects increase with energy of laser pulse and with the concentration of lemon peel in liquid at 15 min period of exposure to plasma discharge where the inhibition rate increased. Study the UV before and after mixing we note increases in the intensity of surface Plasmon resonance.

Disclosure and conflict of interest: The authors declare that they have no conflicts of interest.

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