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Growth and characterization of spherical cinnamon nanoparticles: Evaluation of antibacterial efficacy

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ABSTRACT

Organic nanoparticles with controlled properties are advantageous for diversified biomedical and pharmacological applications. Cinnamon nanoparticles (CNPs) being bioactive and nontoxic can be effective antibacterial agents. Driven by this idea, we prepared spherical CNPs using pulse laser ablation in liquid (PLAL) technique and characterized these NPs. A pure cinnamon target immersed in liquid ethanol (5 mL) was ablated using Q-switched Nd:YAG pulse laser of varying energy (30–180 mJ). Laser energy dependent structure, morphology and optical properties of the as-grown CNPs were determined. Furthermore, the antibacterial activity of such CNPs against four bacterial strains (*Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa*) was evaluated using agar well diffusion and optical density measurements. These CNPs were demonstrated to be beneficial for the development of antibacterial drugs and food processing.

1. Introduction

Organic compounds, with particle size above 100 nm, can be converted by mechanical milling methods. However, these approaches are limited as numerous applications demand particles tinier than 100 nm (An, Kwon, Jung, & Park, 2002; Müller, Jacobs, & Kayser, 2001). Particles of nanometric dimension can be achieved using re-precipitation of organic molecules, where such organic solution can rapidly be injected into a poor solvent with/without a surfactant to form molecular precipitates (An et al., 2002). Formerly, several techniques were used to synthesize various organic NPs including pulse laser ablation in liquid (PLAL), chemical etching, ball milling, sol-gel, molecular condensation, microorganism, etc. Each of these methods has its own advantage to produce NPs with controlled morphology and composition dependent properties (Asahi, Sugiyama, & Masuhara, 2008; Salim & Bidin, 2017). Compared to nanoemulsion (Yildirim, Oztop, & Soyer, 2017; Zhang, Zhang, Fang, & Liu, 2017) and chemical (Ganeev, Baba, Ryasnyansky, Suzuki, & Kuroda, 2004) techniques, PLAL technique is very useful due to its ease, economic, eco-friendly and large-scale reproducibility of contaminant free nanostructures (Sajti, Sattari, Chichkov, & Barcikowski, 2010; Sylvestre, Kabashin, Sacher, Meunier, & Luong, 2004). Moreover, the nature of the nanostructures obtained via PLAL technique is decided by the laser characteristics such as the lasing wavelength, the pulse width, the repetition rate, the fluence and the exposure time (Zhang, Gökce, & Barcikowski, 2017; Sylvestre et al., 2004; Tsuji, Iryo, Nishimura, & Tsuji, 2001).

Research revealed that increase in the laser power can improve the nanoparticles productivity (Wagener, Schwenke, Chichkov, & Barcikowski, 2010). It is established that the laser parameters play a significant role in controlling the nanoparticles morphology by delivering favourable thermodynamic conditions during the growth process (Zhang, Gökce, et al., 2017). In the ablation process, the laser interacts with the material (target) and creates hot and dense plasma on the target surface, wherein an expanding bubble is formed during the optical inactivation period of the plasma (Tsuji et al., 2001). Subsequently, NPs are released in the liquid environment after the bubble is collapsed (Wagener et al., 2010). It is found that the lifetime of the cavitation bubble become longer with the increase of the laser fluence and the repetition rate, which in turn increase the productivity of NPs.

Currently, products from different parts of the aromatic plants have been extensively used for biomedicinal purposes (Ahmad et al., 2010; Jeyaratnam et al., 2016). Compared to metallic nanomaterials, the organic nanoparticles produce from natural herbs are favored due to the presence of rich of active agents, biocompatibility, abundance, easy stabilization and safe handling (Gopinath et al., 2012; Yang et al., 2010). Polyphenol and cinnamaldehyde compounds in cinnamon cassia have been used worldwide as traditional herbal medicine and healthy nutrition element. Unique attributes including safe, bioactivity, non-toxicity and anti-bacterial efficacy make cinnamon compounds suitable for different applications (Chang, Chen, & Chang, 2001; Fatima, Zaidi,

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Amraiz, & Afzal, 2016). Yet, the bioactivity of cinnamon components (liquid or powder) against bacterial cell has not been well understood (Sathishkumar et al., 2009). In-depth understanding of the antibacterial effectiveness may require an accurate synthesis method to produce CNPs with controlled morphology. Subsequent implementation of such CNPs in the bacterial cell is needed to examine their cell wall penetrability and concomitant destruction of DNA (Basniwal, Buttar, Jain, & Jain, 2011).

Earlier, metal nanoparticles using cinnamon as a stabilizing agent for biomedical applications (especially as antibacterial agent) have been widely investigated (Huang et al., 2007; Ma, Davidson, Critzer, & Zhong, 2016; Salim, Bidin, Lafi, & Huyop, 2017; Sathishkumar et al., 2009). Cinnamon leaf with aqueous Au and Ag precursors at ambient temperature has also been synthesized for anticancer drug development (Huang et al., 2007). The presence of polyphenols components in cinnamon was considered to be beneficial for the reduction of Ag and Au ions (Huang et al., 2007). According to Ma et al. (Ma et al., 2016), the ethylenediaminetetraacetate can overcome the antagonistic effect of the lauric arginate and the cinnamon oil combination against Gram-negative bacteria. Using nanoemulsions technique, Zhang et al. (Zhang, Zhang, et al., 2017) investigated the bioactivity of cinnamon oil against some pathogenic microorganisms. The morphological properties of diverse cinnamon nanostructures produced inside different growth media including citric acid, ethanol, methanol and olive oil (Salim & Bidin, 2017; Salim et al., 2017) have been widely studied. So far, an accurate preparation method for CNPs with desired morphology and antibacterial activities is far from being achieved. On top, the structural and antibacterial properties of CNPs produced in ethanol media have never been evaluated. Thus, the main purpose of this paper is to produce better CNPs morphologies inside ethanol as growth media using PLAL technique for examining the feasibility of achieving their enhanced antibacterial effectiveness.

This paper reports the eco-friendly synthesis of CNPs (grown inside ethanol medium) using inexpensive PLAL technique. The influence of changing laser ablation energy (30–180 mJ) on the growth mechanism of CNPs was inspected. As-prepared CNPs were characterized using various analytical tools. Furthermore, the antibacterial efficiency of these CNPs was evaluated on Gram-negative and Gram positive bacterial strains. Agar well diffusion assay and optical density (OD_{600}) measurements were performed to determine the antibacterial effectiveness.

2. Experimental

2.1. Materials used

Cinnamon cassia sticks were purchased from the local supermarket (Aeon, Malaysia). Analytical grade ethanol (C_2H_5OH, 96% purity) was purchased from Sigma Aldrich (utilized as liquid media). Broth media (Merck, Sigma Aldrich) including Nutrient Agar and Mueller Hinton Agar were used for all the bacterial cultures. Penicillin-streptomycin (Sigma Aldrich) was applied for evaluating the antibacterial activity of CNPs. Furthermore, two representative microorganisms of Gram (negative and positive) bacteria strain *Bacillus subtilis* (BS) ATCC 21332, *Pseudomonas aeruginosa* (PA) ATCC 27853, *Staphylococcus aureus* (SA) ATCC 11775 and *Escherichia coli* (EC) ATCC 25923 were acquired from the Microbiology Research Laboratory, Universiti Teknologi Malaysia. Each cinnamon stick was cut into a dimension of (20 mm × 10 mm × 2 mm) and washed using analytical grade (acetone) in an ultrasonic bath for 60 min. Then, these sticks were rinsed using distilled water to remove the organic contaminants.

2.2. Cinnamon nanoparticles synthesis

The PLAL technique (Fig. 1) was used to prepare the CNPs, wherein a Q-switched 1064-Nd: YAG laser of 10 ns pulse duration, 1 Hz repetition rate, and under varying ablation energy (in the range of 30–180 mJ with spot size of 2 mm) was used to irradiate the cinnamon target. The stick was immersed at the bottom of cubic pyrex container of dimension (3 cm × 3 cm × 3 cm) filled with 5 mL of liquid ethanol as growth medium (at room temperature). The laser beam was focused onto the surface of the target at the laser repetition rate of 1000 pulse/sec. To achieve the optimum growth, the separation between the lens and the surface target was kept fixed (17 mm) (Zhang, Gökce, et al., 2017). During the ablation process, the solution was rotated using a magnetic stirrer (at the revolution of 12 rpm) to achieve a homogenous mixture by avoiding the formation of craters on the target surface.

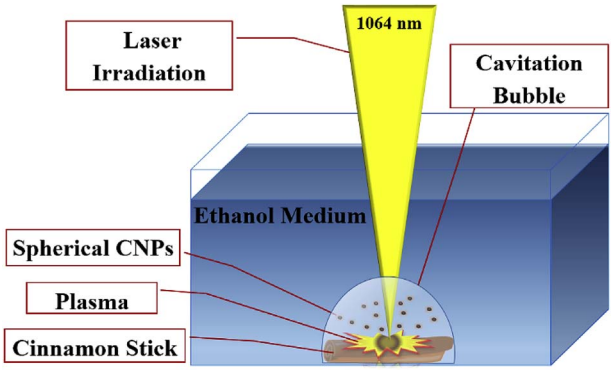

Fig. 1. Schematic diagram of laser ablation development for synthesizing CNPs in ethanol media.

2.3. CNPs characterization

The absorption spectra in the wavelength range of 200–600 nm were recorded on a UV–Vis spectrophotometer (PerkinElmer Lambda 25 Spectrometer). FTIR spectra of the CNPs in the wave number range of 500–4000 cm^{-1} were recorded on a PerkinElmer Frontier™ Spectrometer. The CNPs morphology (size and shape) and selected-area electron diffraction (SAED) pattern was analyzed respectively using a Biological Transmission Electron Microscope (BIO-TEM from Hitachi HT7700) and a High-Resolution Transmission Electron Microscope (HRTEM from JEOL ARM 200F). The samples elemental compositions were detected by Energy Dispersive X-ray (EDX) spectrometer (Field Emission Scanning Electron Microscope, HITACHI SU8020). The Dynamic Light Scattering (DLS) tool was used to measure mean diameter of CNPs. The standard deviation of the CNPs size distribution was determined using the Zetasizer (Nano-ZS90 Malvern Instruments). The chemical degradation of the prepared CNPs was assessed using Liquid Chromatography-Mass Spectrometry (LC-MS operated at 254 nm, Agilent Ion Mobility 6560 Q-TOF system) coupled with the Agilent 1290 Infinity II UHPLC. A reverse-phase C18 column (Poroshell 120 EC-C18, dimension of 4.6 mm × 100 mm × 2.7 μm at 35 °C) was used. In this test, CNPs solution of volume 10 μL was injected into the LC system at the flow rate of 1.0 mL/min. All the characterizations were performed at room temperature.

2.4. Antibacterial activity test of CNPs

Four bacterial cultures were examined using the agar well diffusion method to determine the capacity of CNPs in preventing the bacterial growth. First, the Mueller-Hinton agar was injected into the sterilized Petri dishes and the broth was allowed to solidify. Next, the fresh bacterial cultures (BS, EC, SA, and PA) were swabbed homogeneously over the broth plates using sterile L-shape. Four wells were made (each of diameter 6 mm) on the agar medium plate using sterile cork borer. In each case, about 20 μL of the CNPs solution prepared using different laser ablation energy (30–180 mJ) was poured into the corresponding well. The blank, standard antibacterial agent (penicillin-streptomycin)

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