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Effect of Cinnamon Nano/Micro Particles at Antibacterial Activity

Saif Khalel Jasim,^{1,2} Wan Maryam Wan Ahmad Kamil,^{1*} Ammar Ayesh Habeeb,³ Eskander Ali Azeez⁴ and Zahraa Luay Youssef²

¹School of Physics, Universiti Sains Malaysia, 11800 Penang, Malaysia ²Department of Radiological Tech., Bilad Alrafidain University College, 32001 Diyala, Iraq ³Department of Physics, College of Science, University of Diyala, 32001 Diyala, Iraq ⁴Al farahidi University, Radiography and Sonar Department, Baghdad, Iraq

Corresponding author: wanmaryam@usm.my; saifalaosy@gmail.com

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ABSTRACT: This study utilised the pulsed laser ablation in liquid (PLAL) technique with a switched neodymium-doped yttrium aluminium garnet (Nd $^{+}$:YAG) laser to produce cinnamon materials in nano and micro particle forms. The laser parameters included a 1,064 nm wavelength, a 10 ns pulse duration, an ablation energy of 600 mJ and varying pulse numbers (400 and 800 pulse/second). Methanol was used as the liquid medium. The size of nano and micro particles in resected materials was determined using field-emission scanning electron microscopy (FESEM) measurements, the results revealed average nanoparticle diameters of 68 nm and 56 nm and average microparticle frame sizes of 4 μ m and 3 μ m, respectively. Ultraviolet-visible (UV-Vis) spectra measurements showed two peaks for the nano-material and two irregular peaks for the micro material. The prepared material, in both nano and micro forms, was utilised to inhibit two types of bacteria through 800 pulse/sec. It was observed that the nano-material exhibited a significant antibacterial effect against these bacteria.

Keywords: cinnamon, organic, nanomaterials, laser, antibacterial

1. INTRODUCTION

Since their inception, nanomaterials, which are characterised by having at least one dimension within the nanometer size range (1 nm–100 nm) or being composed of such units in three dimensions, have garnered significant interest.¹ Nanoparticles (NPs) possess distinctive attributes that distinguish them from their bulk counterparts, particularly in their application within the field of catalysis.^{1,2} Nanosised crystals typically demonstrate enhanced exposure of catalytic sites due to their increased surface area.^{3,4} Moreover, nanosised catalysts generally exhibit higher catalytic activity than their bulk analogues owing to the facilitated interaction between substrates and catalytic centres resulting from shorter diffusion distances within nanocrystals.⁵

Additionally, nanomaterials with minute size effect, quantum size effect and macroscopic quantum tunnelling effect can generate significantly novel acoustic, optical, electrical, magnetic and thermal properties. They are widely employed across various industries, such as biotechnology, aerospace, aviation and nanoelectronics, as well as in the fields of medicine, health, aerospace and space exploration.⁷⁻⁹ The benefits of this "miniaturisation" are remarkable from multiple perspectives and for diverse purposes.¹⁰ Over the past two decades, significant progress has been achieved in the realm of metal organic frameworks (MOFs), a novel category of porous hybrid materials formed through the self-assembly of metal ions/clusters and organic ligands. 11-13 MOF materials have potential uses in catalysis because of their distinctive qualities, such as their high surface area, structural variety, and tailorability, among others. 14,15 In the top to bottom approach, the two techniques are commonly used in nanocrystals manufacturing including media milling and high-pressure homogenisation. Media grinding process needs five of the six commercial products in fine plates or in low-volume vials. 16 Presence wear or deformation of the wells limits their applicability, as surface adhesion is mediated by incomplete recovery of the drug from the grinding media and the internal grinding chamber occurs. 17,18 Compared with ion implantation in solids and nanoemulsion technology. 16,19 Laser ablation and fractionation in liquid are popular methods due to their simplicity, cost effectiveness and rapid reactive cooling of degraded species at the interface between plasma and liquid. Plasma liquid interface, environmental friendliness, reproducibility of contaminant free sample results on a large scale and ease of production of inorganic and organic NPs.²⁰ In pulsed laser ablation in liquid (PLAL), a solid target is irradiated and the ejected material forms NPs within the immersed liquid media. In laser fractionation in liquid (LFL), microparticles are suspended excitingly irradiated to rupture them into NPs. 21 Previous studies revealed that PLAL is advantageous than LFL because the NPs morphology (size and phase) can be better controlled by adjusting the laser pulse width, fluence, wavelength and pulse repetition rate. ^{22,23} It has been shown that higher fluence resulted in larger NPs and the size can change significantly depending on the pulse width, laser wavelength and flux can significantly alter the phase of the formed NPs. ^{24,25} The pulsed laser ablation method is biologically safe, environmentally friendly, inexpensive, fast and can control the size of the resulting NPs by controlling the laser parameters.²⁶

2. METHODOLOGY

2.1 Materials and Methods

Cinnamon sticks with dimensions of (80 mm \times 20 mm \times 2.5 mm) were obtained from a local supermarket, and were identified as cinnamon of (Chinese origin) with the chemical formula (C_9H_8O). To produce the cinnamon nanoparticles (CNPs),

a liquid medium containing analytical grade ethanol (C_2H_5OH , 96% purity from Sigma Aldrich) was utilised (Indian origin). Each small piece of cinnamon stick was rinsed with filtered water after being cut to a size of (20 mm × 10 mm × 3 mm) and cleaned using an ultrasonic bath with acetone solvent to eliminate any impurities. CNPs were synthesised using the PLAL technique and were cultivated in ethanol liquid medium. In this study, a Q-switched 1064-Nd $^+$:YAG laser was employed, operating at pulse durations of 10 ns, a repetition rate of 1 Hz and with variable laser pulses at rates of 400 and 800 pulses per second and an energy level of 600 mJ. Two categories of bacteria (gram-positive and gram-negative) were selected for the biological examination using prepared NPs and microparticles. The Mueller-Hinton method was utilised to analyse the impact of CNPs and microparticles on bacteria, with the aim of exploring their potential as an alternative to chemical agents.

2.2 Synthesis Cinnamon Nano/Micro

CNPs were synthesised using PLAL technology, with the Q-switched Nd⁺:YAG Laser set at a repetition rate of 1 Hz, a pulse duration of 10 ns and a wavelength of 1,064 nm. The energy of the laser was 600 mJ, and the number of pulses was varied between 400 and 800 pulses/sec. The target material used was a cinnamon stick placed at the bottom of a 27 cm³ beaker filled with 5 mL of liquid methanol, with the liquid level maintained at about 5 mm above the cinnamon, by directing the laser beam at the cinnamon target surface through a 70 mm distance as Figure (1). NPs were synthesised using these parameters. Additionally, by maintaining the same laser parameters but adjusting the distance between the laser lens and the objective to 130 mm, micro particles were obtained.

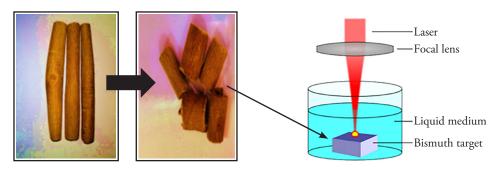


Figure 1: Illustrative diagram procedure of PLAL method.

3. RESULT AND DISCUSSION

3.1 Field-Emission Scanning Electron Microscopy Analysis

Figure 2 displays the Field-emission scanning electron microscopy (FESEM) image depicting the morphology of CNPs. The sample prepared with a frequency of 400 pulse/sec exhibited spherical NPs ranging in size from 55 nm to 92 nm, with an average particle size of 68 nm, as Figure 2(a). In contrast, the sample prepared at 800 pulse/sec also showed predominantly spherical particles, with sizes ranging from 40 nm to 78 nm and an average particle size of 56 nm, as Figure 2(b). The energy dispersive spectroscopy (EDS) analysis presented in Figures 2(c and d) indicated the presence of various elements such as carbon (C), calcium (Ca), oxygen (O), sodium (Na), magnesium (Mg), bromine (Br), silicon (Si), aluminium (Al) and potassium (K) in the cinnamon compounds. These elements are attributed to cinnamon's organic nature and their natural occurrence in the environment.²⁶

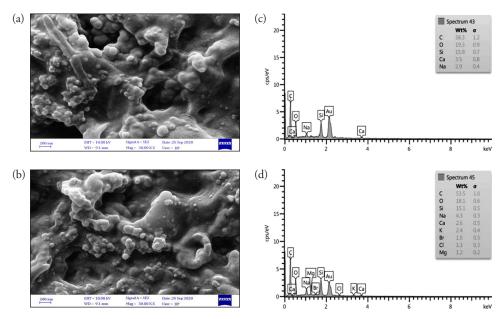


Figure 2: (a and c) FESEM image at (400 and 800) pulse/sec, (b and d) energy dispersive X-ray (EDX) result.

Figure 3 displays the FESEM images depicting the morphology of cinnamon microparticles. Image (a) illustrates the microparticles obtained from the sample prepared with 400 pulse/sec, exhibiting spherical NPs ranging in size from 2 μ m to 7 μ m. Similarly, image (c) shows spherical particles with a size range of 1 μ m to 7 μ m for the sample prepared at 800 pulse/sec. The EDS analysis, represented in images

(e and g), identified the presence of C, O, Na, Al and chlorine (Cl) in the cinnamon compounds. These elements are attributed to cinnamon's organic nature and their origin from natural sources. Additionally, mapping results in images (b, f and d, h) demonstrate the distribution of elements and their corresponding colours relative to the number of pulses used.

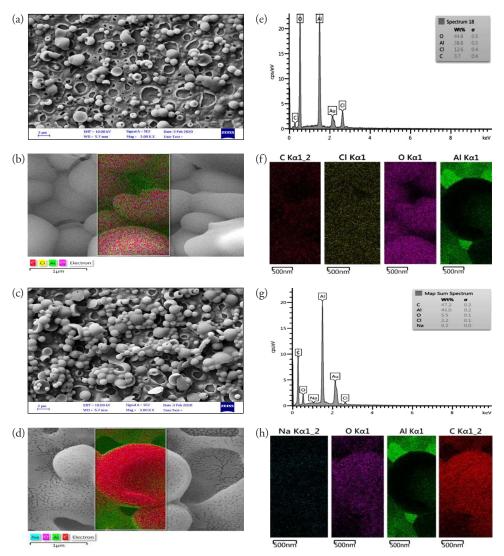


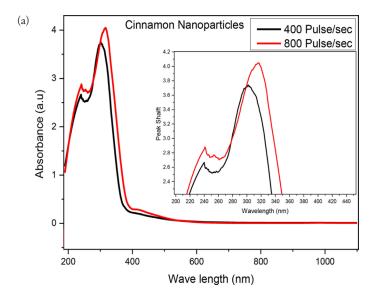
Figure 3: (a and c) FESEM image, (e and g) EDX result and (b, f and d, h) MAP result, at (400 and 800) pulse/sec, respectively.

3.2 Ultraviolet-visible Spectroscopy Analysis

The ultraviolet-visible (UV-Vis) spectrometer was utilised to measure the absorbance of cinnamon nano/micro particles. The NPs and microparticles were prepared with (400 and 800) pulse/sec, and their absorbance was measured separately. The nanosamples exhibited a gradual colour change from colourless to light brown as the number of pulses increased. In contrast, the micro samples displayed a gradual colour change from colourless to yellow, indicating a change in shape and size dispersion. The CNPs produced two distinct peaks at wavelengths (204 nm, 241 nm and 302 nm, 317 nm) with absorbance values of (2.6, 2.8 and 3.7, 4) (a.u.) as Figure (4a), while the cinnamon microparticles had irregular peaks at wavelengths (225 nm, 292 nm and 235 nm, 290 nm) with absorbance values of (3, 4 and 3.3, 4.1) (a.u.) as Figure (4b) due to irregularly sized particles. Additionally, the colour of the liquid suspension containing CNPs remained unchanged even after a 2-month incubation at room temperature, confirming the excellent stability of the implanted CNPs.²⁶ On the microscopic level, the particles in the samples settled at the bottom of the tube over time, and the colour of the liquid returned to its original semi-colourless state. While NPs form from the condensation of a laser-ablated plasma plume on a substrate surface, the development of carbon NPs in terms of density and particle size distribution through vacuum pulse laser deposition is notably different.²⁸ This difference was linked to the effects of quantum size and the presence of aromatic amino acids in the protein structure of CNPs, as previously reported.^{29,30} The presence of phenolic acids in cinnamon and its derivatives (flavanols, cinnamaldehyde, phenylpropenes and eugenol) was identified as a potential mechanism for stabilising CNPs for up to 2 months after their synthesis, leading to an increase in peak intensity. 31,32

3.3 Antibacterial Tests by Nano/Micro Particles

The study evaluated the biological efficacy of CNPs and microparticles produced at a frequency of 800 pulses per second, with cinnamon added in three different concentrations (25 mL, 50 mL and 75 mL). The agar well diffusion assay was utilised to qualitatively assess the antibacterial potential of the freshly synthesised CNPs in methanol medium. Each methanol suspension contained CNPs of varying sizes (less than 100 nm). The produced NPs demonstrated inhibitory activity against both gram-positive and gram-negative bacterial strains (Bacillus subtilis [BS], Pseudomonas aeruginosa [PA], Escherichia coli [EC]). Notably, the sample S2-50 mL exhibited the highest antibacterial activity, as evidenced by the widest diameter of the inhibitory zone (8 mm) observed in Figure 5 and Table 1. The antibacterial effectiveness demonstrated by microparticles was found to be minimal. Figures 5 (a), (b) and (c) display the measurements and comparisons with the antibiotic. It was proposed that the efficient inhibition of the bacterial surface protein sortase and subsequent



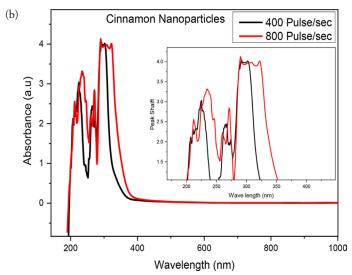


Figure 4: UV-Vis spectroscopy result (a) cinnamon nanoparticles and (b) cinnamon microparticles.

prevention of cell adhesion to fibronectin could be potential mechanisms underlying the antibacterial properties of CNPs. It was suggested that CNPs have the ability to bind to bacterial cell walls, modify membrane structure and subsequently penetrate cells to disrupt the organisation of cell organelles.³³

Table 1: Biological application results.	Table	1:	Biol	ogical	app	olication	results.
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	The inhibition zone (mm)						
Bacterial cell	S1 (25 mL)	S2(50 mL)	S3 (75 mL)	Control S4			
	Cinnamon Nanoparticles						
Bacillus subtilis	6	3	2	1			
Pseudomonas aeruginosa	5	6	5	0			
Escherichia coli	3	8	4	0			
	Cinnamon Micro particles						
Bacillus subtilis	0	0	0	0			
Pseudomonas aeruginosa	0	0	0	0			
Escherichia coli	0	1	0	0			

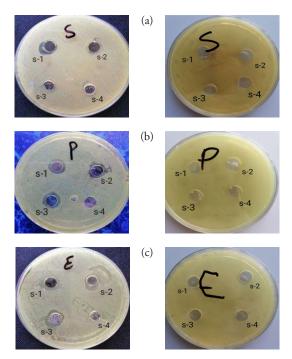


Figure 5: Biological application results (a) *Bacillus subtilis* (b) *Pseudomonas aeruginosa* and (c) *Escherichia coli*.

4. CONCLUSON

This study utilised the pulsed laser ablation technique in liquids to determine that the size of particles produced can be managed by adjusting the distance between the laser lens and the target. It was observed that this method yielded NPs and microparticles at different distances. In this study, the physical properties such as the size of nano

and micro particles were studied using FESEM and the absorbance of nano and micro particles using UV-Vis spectroscopy. Furthermore, it was established that NPs exhibit a notable impact in biological contexts. Testing against bacterial strains revealed that NPs effectively inhibit infected cells, whereas microparticles showed no effect.

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