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Research Article

Green Synthesis, Characterization, and Evaluation of the Antimicrobial Activity of *Camellia sinensis* Silver Nanoparticles

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An extremely worrying and alarming increase in the level of multiple drug resistance is reported in Sudan, in which bacterial strains are becoming resistant to many commonly available antibiotics. Eventually, it is becoming extremely difficult to treat debilitating infections. In search of promising solutions to this arising crisis, *Camellia sinensis* silver nanoparticles were synthesized using the green synthesis method. The synthesis of the *Camellia sinensis* silver nanoparticles is confirmed using analytical methods as ultraviolet-visible spectroscopy, X-ray diffractometer, and scanning electron microscopy. Using the ultraviolet-visible spectroscopy, an absorption band of 412 nm was observed. Furthermore, scanning electron microscopy revealed the presence of silver nanoparticles which fell within the range of 1–100 nm, and X-ray diffractometer analysis showed three intense peaks with a maximum intense peak at 24.3 theta. Nanoparticles distribution between 12 nm and 64 nm was observed with an average diameter of 18.115 nm. It also revealed orthorhombic-shaped nanoparticles. The synthesized nanoparticles showed antimicrobial activity against *Staphylococcus aureus* with a zone of inhibition of 7 mm, but none was detected against *Escherichia coli*. The obtained physicochemical properties were correlated with the antibacterial activity of the silver nanoparticles.

1. Introduction

Nanotechnology is a rapidly growing field, and its benefits in the pharmaceutical industry during the last decade are undeniable. Since nanoparticles are diverse in shape, size, and physical properties, they are finding new ways to become one of the major trends used nowadays in pharmaceutical practice. Latest research trends greatly advise the use of nanoparticles, and this would be attributed to their nanosize which is estimated to be one billionth (10^{-9}). Nanoparticles are known to have at least one dimension in the nanoscale range of 1–100 nm [1]. The unique physicochemical properties of the silver nanoparticles have led to their diverse range of uses in fields as biomedicine, catalysis, and energy storage. Additionally, silver nanoparticles are well known for their broad-spectrum and potent antimicrobial and anticancer properties. Various other medical applications would be implicated as in wound repair, bone healing, dental applications, vaccine adjuvant, and antidiabetic agents [2].

Silver nanoparticles would be synthesized using chemical, physical, and biological methods. However, the use of biological methods for the synthesis of silver nanoparticles have an established economic and environmental value, namely, the green synthesis method [3]. Biological molecules obtained from plants and microorganisms such as bacteria and fungi have been extensively used for the synthesis of nanoparticles [4]. The biological molecules act as in situ reducing and capping agents which are capable of forming well-dispersed nanoparticles with capping agents instilling properties as reduced nanoparticle agglomeration and better antimicrobial action [4]. The silver nanoparticles are well known for their high antimicrobial activity and are capable of eradicating various microorganisms at significantly low doses [5]. This intrinsic property of the silver when combined with the capping properties of the biologic moiety in the plants offers synergistic and enhanced antimicrobial activity.

Additionally, significant antibacterial spectrums of activity have been observed against Gram-positive and Gram-negative bacteria. The Gram-negative bacteria were found to be more sensitive to silver nanoparticles [6]. The antibacterial activity of the silver nanoparticles is greatly impacted by physicochemical properties such as morphology and size. In several studies, it was determined that the smaller the size of silver nanoparticles, the larger the surface area and silver ion release [7]. The antimicrobial activity is attributed to the large surface-to-volume ratio of the nanoparticles [8]. Better antimicrobial activity has been demonstrated when the nanoparticle size fell below 10 nm, thus implying an important association between the size of the nanoparticle and antibacterial activity [2].

Tea (*Camellia sinensis* L.) is an evergreen plant, which was primarily harvested from China and later spread to India and Japan, followed by Europe and Russia. Green, oolong, and black tea are all made from the same plant species, *C. sinensis* L., but differing in properties as their appearance or organoleptic taste [9]. Significant antioxidant, antibacterial, and anticancer properties related to *Camellia sinensis* have been established in many previous works [10]. These beneficial properties were greatly attributed to the presence of phenolic compounds [11]. Polyphenols which include flavonoids such as catechins, catechin gallates, and proanthocyanidins have been widely implicated along with other chemicals as caffeine, methylxanthines, lignin, and organic acids as the major contributors to the properties of tea [12]. It also comprises free catechins which serve as potent antioxidants such as (+) catechin, (+) gallocatechin, (−) epicatechin, and (−) epigallocatechin and the galloyl catechins [12].

The importance of this study was to synthesize, characterize, and determine the antimicrobial activity of the *Camellia sinensis* silver nanoparticles formed using the green synthesis method. Furthermore, the study aimed to establish a link between the morphology and size of the silver nanoparticles and the corresponding antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

2. Methodology

2.1. Preparation of Silver Nitrate Stock Solution. The stock of silver nitrate ($AgNO_3$) solution was prepared by weighing 54 mg of silver nitrate powder (India) and was dissolved in 200 ml of distilled water. The prepared stock solution was stored in a dark place at room temperature.

2.2. Preparation of Camellia sinensis Solution. The contents of a tea bag were emptied and visually inspected for any impurities. 1.5 g of black tea (obtained from a local market in Khartoum, Sudan) was weighed on a sensitive balance, and 250 ml of distilled water was added. It was then heated to 20 minutes on a waterbath maintained at 80°C. The solution was then filtered. The filtrate was obtained and allowed to cool at room temperature. The pH of the resulting solution was neutral and was made alkaline by the addition of a small amount of solid potassium carbonate (K_2CO_3) particles (India) until a pH of 10 was reached. This was confirmed by the use of a pH meter.

2.3. Synthesis of Silver Nanoparticles (AgNPs). At pH 10, 80 ml was withdrawn from the alkalinized stock solution of black tea. It was then transferred into a magnetically stirred flask. Stirring was maintained at the minimum speed. The stock solution of silver nitrate ($AgNO_3$) was added in portions of 5 ml. After each addition, the solution was stirred to optimize the reduction of silver and the resulting electronic absorption was recorded using ultraviolet-visible spectroscopy.

2.4. Characterization of Synthesized Silver Nanoparticles

2.4.1. Ultraviolet-Visible Spectroscopy (UV-Vis Spectroscopy). *Camellia sinensis* silver nanoparticles formation was determined using a UV spectrophotometer (Shimadzu, Japan) at a wavelength ranging from 300 nm to 800 nm. 5 ml of $AgNO_3$ was added consecutively, and the reduction of the silver ions by the green tea infusion was monitored [13].

2.4.2. Scanning Electron Microscopy (SEM). The prepared silver nanoparticles (AgNP) were centrifuged at 3000 rpm, and the obtained filtrate was dispersed in distilled water and recentrifuged. The filtrate was spread over plastic Petri dishes that were taken to the oven to dry for 15 minutes. The dried brown deposit of silver nanoparticles was scraped off and was taken for analysis using scanning electron microscopy (TESCAN MIRA-Brno, Czech Republic).

2.4.3. X-Ray Diffractometry (XRD). The prepared silver nanoparticles were centrifuged using eppendorf tubes, and the obtained supernatant layers were collected using a micropipette. The *Camellia sinensis* silver nanoparticles were characterized in powdered form using X-ray diffractometry (XRD, 7000 s/7000 L, Shimadzu, Japan) located at the forensic laboratories, Sudan.

2.5. Antimicrobial Activity of Synthesized Silver Nanoparticles. The synthesized silver nanoparticles were tested for their antimicrobial activity against two bacterial strains: *Staphylococcus aureus* and *Escherichia coli*. The disc diffusion method was used to evaluate the antimicrobial activity. The bacterial species were subcultured on Muller Hinton media. Sterile filter paper discs were loaded with the *Camellia sinensis* AgNP solution (20 mg/ml) which was placed on top of the Mueller Hinton agar plates. Ciprofloxacin was used as a positive control. The plates were then kept in the fridge at

5°C for 2 hours. The plates were then incubated overnight at 35°C. Inhibition zones were then measured and considered as an indication for antibacterial activity.

3. Results

3.1. Visual Appearance and UV-Visible Spectroscopy. The green synthesis of *Camellia sinensis* nanoparticles was characterized by brownish-orange color. The nanoparticles formation and growth was further confirmed using UV-visible spectroscopy. It gave a characteristic absorbance at a wavelength of 412 nm. This absorption band is mainly known as the surface plasmon resonance absorption band, as shown in Figure 1 [14].

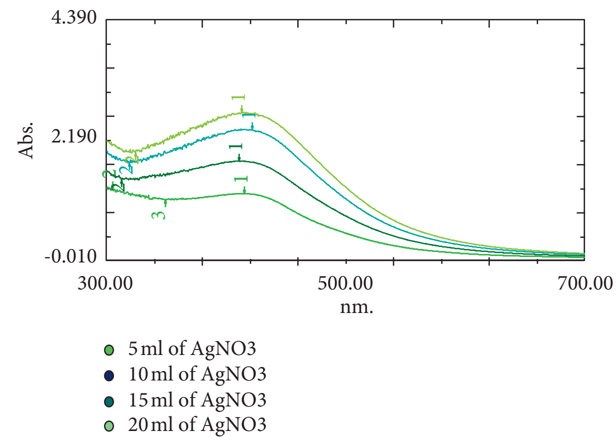

Figure 1: The electronic absorption spectrum of *Camellia sinensis* silver nanoparticles upon addition of 5 ml, 10 ml, 15 ml, and 20 ml of $AgNO_3$.

3.2. SEM Analysis of Camellia sinensis Silver Nanoparticles. Using the scanning electron microscopy technique (SEM), the morphological features of the synthesized silver nanoparticles were examined. Figure 2 shows the SEM micrographs of the synthesized silver nanoparticles. When a voltage of 25 kV was applied, the size was found to be within the actual size range of nanoparticles, which is 1–100 nm. The images with the smaller particles size were blurred as the SEM present at the institute was not able to detect images in clear shapes.

3.3. XRD Analysis of Camellia sinensis Silver Nanoparticles. The X-ray diffractometry–XRD analysis was carried out for the determination of the size distribution and crystal structure. The following results were obtained by the XRD analysis results (Figure 3), and the synthesized nanoparticles revealed the presence of an orthorhombic shape. In addition to this, three characteristic peaks were observed using XRD: Peak A [$Ag_2\ In_4\ La_4\ S_{13}$], peak B [$Ag\ F_{11}\ Sb_2$], and peak C [$Ag_{0.9}\ Al_{1.06}\ Co_{2.94}\ Mo_5\ O_{20}$]. Peak A was found to be the most abundant and intense peak appearing at about 24.3 theta. In addition, the silver nanoparticles size distribution was found to fall in the range of 12–64 nm. The average nanoparticle size was found to be 18.115 nm. The other peaks which are detected by XRD were being considered to be for the other components present in the silver nanoparticles solution from the plant extract.

3.4. Antimicrobial Activity of AgNP. The antimicrobial activity of the AgNP was demonstrated using two bacterial strains, namely, *Escherichia coli* and *Staphylococcus aureus.* Muller Hinton plates were used to demonstrate the antibacterial properties of the synthesized AgNP, as shown in Figures 4 and 5 . Specifically, the disc diffusion method has been utilized for the determination of antimicrobial activity. After an incubation period of 24 hours, a zone of inhibition of 7 mm was obtained against *Staphylococcus aureus*. Regarding *Escherichia coli*, which is a Gram-negative organism, silver nanoparticles were not found to be effective, and no zone of inhibition was observed. Using the positive control antibiotic (ciprofloxacin), a zone of inhibition of 35 mm was observed with *S. aureus* species and 37 mm with *E. coli* species, respectively. The obtained zones of inhibitions for each of the two bacterial strains were illustrated in the form of a pie chart as shown in Figure 6.

4. Discussion

In this research, *Camellia sinensis* silver nanoparticles were synthesized using the green synthesis method. When compared to physical or chemical means of synthesis, this method was found to confer major advantages in terms of simplicity, efficacy, less toxicity, and cost-effectiveness [4]. This manuscript also described the ability of the plant phytochemicals to detoxify heavy metals, therefore, reducing the undesirable toxic effects of silver nanoparticles making them a preferable choice [4]. The utilization of the green synthesis method is mainly attributed to the reducing and capping properties of the phytochemicals. This was indicated by multiple literatures as in Onitsuka et al. where it was stated that the main biomolecules responsible for the reduction of nanoparticles are the polyphenols and flavonoids found in black tea [14]. Furthermore, it is a widely accepted fact that the biocompounds responsible for the reduction of silver ions have a major influence on the size and size distribution of the synthesized silver nanoparticles. Stronger reducing properties of the phytochemicals have been correlated with the formation of smaller-sized silver nanoparticles [4]. However, very little is known about the effect of phytochemicals on the size and morphology of the silver nanoparticles, and similarly, not much has been reported on the effects of the size and morphology on the antibacterial activity.

The synthesis of the *Camellia sinensis* silver nanoparticles was initiated by the reducing capabilities and capping properties of black tea. This process was further confirmed by the appearance of a dark brown color. Ultraviolet-Vis spectroscopic analysis revealed a characteristic absorption band of 412 nm upon the consecutive addition of the metal salt. Several previous literatures, as in Cataldo et al., obtained a similar characteristic absorption wavelength [13]. Rapid oscillations of electrons on the

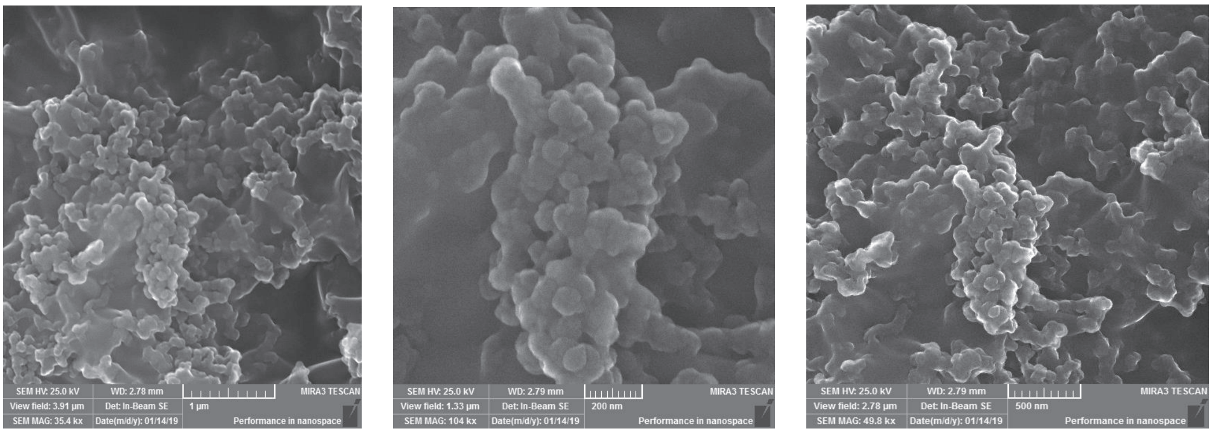

Figure 2: SEM analysis of AgNP synthesized from *Camellia sinensis*.

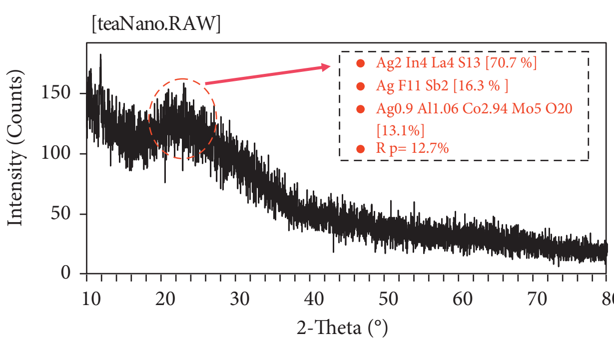

Figure 3: XRD analysis of *Camellia sinensis* silver nanoparticles.

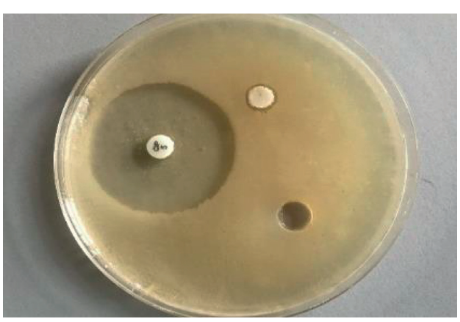

Figure 4: Antibacterial activity of *Camellia sinensis* AgNP against *Staphylococcus aureus.*

nanoparticle surface termed the surface plasmon resonance phenomena have been referred to as the major cause of this characteristic wavelength. This interaction relies on the size and shape of the silver nanoparticles [15].

Scanning electron microscopy was used to investigate the morphology and to determine the stability of the *Camellia sinensis* silver nanoparticles. Characteristic spherical clusters with a diameter of 26.9 nm were obtained in this study. Similar morphological features were found in Asghar et al. in which *Camellia sinensis* silver nanoparticles with a size in the range of 10–20 nm were synthesized and demonstrated antimicrobial activity against methicillin-resistant *Staphylococcus aureus* [16].

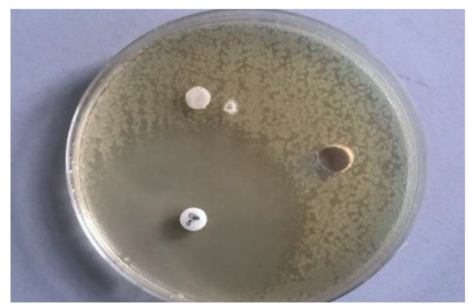

Figure 5: Antibacterial activity of *Camellia sinensis* AgNP against *Escherichia coli.*

X-ray diffractometry analysis was conducted to investigate the nanoscale crystallographic structure [17]. Three characteristic peaks were shown, and a reliable distribution between 12 nm and 64 nm was obtained. Crystallite size of 18 nm was also determined by computing the average size. Additionally, an orthorhombic structure has been revealed. Upon the characterization of the synthesized silver nanoparticles, it is notable to state that the antimicrobial activity has been established using the disc diffusion method. Interestingly, the coupling of the capping properties of black tea with the intrinsic antibacterial capabilities of the silver nanoparticle would result in greater antibacterial activity against Gram $+^{ve}$ and Gram $-^{ve}$ bacteria. However, the individual physicochemical properties of the silver nanoparticles may have an impact on the resulting biological activity.

In our study, *Camellia sinensis* silver nanoparticles with a particle size of 26.9 nm demonstrated antimicrobial activity against Gram $+^{ve}$ *Staphylococcus aureus*, and minimal antimicrobial activity was found against Gram $-^{ve}$ *Escherichia coli*. The anticipated antimicrobial activity of the plant-mediated silver nanoparticles was limited in the face of the obtained physicochemical properties. A particle size greater than 20 nm was obtained in our study. According to the research conducted by Tang et al., it was elucidated that the particles' physicochemical properties will greatly influence the antibacterial activity [18]. In compliance with the

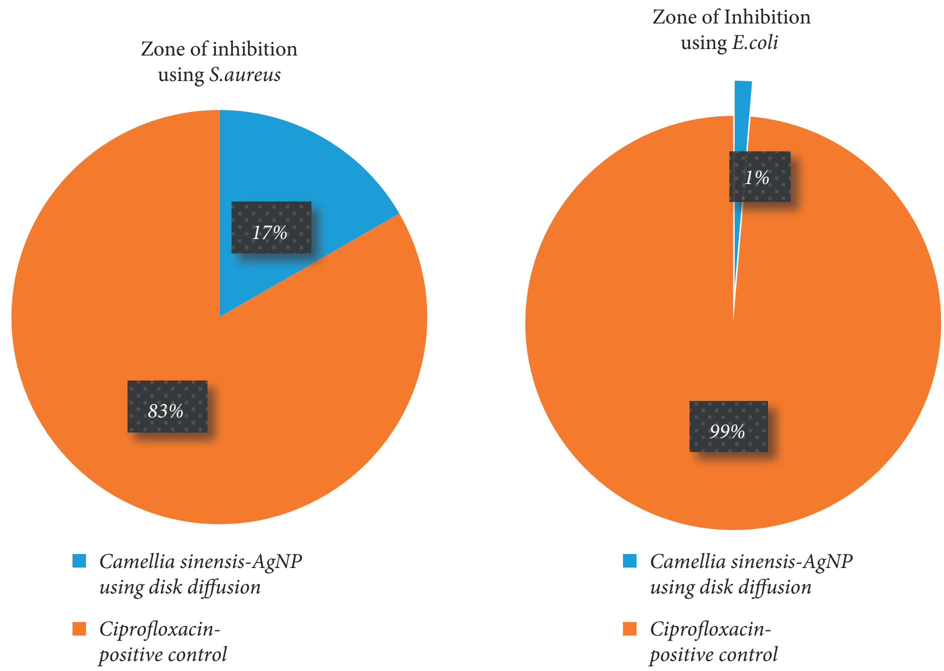

Figure 6: The zone of inhibition obtained against *Staphylococcus aureus* and *Escherichia coli* illustrated in the pie charts.

previous statement, this study also concluded that nanoparticles with a size less than 10 nm and a plate-like morphology exhibited maximum antibacterial activity. An explanation of this phenomenon has been well demonstrated by Raza et al., in which greater antibacterial activity was correlated with smaller nanoparticles as they have greater stability and a larger surface area, thus leading to better interactions and improved intracellular penetration of the bacterial cell walls [19]. Furthermore, the impact of the particle size was indicated by another research, in which smaller size particles had greater activity against *Escherichia coli*. [20]. Gol et al. found that black tea silver nanoparticles with a particles size in the range of 14.9 ± 1.4 nm showed antibacterial activity against *Escherichia coli, Staphylococcus aureus*, *Bacillus subtilis*, and methicillin-resistant *Staphylococcus aureus* [21]. Another interesting correlation would be found in a study conducted by Ismail et al. where the antibacterial activity was mainly observed against the Gram-negative rather than the Gram-positive bacteria; whereas, in our study, the antibacterial activity was mainly against the Gram-positive *Staphylococcus aureus*. A possible explanation for this event would be attributed to the high concentration of silver nanoparticles used in our study, keeping in mind that a similar range of particle size and shape was obtained in the two studies. Thus, the difference in the concentrations and the methods of synthesis may have led to a different level of interaction of AgNP with the cell wall of the microorganisms leading to variations in the spectrum of the antimicrobial activity [22].

Regarding the morphology of the *Camellia sinensis* silver nanoparticles, scanning electron microscopy analysis revealed the presence of roughly spherical silver nanoparticles. Some literature has established a link between the shape of the nanoparticles and the resultant antibacterial activity. A study carried out by Hong et al. stated that the shape of the silver nanoparticle has an undeniable effect on the antibacterial activity. Silver nanoparticles of various shapes were synthesized, and each shape was found to have different antimicrobial activities against *Escherichia coli*. Nanocubes and nanospheres had a greater antibacterial activity due to the larger specific area, unlike nanowires which demonstrated low antibacterial activity [23]. Cheon et al. affirmed that it is possible to control the antimicrobial activity by controlling the shape and size of the silver nanoparticles. Spherical, triangular plate, and disk-shaped nanoparticles were synthesized, and the corresponding antibacterial activity was evaluated against *Escherichia coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Results imply that the antimicrobial activity was the highest in order of silver spheres > silver disks > silver triangles [24].

Shockingly, few studies reported that the shape of the silver nanoparticle may not have a profound effect on the antimicrobial activity as in Actis et al. where spherical, triangular, and cubic silver nanoparticles did not influence the activity against methicillin-susceptible and resistant *Staphylococcus aureus* [25].

5. Conclusion

Camellia sinensis silver nanoparticles were synthesized using the green synthesis method. Evaluation of the synthesized nanoparticles was conducted using ultraviolet-visible spectroscopy, scanning electron microscopy, and X-ray diffractometry. The antimicrobial activity was demonstrated against *Staphylococcus aureus* and *Escherichia coli*.

It is evident that nanoparticles with a size less than 10 nm had a greater surface area and better activity. It should be stated that the physicochemical properties of the silver nanoparticles have a positive strong impact on the intrinsic antimicrobial activity of the silver nanoparticles. The study provides additional insight into the effects of size and shape of the nanoparticles on the claimed antimicrobial activity of the *Camellia sinensis* silver nanoparticles; however, further future advancements in the possible effects of size and morphology are strongly recommended. Furthermore, the effects of the phytochemicals on the physicochemical properties should be explored to enable greater optimization of the antimicrobial properties of silver nanoparticles.

Data Availability

The data used in this study were generated with the assistance of several corporations located in Khartoum, Sudan. UV analysis data were produced by the Alawia Imam Pharmaceutical Development Center located at the University of Medical sciences and Technology. SEM analysis data were retrieved from the Military Industry corporation. XRD data were conducted and generated from the Forensic Laboratories located. The authors further confirm that all the relevant data are included in this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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