



Green chemistry synthesis and *Escherichia coli* antibacterial activity of silver and zinc oxide nanoparticles

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Abstract

Ag and ZnO nanoparticles (NPs) were synthesized by a green synthesis from a *Pinus latteri* tree extraction. Characterizations of the as-prepared Ag and ZnO NPs were determined by DSC/TGA analyses, XRD patterns, and FE-SEM images. The average particles of the as-prepared materials, including Ag NPs and ZnO NPs, are approximately 25 nm and 45 nm, respectively. The XRD patterns confirmed the presence of the Ag metal and ZnO wurtzite crystalline phases. In addition, an agar-well diffusion method was used to evaluate the *Escherichia coli* (*E. coli*) antibacterial activity of as-prepared materials. Results show that Ag NPs and ZnO NPs have a high inhibitory ability against *E. coli* bacteria. Specially, *E. coli* antibacterial activity of Ag NPs is higher than ZnO NPs under the same conditions.

Keywords Ag nanoparticles · ZnO nanoparticles · Antibacterial · Green chemistry

Introduction

Nanomaterials, i.e., Ag and ZnO NPs, are widely studied and applied in industry, medicine, pharmaceutical, environmental treatment, renewable energy, and antibacterial applications [1–7]. Recently, Ag NPs are most used the antibacterial fields because of their strong antimicrobial effect and low cytotoxicity [8–10]. Furthermore, Ag NPs can against gram-negative and -positive bacteria through protein dysfunction and destruction of cell membranes and DNA of bacteria [4, 11, 12]. Also, ZnO NPs can kill both gram-positive and gram-negative bacteria, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Vibrio cholerae* [5, 13]. Many studies reported that reducing the particle size enhanced antibacterial activity

of Ag and ZnO NPs [14–18]. In addition, the antibacterial ability of ZnO NPs is explained by the release of ions Zn^{2+} [19, 20] or contributing ROS radicals to the destruction of bacterial cell membranes [21, 22]. Ag NPs and ZnO NPs generally expressed many advantages in the antibacterial, prevention, and growth inhibition of bacteria, which can be applied in coating technology, medical devices, etc. Furthermore, Ag and ZnO are inexpensive materials that are easy to synthesize and scale up synthesis for practical applications [5, 23, 24].

There have many synthesis methods of Ag NPs and ZnO NPs, such as sol-gel [25, 26], wet chemical route [16, 27], electrochemical reduction [28, 29], chemical vapor deposition [30, 31], microemulsion [32, 33], high-energy irradiation [34], etc. Recently, the green chemistry synthesis method from a plant extraction was used to replace harmful substances and reduce byproducts' exhaustion to the environment, which is the current development trend [15, 35]. Synthesizing nanomaterials from natural origin is considering an environmentally and economically sustainable pathway [36, 37]. For instance, ZnO NPs were synthesized by a green method from *pineapple peel* extract with diameters of 73–123 nm [38]. Mohammadi Arvanag et al. used *Silybum marianum* L. seed to synthesize ZnO, which can ultimately kill *E. coli* bacteria [39]. Kaviya synthesized hexagonal ZnO NPs from pomegranate peel (*Punica granatum*) for *E. coli* and *Staphylococcus aureus* antibacterial [40]. Similarly, Ag NPs were synthesized by a green

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method from *Garcinia mangostana* L. extraction, expressing a higher antibacterial activity on both gram-negative and -positive bacteria [41]. The leaf extract, i.e., *Capsicum annum* L., *Camellia sinensis*, and *Datura metel*, was used as reducing and stabilizing agents for synthesizing Ag NPs that achieved effective antibacterial performance [42–44].

Recent literature reviews indicated that metal and metal oxide NPs have many achievements in the antimicrobial field. However, the toxicity and the optimization dosage of NPs in the antimicrobial is still a problem, which can decrease the minimum toxicity of metal and oxide metal NPs for biomedical applications shortly [45–47]. Based on this problem, a green chemistry synthesis of Ag and ZnO NPs was conducted toward a highly antimicrobial performance. Besides, a rosin was extracted from the *Pinus latteri* tree which obtains more than 95% acid compounds. The synthesized Ag NPs and ZnO NPs were evaluated for their antibacterial activity against *E. coli* at the same conditions.

Materials and methods

Materials

Rosin, having the general formula $C_{19}H_{29}COOH$, was purchased from Loc Thien company, Vietnam. Zinc chloride ($ZnCl_2$), silver nitrate ($AgNO_3$), and sodium hydroxide ($NaOH$) were supplied by Xilong Scientific Co., Ltd, China. Nutrient Broth and Agar powder were provided by Titan Co., Ltd. The *Escherichia coli* gram-negative bacteria (VTCC–B–482) was used by the Institute of Microbiology and Biotechnology, Vietnam National University, Hanoi.

Synthesis of Ag NPs and ZnO NPs

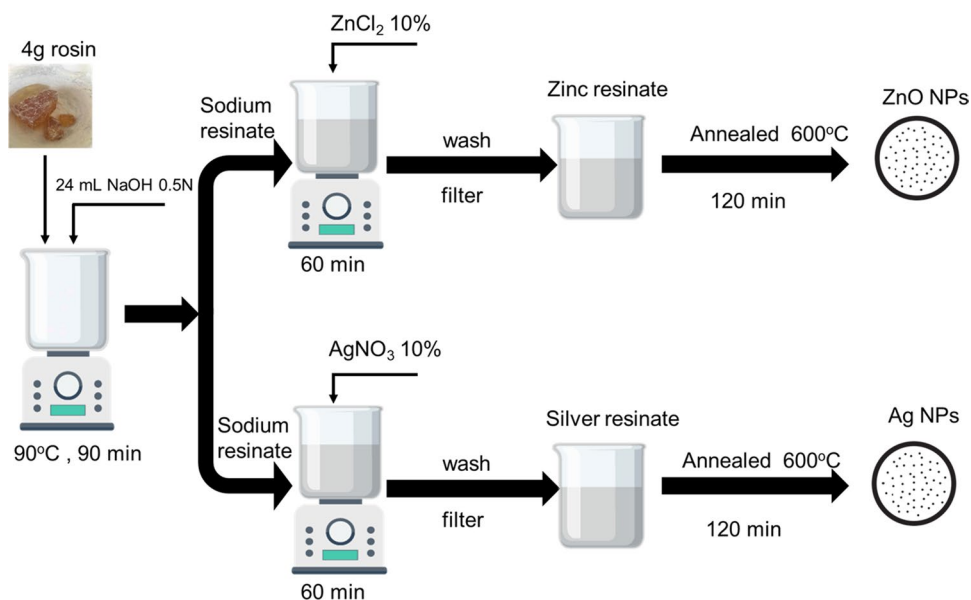
The synthesis process of the ZnO NPs and Ag NPs samples is shown in Fig. 1 in two steps with a following procedure: firstly, 4 g of rosin was placed into a beaker and 24 mL of 0.5N $NaOH$ solution was slowly added to form sodium resinate ($C_{19}H_{29}COONa$) at 90 °C for 90 min. The amount of $NaOH$ was added slowly to avoid excessive alkalinity causing dissociation of soap. After that, to synthesize ZnO NPs, 8.16 g of 10% $ZnCl_2$ solution was added to the $C_{19}H_{29}COONa$ mixture and magnetically stirred for 60 min. The obtained precipitate was washed several times with hot distilled water, then dried at 100 °C for 12 h to get zinc resinate ($(C_{19}H_{29}COO)_2Zn$). Then, the mixture was heated at 600 °C with a heating rate of 5 °C/min, remaining at 600 °C for 2 h.

To synthesize Ag NPs, 20.40 g of 10% $AgNO_3$ solution was added to the above mixture ($C_{19}H_{29}COONa$) and magnetically stirred for 60 min. The obtained precipitate was washed several times with hot distilled water (60 °C), then dried at 100 °C for 12 h. Next, the mixture was annealed at 600 °C for 2 h with a heating rate of 5 °C/min.

Characterization of materials

The structure of prepared materials was characterized by X-ray diffraction (XRD) pattern in the range of 20–80° with a scan rate of 0.02°/s, using a Philips with $Cu K\alpha$ radiation ($\lambda = 1.5418 \text{ \AA}$). The surface morphology and size of materials were determined by field emission scanning electron microscope (FE-SEM) on Hitachi S-4800 equipment. The thermal properties and the weight change of $(C_{19}H_{29}COO)_2Zn$ and silver resinate

Fig. 1 Schematic illustration of the synthesis processes of Ag NPs and ZnO NPs



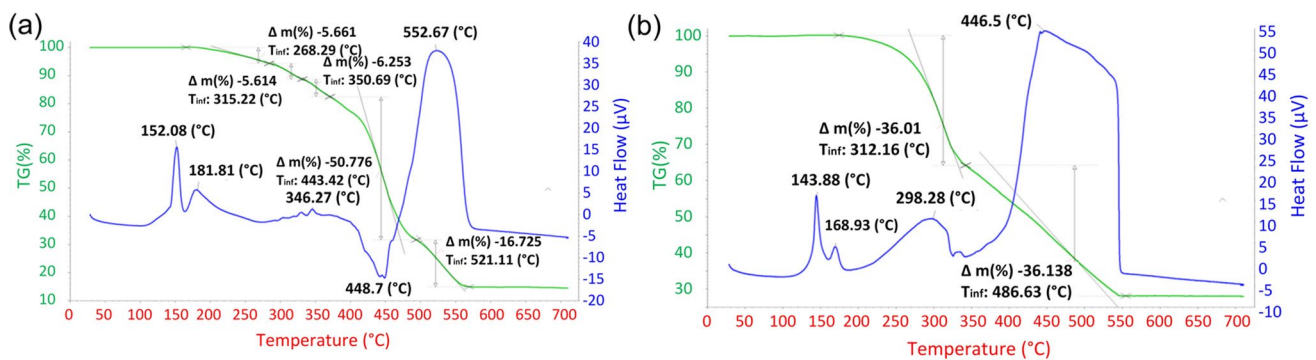


Fig. 2 The TG-DTA curves of $(C_{19}H_{29}COO)_2Zn$ (a) and $C_{19}H_{29}COOAg$ (b)

$(C_{19}H_{29}COOAg)$ were determined using thermogravimetry differential thermal analysis (TG/DTA) on Labsys Evo (TG-DSC 1600 $^{\circ}C$) device.

Escherichia coli cell preparation

Escherichia coli culture medium consisting of 2.6 g of Nutrient Borth and 2 g of Agar was added to a beaker and dissolved with 200 mL of distilled water; then, the mixture was sterilized at 120 $^{\circ}C$ for 15 min. Next, petri dishes were sterilized at 120 $^{\circ}C$ for 15 min to remove all bacteria. Then, 20 mL of culture medium (40–45 $^{\circ}C$) was poured into a petri dish in a microbiological incubator which was sterilized under UV light for 30 min, and continuously incubated at 37 $^{\circ}C$ for 24 h.

Escherichia coli antibacterial test

ZnO NPs and Ag NPs were prepared at various contents: 1, 5, and 10 mg/mL. Adding 1 mL of the *E. coli* cells with

different concentrations was adjusted 5.10^8 , 5.10^7 , 5.10^6 , 5.10^5 , and 5.10^4 CFU/mL and let the bacteria contact the material at intervals of 0, 1, 3, and 6 h, respectively. Then, 10 mL of the above mixture was taken on the agar plate prepared above and incubate at 37 $^{\circ}C$ for 24 h. After incubation, the number of *E. coli* colonies grown on the petri dish was counted with a colony counter, and the results were recorded and repeated three times.

Results and discussion

The TG-DTA curve results of $(C_{19}H_{29}COO)_2Zn$ (Fig. 2a) show that the weight loss of $(C_{19}H_{29}COO)_2Zn$ occurred in three steps. The first weight loss was about 5.7% at the temperature range of 30–280 $^{\circ}C$. There have two peaks at 152 $^{\circ}C$ and 181 $^{\circ}C$ of exothermic effect, related to the physical dehydration and desorption of chemically absorbed water on the $(C_{19}H_{29}COO)_2Zn$, respectively. The second weight loss occurs between 280 and 480 $^{\circ}C$, corresponding to a weight loss of about 64%, with a little exothermic DTA peak at 346.27 $^{\circ}C$ related to organic combustion. The third step takes place between 480 and 580 $^{\circ}C$. There is a robust exothermic peak at 523 $^{\circ}C$ and a weight loss of approximately 16%. This process corresponds to the oxidation of Zn to ZnO and continues organic combustion.

The TG-DTA results of $C_{19}H_{29}COOAg$ (Fig. 2b) shows that with a 2% weight loss, the exothermic effect has two typical peaks at 143 $^{\circ}C$ and 168 $^{\circ}C$, indicating a physical water loss on the material's surface). A typical peak for the exothermic effect in a temperature range of 200 to 340 $^{\circ}C$ and a weight loss of 32% indicate that this process has a significant decomposition of organic matter. Moreover, the weight loss decreases by around 18% between the 340 and 550 $^{\circ}C$ range, and the process is substantially exothermic, demonstrating that the burning of the organic materials continues. The TG-DTA results of both $(C_{19}H_{29}COO)_2Zn$ and $C_{19}H_{29}COOAg$ show physical dehydration at the temperature range of a 30–250 $^{\circ}C$, the decomposition of the organic matter at the

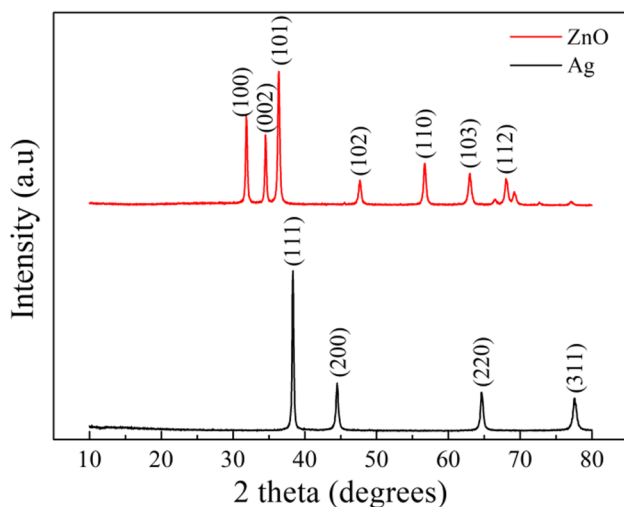


Fig. 3 The X-ray diffraction patterns of Ag NPs and ZnO NPs

Fig. 4 FE-SEM images and particle size distributions of Ag NPs (a, b) and ZnO NPs (c, d)

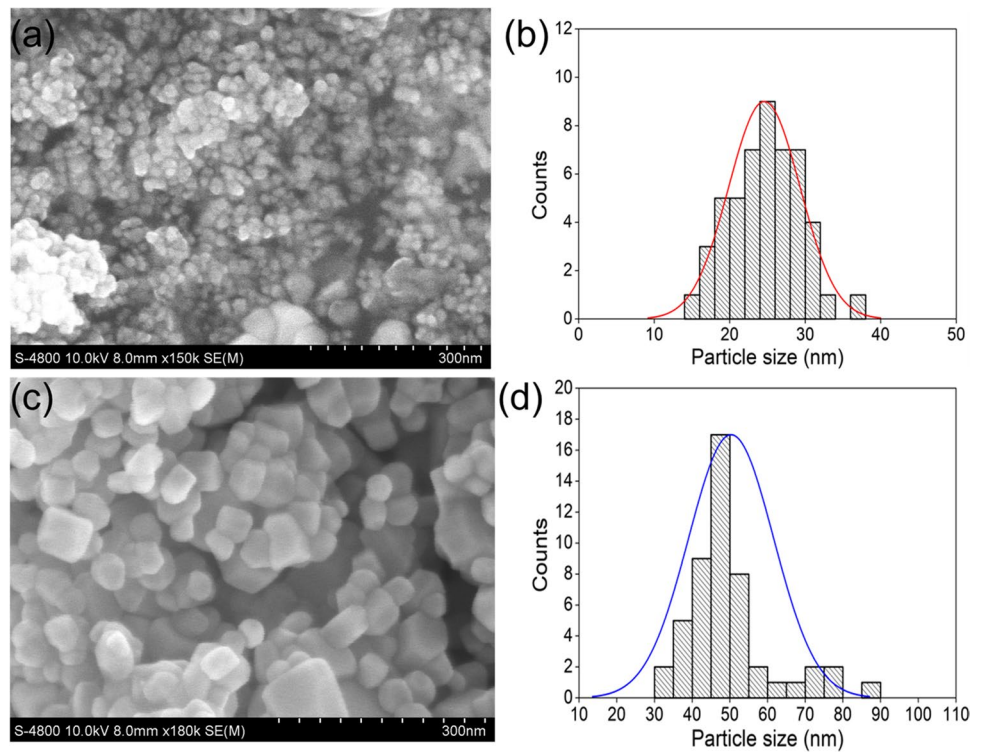


Fig. 5 *Escherichia coli* inhibition percentage by Ag NPs and ZnO NPs with different initial bacterial concentrations, material content used: 0.01 g/L, exposure time: 1 h (a), 2 h (b), and 6 h (c)

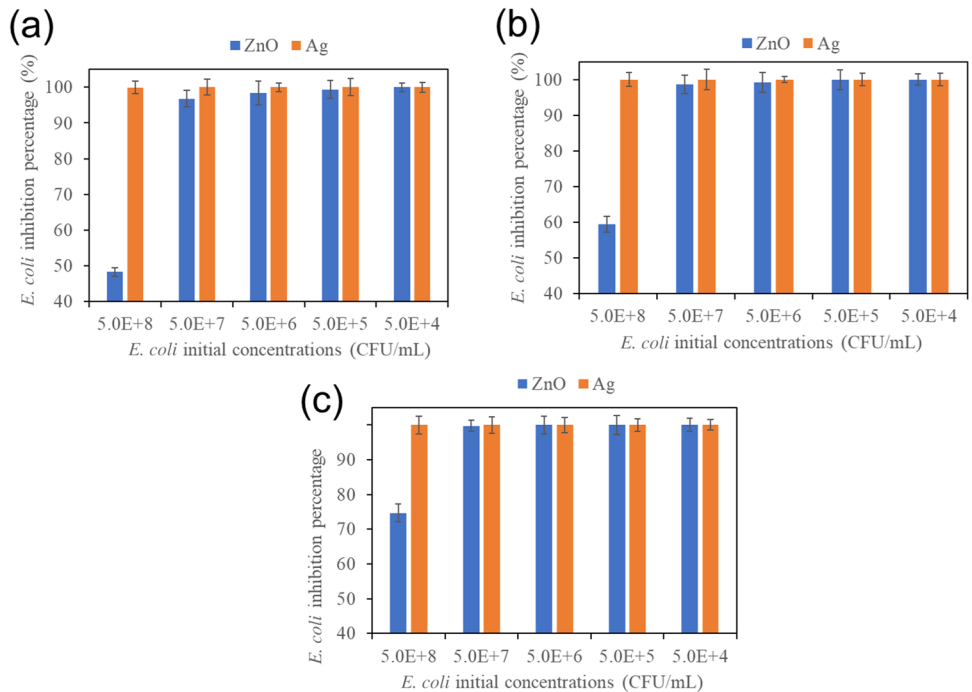
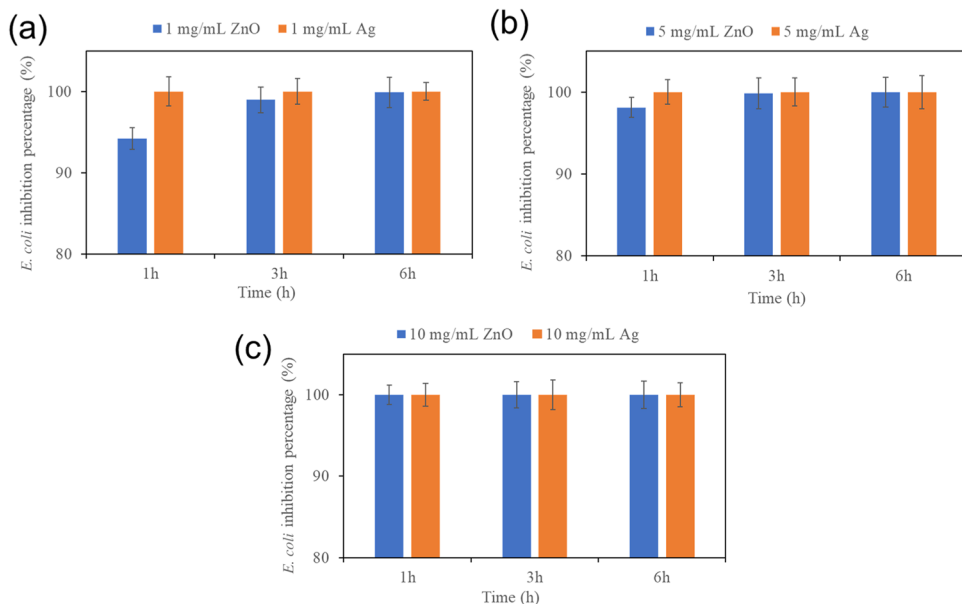


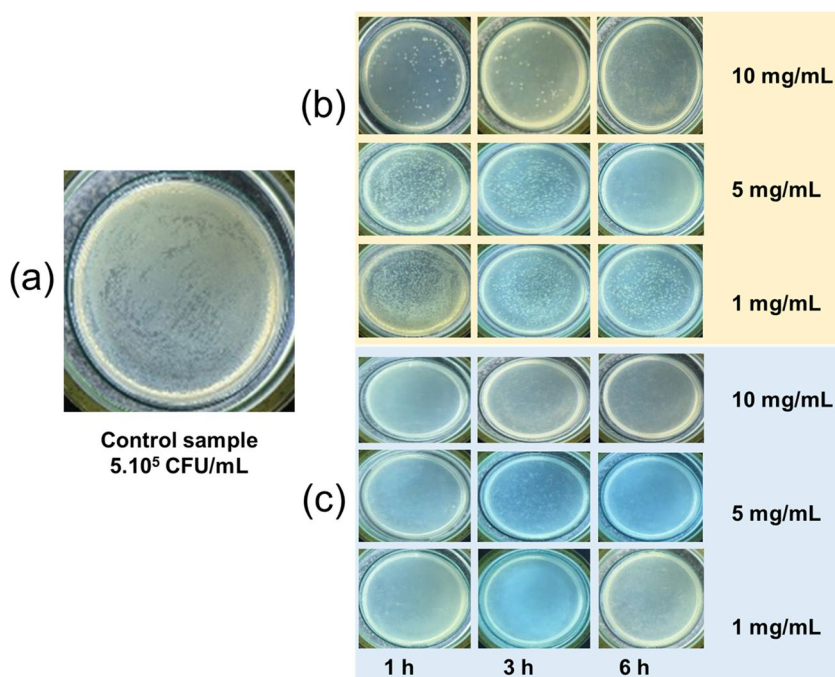
Fig. 6 *Escherichia coli* inhibition percentage by Ag NPs and ZnO NPs with material contents of 1 mg/mL (a), 5 mg/mL (b), and 10 mg/mL (c). *Escherichia coli* initial concentration: 5.10^4 CFU/mL



temperature range of a 300–580°C, and the oxidation to form metal oxides also occurs at this temperature. Therefore, to completely decompose organic matter and the product developed is an entirely crystalline phase, choosing the calcination rate of $(C_{19}H_{29}COO)_2Zn$ and $C_{19}H_{29}COOAg$ at 5 °C/min with a maximum calcination temperature of 600 °C.

XRD patterns of Ag NPs and ZnO NPs (Fig. 3) show diffraction peaks at $2\theta = 31.85^\circ; 34.52^\circ; 36.37^\circ; 47.66^\circ; 56.65^\circ; 62.99^\circ; 66.50^\circ; 68.01^\circ; 69.22^\circ; 72.69^\circ; \text{ and } 77.09^\circ$, corresponding to (100); (002); (101); (102); (110); (103); (200); (112); (201); (004); and (202) planes of hexagonal wurtzite structure of ZnO (JCPDS card no. 36-1451). Also, the XRD pattern of

Fig. 7 Photographs of *E. coli* bacterial culture plates formed (a) and *E. coli* palates were exposed to ZnO NPs (b) and Ag NPs (c)



the Ag NPs displays diffraction peaks at positions $2\theta = 38.11^\circ$; 44.28° ; 64.45° ; and 77.35° , corresponding to (111), (200), (220), and (311) planes of Ag metal (JCPDS card no. 04-0783).

Furthermore, the FE-SEM images and distributions of ZnO and Ag NPs were expressed in Fig. 4. It can be found that the Ag NPs have a uniformly spherical structure and a particle size of about 25 nm (Fig. 4 a–b). The ZnO materials have a tetragonal shape and a larger particle size with an average length of about 45 nm (Fig. 4 c–d).

To determine the *E. coli* antibacterial activity of Ag NPs and ZnO NPs, an experimental work was conducted on the efficiency of inhibiting *E. coli* bacteria by Ag and ZnO NPs with the same amount of material (10 mg/mL). The initial bacterial concentration was from 5.10^4 to 5.10^8 CFU/mL; the exposure time between bacteria and materials was 1 h, 3 h, and 6 h as in Fig. 5. For the initial bacterial concentration was 5.10^8 CFU/mL and the exposure time was 1 h, the bacterial inhibition percentage was only 48.25% for ZnO NPs and 99.98% for Ag NPs. When the exposure time increased to 3 h and 6 h, the inhibition percentage of *E. coli* increased, reaching 59.37% and 74.65% for ZnO NPs and 99.99 and 100% for Ag NPs, respectively. When the initial bacterial concentration decreased by 10, 100, 1000, and 10000 times, representing the *E. coli* concentration of 5.10^7 , 5.10^6 , 5.10^5 , and 5.10^4 CFU/mL, respectively, the antibacterial performance of ZnO NPs increased significantly and achieved 96.76%, 98.43%, 99.39%, and 99.99% in 1-h exposure. Moreover, Ag NPs only need 1-h exposure to completely inhibit *E. coli* bacteria at concentrations of 5.10^5 and 5.10^4 CFU/mL while ZnO NPs completely inhibited *E. coli* with a concentration of 5.10^4 in 6 h of exposure.

To evaluate the effect of material contents on *E. coli* antibacterial ability, an investigation of *E. coli* antibacterial activity with the initial concentration of 5.10^4 CFU/mL over Ag and ZnO NPs with the range varying from 1, 5, and 10 mg/L for 1 h, 3 h, and 6 h exposure periods were investigated (Fig. 6). At 1 mg/mL of the material content, the inhibitory percentage against *E. coli* of ZnO NPs was 94.25, 98.96, and 99.97% for the exposure time of 1 h, 3 h, and 6 h, respectively. Meanwhile, Ag NPs with a content of 1 mg/L could completely inhibit an *E. coli* concentration of 5.10^4 in 1 h. Continuing to perform the antibacterial experiment, when increasing the material content to 5 mg/L and 10 mg/L, the bacterial inhibition rate increased. Specifically, the inhibitory rate of *E. coli* bacteria in the exposure time of 1, 3, and 6 h reached 98.12, 99.86, and 100% when using 5 mg/L ZnO nanomaterials and 99.99, 99.99, and 100% when using 10 mg/L ZnO NPs. Thus, Ag NPs can be killed completely bacteria even at low concentrations and exposure times (1 mg/L and 1 h exposed), while ZnO NPs can be inhibited entirely at 6 h exposed and 5 mg/L.

The difference in the *E. coli* antibacterial activity over Ag NPs and ZnO NPs was also illustrated based on the digital photographs of *E. coli* bacterial culture plates in the control sample and after existing of Ag NPs and ZnO NPs (Fig. 7). As we can see in Fig. 7, the decrease of the amount of *E. coli* bacteria in the plates when the existence of Ag and ZnO NPs when their weight content of these materials as well as the increase of the exposure time. This expressed a high antibacterial activity, and the quantitative performance was also indicated in Fig. 5 and Fig. 6. Besides, a comparison of the antibacterial ability of Ag NPs and ZnO NPs is also

Table 1 A comparison of the antibacterial ability of Ag NPs and ZnO NPs

Type	Size (nm)	Synthesis method	<i>Escherichia coli</i> concentration (CFU/mL)	Concentration of NPs (mg/L)	Exposure time (h)	<i>Escherichia coli</i> efficiency (%)	Ref.
Ag NPs	5	Purchased from Shanghai Huzheng Nanotechnology Company Limited.	10^7	10	168	100	[48]
	10–40	Biosynthesis method using <i>Lycopersicon esculentum</i> extract	10^6	50	4	100	[49]
	N/A	Manufactured by Thermolon Korea, Inc. (Busan, Korea)	10^5	100	6	100	[50]
	16	Inert gas condensation method	10^4	60	24	100	[51]
	25	Green method using rosin extract	5.10^7	10	3	100	This study
ZnO	500–1000	Hydrothermal method	10^7	25	0.5	48.7	[52]
	18–21	Sol-gel method	10^6	18	24	24	[46]
	~ 30	Purchased from Inframat Advanced Materials LLC (Manchester, CT)	10^4	240	16	100	[53]
	4–5	Chemical precipitation	10^5	1500	24	100	[54]
	12–95	Green synthesis using orange fruit peel extract	10^6	25	8	99.96 to 99.99	[55]
	45	Green method using rosin extract	5.10^7	10	6	99.78	This study

presented in Table 1. Therein, the *E. coli* antibacterial of the Ag NPs and ZnO NPs, synthesized by the green chemistry method from rosin agent in this study, expressed a highly efficient performance (nearly 100%) even at a very short exposure time (3 h for Ag NPs and 6 h for ZnO NPs).

Conclusion

In summary, we successfully fabricated Ag and ZnO NPs by a green synthesis from inexpensive precursors and a non-toxic rosin compound. The particle size of the synthesized Ag NPs are smaller than the ZnO NPs with the average size of Ag NPs being 10–40 nm while the ZnO is 30–90 nm. The *E. coli* antibacterial activity of Ag NPs is higher than that of ZnO NPs because Ag NPs are smaller particle size than ZnO, leading to Ag NPs easily penetrate the cell membrane and kill bacteria. In 6 h of exposure, the synthesized Ag NPs completely inhibited *E. coli* bacteria at the concentration of 5.10^8 , while ZnO NPs inhibited *E. coli* bacteria at the concentration of 5.10^4 CFU/mL.

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Data Availability The raw/processed data required to reproduce the above findings cannot be shared at this time as the data also forms part of an ongoing study.

Declarations

Competing interest The authors declare no competing interests.

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