CREATIVE SCIENCE

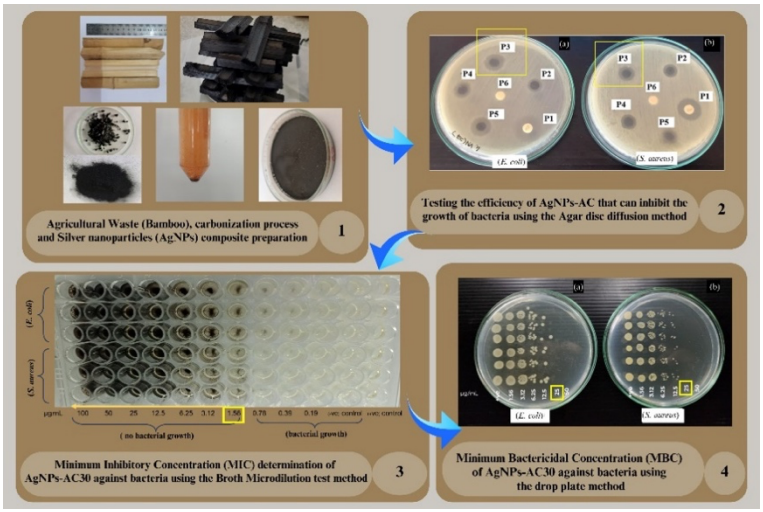
Journal home page: csj.snru.ac.th

In vitro anti-pathogenic activity of composite materials made of activated carbon from bamboo containing silver nanoparticles

Wittaya Suwonnachot[1], Konkanok Chaisen[1*] and Sasiporn Audtarat[1]
[1]Faculty of Interdisciplinary Studies, Khon Kaen University, Nong Khai, 43000 Thailand
[*]**Corresponding Author:** konkch@kku.ac.th
DOI: 10.55674/cs.v17i1.258193

Received: 4 September 2024; **Revised:** 22 November 2024; **Accepted:** 29 November 2024; **Available online:** 2 December 2024

Abstract



Pathogenic bacterial contamination has become a major concern for public health and the environment. However, the development of anti-pathogenic materials remains exceptionally challenging. This study examined the anti-pathogenic activities of composite materials by combining silver nanoparticles with activated carbon from bamboo charcoal. This was done to improve the overall qualities of the materials, avoid agglomeration, and maintain their original properties. The experiments sought suitable conditions for producing activated carbon from bamboo charcoal using physical reactivation under high-temperature steam. This greatly increased the specific surface area. Then, the material was combined with silver nanoparticles to test its anti-pathogenic activity against two common pathogenic bacteria, *Escherichia coli* DMST 12743 and *Staphylococcus aureus* DMST 19381. The findings revealed that the optimum time for producing activated carbon under high-temperature steam at 650 °C was 30 min. Anti-pathogenic activity was determined using an agar disc diffusion method. Testing included determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using broth microdilution and drop plate methods. These tests showed a greater anti-pathogenic effect against the Gram-negative bacterium. The zones of inhibition (ZOI) were 12.00±1.00 mm and 10.67±0.29 mm, respectively, against *E. coli* and *S. aureus*. MIC values of 1.56 µg/mL resulted with MBC values of 25 µg/mL. These composite materials have antimicrobial properties. This promotes nanotechnology and activated carbon use from agricultural waste for commercial products. These products can be employed in various treatment systems and medical devices for environmental safety and public health enhancement. Our approach demonstrates the development of nanotechnology and biomedical science integrated with green technology.

Keywords: Nanotechnology; Bamboo charcoal; Composite materials; Physical Reactivation

1. Introduction

Currently, nanotechnology is a critical area of research with broad applications across various disciplines [1]. Over the past decade, silver nanoparticles (AgNPs), have received considerable interest owing to their strong antibacterial capabilities and diverse biological activities [2]. Biological synthesis is a rapidly advancing and environmentally friendly method to produce AgNPs. Their use reduces reliance on hazardous chemicals and aligns with green chemistry principles [3]. Reports have documented successful biological synthesis of AgNPs using a variety of biological entities, including bacteria, fungi, yeast, algae, and plants [4]. AgNPs are particularly notable for their effectiveness as antimicrobials against a range of microorganisms, including viruses, fungi, and bacteria. Their nanoscale size, typically between 1 and 100 nm, endows them with a large surface area, facilitating extensive interaction with microbial cells [5]. This property has led to their application in diverse fields, including medical device coatings, wound dressings, textile fibers, paints, and food packaging materials [6]. Additionally, they have been integrated into dental restorative materials to prevent tooth decay [7]. Theories suggest they interact with bacterial cell walls, cause membrane damage, and release reactive oxygen species, disrupting essential functions. Additionally, they interfere with DNA replication, leading to cell death [8].

Pathogenic bacterial contamination poses a significant threat to public health and environmental safety, as these microorganisms can cause severe infections, propagate antibiotic resistance, and disrupt ecosystems. Gram-positive and Gram-negative bacteria have evolved antibiotic resistance in different ways [9]. The emergence of antimicrobial-resistant bacteria has become a global threat. Hospital-acquired infections (HAIs) caused by drug-resistant pathogens are particularly concerning. The five major bacterial pathogens contributing to HAIs are *Staphylococcus aureus, Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* [10]. Researchers and healthcare professionals are increasingly focused on finding innovative solutions to address severe bacterial infections in this evolving landscape.

AgNPs and bamboo charcoal can be used to fabricate enhanced composite materials, providing improved properties for various applications. Activated carbon, derived from bamboo charcoal, is an effective adsorbent for removing pollutants and conforms to environmental regulations of the pharmaceutical and chemical industries [11]. Bamboo is rich in high-quality cellulose fibers. Its charcoal exhibits a large surface area, and a porous structure [12], making it an ideal candidate for composite materials. Bamboo is an abundant, inexpensive material. Bamboo charcoal can be integrated into composites due to its porosity and high surface area, which ranges from 600 to 2,000 m^2/g [13]. Although there are other materials with antibacterial properties, such as gold nanoparticles (AuNPs), titanium dioxide nanoparticles (nanoTiO), and zinc oxide nanoparticles (nano ZnO), the study of composite materials containing AgNPs remains of great interest to researchers. This is due to its foundation on past research in which biological synthesis techniques were developed for AgNPs.

The current research combines AgNPs with activated carbon using physical activation with no chemicals to improve the material structure yielding high porosity and large surface area. It is a new composite material biologically synthesized with antibacterial AgNPs produced through an environmentally friendly process. The study team obtained the AgNPs from graduate research of the Interdisciplinary Faculty, Khon Kaen University. They were synthesized from crude extracts of moringa leaves using a biological method. Material characterization was done using transmission electron microscopy (TEM), UV-visible spectroscopy, and X-ray diffraction (XRD) analysis, per the methodology of Audtarat et al. [14] The effectiveness of the composite materials against *Escherichia coli* and *Staphylococcus aureus* was then tested. It is hypothesized that this composite material will have excellent antibacterial properties.

This research aims to produce a composite material made of bamboo-activated carbon and AgNPs, utilizing a green synthesis approach throughout the entire process. Its goal is to find the optimal conditions for producing activated carbon through high-temperature physical activation with steam, and then mix the resulting product with AgNPs. A further goal is to quantify the antibacterial activities of these composites against Gram-negative *E. coli* and Gram-positive *S. aureus*.

2. Materials and Methods

Materials

Commonly used laboratory glassware and equipment were employed. These included an autoclave sterilizer (FLS-1000, Tommy, Japan); an oven (Cosmos CO-9919, North Jakarta, Indonesia); a furnace (Vulcan 3-130, USA); a hot air oven (Binder, Germany); an ultrasonic cleaner (standard, ISOLAB, Germany); a biosafety cabinet, Class II (ESCO, SC2-4E1, Singapore); a micro-pipette (NPX2, Nichiryo, Japan); and an incubator (Memmert, Germany).

Preparation of activated carbon

Bamboo samples were collected in Nong Kom Ko Subdistrict, Mueang District, Nong Khai Province, Thailand. A saw was used to cut the samples into small pieces, as shown in Figure 1a. The samples were washed, cleaned, partially dried in the sun, and put into a hot air oven at 70 °C until the sample weights were constant. Then, they were carbonized in a high-temperature furnace at 400 °C for 1 h to produce charcoal, as shown in Figure 1b. Sample 1 was referenced as CC (charcoal), and samples 2-4 were referred to as AC (activated carbon). The second set of bamboo charcoal samples was physically reactivated to increase porosity under steam activation at 650 °C for three different times, 30, 45, and 60 min. These samples were respectively referenced as AC30, AC45, and AC60, as shown in Figure 1c. After that, they were thoroughly ground to pass a 500 μm sieve to yield powder-activated carbon, as shown in Figure 1d. CC and AC (AC30, AC45, and AC60) samples were stored in a desiccator, before processing them into composite materials with AgNPs and subsequent property testing.

Compositing nanomaterials

Compositing with activated carbon and AgNPs was done as outlined by Audtarat et al. [14] This was done by mixing solutions of 0.2% (w/v) AgNPs with 1 g of each of the three activated carbon samples, as shown in Figure 1e. Mixing was done at room temperature for 18 h using a magnetic stirrer. The samples were referenced as AgNPs-AC30, AgNPs-AC45, and AgNPs-AC60, where the final two digits represent processing time under 650 °C steam. The samples were placed in an ultrasonic bath for 1 h and dried in a hot air oven at 90 °C, as depicted in Figure 1f. The silver nanoparticle composite powders with activated carbon (AgNPs-AC) were kept in a tightly closed container.

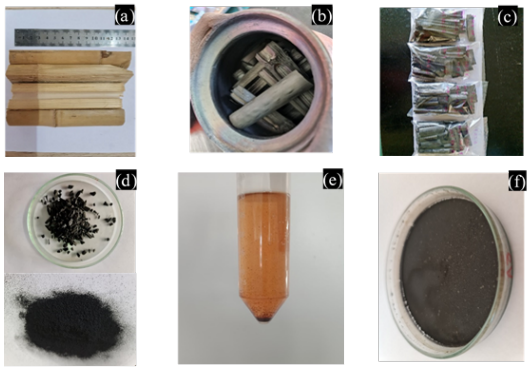


Fig. 1 Agricultural waste (Bamboo) (a), preparation of activated carbon: carbonization process (b), activation step (c), and final activated carbon product (d), AgNPs composite preparation: AgNPs synthesis (e), and characterization (f).

Effect of silver nanoparticle composites on bacterial growth

AgNPs-AC growth inhibition of bacteria determined by agar disc diffusion

This was a qualitative test. The AgNPs-AC samples were tested against two types of pathogenic bacteria, *E. coli* and *S. aureus*. The

test adapted an agar disc diffusion method from the Clinical and Laboratory Standards Institute (CLSI, 2012) [15]. First, two pure bacterial cultures grown in nutrient broth (NB) were incubated at 35±2 °C under aerobic conditions for 18 h. Their turbidity was adjusted to an optical density at a wavelength of 600 nanometers (OD_{600}) equal to 0.4 (or ~1 x 10^8 CFU/mL) before spreading an inoculum over the surface of nutrient agar (NA) using a sterile cotton swab. A sterile paper disc was placed on and gently pressed into the NA agar to ensure contact. Then, 10 µL of the test samples, AgNPs-AC30, AgNPs-AC45, and AgNPs-AC60, were dropped onto the discs. Dimethyl sulfoxide (DMSO) dropped onto a disc served as a negative control and streptomycin was the positive control. Each set was tested in triplicate to ensure the accuracy and reliability of the data. The samples were incubated at 35±2 °C for 18 h under aerobic conditions. After that, the inhibition or clear zones were examined, and their widths were measured using Vernier calipers. These results were recorded, and the averages and standard deviations were calculated.

Minimal inhibitory concentration (MIC) using a broth microdilution test method

This was a quantitative test adapted from a method from the Clinical and Laboratory Standards Institute (CLSI, 2012) [15]. This determination provides the MIC value. The test was done in a 96-well microtiter plate. Two pure bacterial cultures, *E. coli* and *S. aureus*, were grown in nutrient broth (NB) and incubated at 35±2 °C for 18 h under aerobic conditions. After that, AgNPs-AC30, AgNPs-AC45, AgNPs-AC60, and CC (charcoal) were prepared. Their 100 µg/mL concentrations were diluted via a 2-fold serial dilution to achieve 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391, and 0.195 µg/mL levels. Nutrient broth cultures of *S. aureus* and *E. coli* were sampled (100 µL). Their turbidity was adjusted to an OD_{600} of 0.4. Ten µL of each bacterial culture were added to the 96-well microtiter plate and their growth compared with positive and negative controls. Each set was tested in triplicate to ensure the accuracy and reliability of the data. Then, the samples were incubated at 35±2 °C for 18 h under aerobic conditions. The MIC was read by determining the well with the lowest concentration that is clear and free of sediment. Results were recorded, and the means and standard deviations were determined.

Minimum bactericidal concentration (MBC) using a drop plate method

The solution in a well that is clear and free of sediment at the bottom from the process of finding the MIC value was retrieved. Then, 5 µL of the solution was placed onto a TSA medium using a drop plate method. The cultures were incubated at 35±2 °C for 18 h under aerobic conditions. Experiments were done in triplicate to ensure the accuracy and reliability of the data. The results were recorded by observing the growth of the tested bacteria on the TSA medium. Mean and standard deviation values were calculated. The minimum bactericidal concentration (MBC) is defined as the lowest concentration of a substance that can inactivate a test microorganism, resulting in no growth on the surface of the medium.

Statistical data analysis

Quantitative data analysis was done in triplicate to ensure the accuracy and reliability of the data. Descriptive statistics were employed, including percentage, arithmetic mean, and standard deviation.

3. Results and Discussion

Preparation of activated carbon and composites with AgNPs

Activated carbon composites with AgNPs appear as dry, black charcoal powders.

AgNPs-AC inhibition of bacterial growth using an agar disc diffusion method

Materials composited with AgNPs could inhibit the growth of pathogenic bacteria in agar diffusion tests. Specifically, AgNPs-AC30 exhibited superior antibacterial activity

against both Gram-negative and Gram-positive bacteria, respectively showing inhibition zones with diameters of 12.00±1.00 mm and 10.67±0.29 mm, as presented in Table 1. The zones of inhibition (ZOI) are shown in Figure 2.

Table 1 Diameters of sample inhibition zones against *E. coli* and *S. aureus*

		Inhibition zone diameter (mm)	
No.	Materials	*E. coli*	*S. aureus*
P1	Streptomycin	14.67±0.58	15.33±0.58
P2	AgNPs-CC	9.33±1.15	10.00±0.00
P3	AgNPs-AC30	12.00±1.00	10.67±0.29
P4	AgNPs-AC45	10.50±0.50	10.00±0.00
P5	AgNPs-AC60	12.17±0.76	8.67±0.58
P6	DMSO	0	0

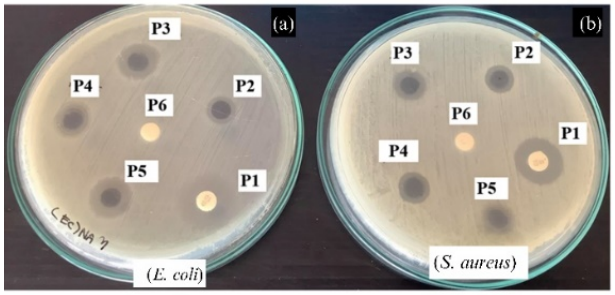


Fig. 2 Inhibition zone of Streptomycin (P1), AgNPs-CC (P2), AgNPs-AC30 (P3), AgNPs-AC45 (P4), AgNPs-AC60 (P5), and DMSO (P6) against (a) *E. coli* and (b) *S. aureus*

Minimum inhibitory concentration (MIC) using a broth microdilution test

The MIC of silver nanoparticle composites was determined against the growth of bacteria at concentrations ranging from 0.19 to 100 µg/mL. Wells were observed that were clear and free of sediment and had the lowest concentration of active materials. The MIC value was 1.56 µg/mL, as shown in Figure 3.

Minimum bactericidal concentration (MBC) using a drop plate method

The results of a test to determine the MBC value of silver nanoparticle composites against the growth of bacteria was done by observing the lowest concentration with no bacterial growth on the surface of a TSA medium, as shown in Figure 4. This test revealed that the MBC value was 25 µg/mL, as shown in Fig. 4 and Table 2.

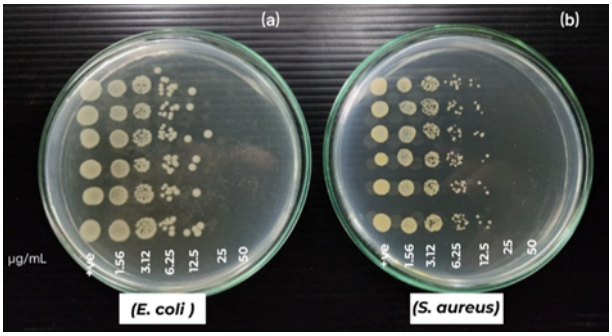


Fig. 4 Minimum bactericidal concentration (MBC) of AgNPs-AC30 against the growth of (a) *E. coli* and (b) *S. aureus* using a drop plate method

Table 2 Comparison of MIC and MBC values of AgNPs-AC30 samples against *E. coli* and *S. aureus*

No.	AgNPs-AC30	MIC (µg/mL)	MBC (µg/mL)
1	*E. coli*	1.56	25
2	*S. aureus*	1.56	25

Bamboo fibers contain high-quality cellulose, making bamboo charcoal very porous, which provides a large surface area

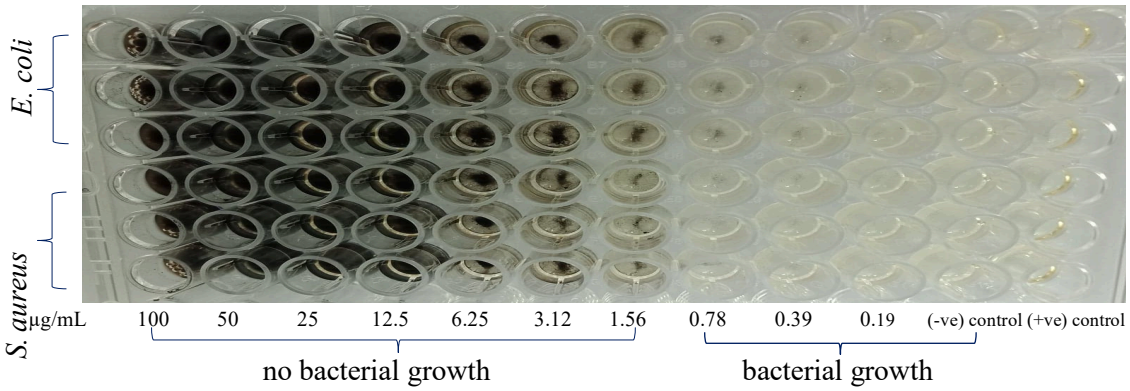


Fig. 3 Minimum inhibitory concentrations (MIC) of AgNPs-AC30 against *E. coli* and *S. aureus* obtained using a broth microdilution method

[12], [13]. This makes it highly suitable for producing activated charcoal. When bamboo charcoal undergoes physical regeneration and is mixed with composite materials coated with AgNPs, these composites exhibit significant antibacterial action. Notably, they continue to show a remarkable growth inhibition of pathogenic bacteria compared to untreated conditions with a greater inhibitory capacity against Gram-negative bacteria. Increasing the activation time did not affect the antimicrobial inhibition of the composite materials. Therefore, when applying these conditions, they can be standardized and potentially used for unit cost calculations. This observation aligns with the study by Liu et al. [16]. They developed bamboo-plastic composites (BPCs) consisting of biodegradable plastic mixed with bamboo fibers and reinforced with ZnO, another material with antimicrobial properties. These composites are biodegradable and strong, with antimicrobial activity that provides an environmentally friendly alternative to traditional plastics. Additionally, the research of Audtarat et al. [14] examined nanocomposites of AgNPs and bacterial cellulose. These materials demonstrated greater antimicrobial activity of AgNPs against *E. coli* than *S. aureus* in the current study. The reason for this difference is the variation in the thickness and composition of the bacterial cell membrane [14]. This composite material shows high efficacy due to its activation and nanoscale size. Experimental results indicate that when AgNPs are mixed with bamboo charcoal, the resulting material possesses antimicrobial properties against both Gram-positive and Gram-negative bacteria. The antimicrobial mechanism of silver is due to its capability to release silver ions, which damage cell membranes by binding to proteins, negatively impacting the membrane structure and function. Additionally, silver ions can also penetrate cells and inhibit various enzymes, such as those involved in DNA and RNA synthesis. This results in disruption of growth processes. Additionally, silver ions can directly bind to microbial DNA, inhibiting DNA replication, which disrupts cell division and structural protein synthesis. This also triggers generation of free radicals within cells, which can damage various cellular components. AgNPs exhibit high toxicity to microbial cells, causing clear DNA strand breakage. This genetic damage is correlated with the quantity of AgNPs used [17].

Although new nano-materials such as AgNPs have garnered significant attention from scientists, confirming both their physical and chemical superiority as well as their antimicrobial efficacy is ongoing. Some researchers raise concerns about their use in food and contact with people. AgNPs can be used in food packaging for their functional capabilities, but there remain concerns regarding their safety and toxicity. Human cells exhibit cytotoxicity, genotoxicity, and inflammatory reactions when exposed to AgNPs. Ingestion, inhalation, and skin contact are three ways nanoparticles can be encountered. The primary risk of human nanoparticle contact is their ingestion. AgNPs can negatively impact people and the environment. Concerns regarding the biosafety of AgNPs, including toxicity to organs, cells, and other tissues, persist. Even repeated exposure over time can still cause damage to mammalian cells, as seen in studies of rat liver cells, nerve cells, rat stem cells, and human respiratory epithelial cells. The acute toxic effects of AgNPs at various concentrations have been studied. Their impacts on plant roots at concentrations of 50-75 ppm showed considerable oxidative harm and decreased root development. Additionally, AgNPs decreased the hatching rate of zebrafish eggs and caused abnormalities in their spinal cords, eye damage, and bent tails in their embryos. AgNPs spread throughout various systems, including the heart, brain, and blood of zebrafish embryos. This highlights the importance of in vivo studies of AgNPs, which can assist us in more accurately evaluating the acute and long-term toxicity of these nanomaterials [18]. These risks must be clarified through detailed toxicological investigations. Establishing the maximum allowable concentration of nanoparticles in food is crucial for consumer protection, and the biggest challenge is complying with legal standards [19]. Guidelines for reducing

toxicity risks include using the lowest concentration that still exhibits the properties of nanoparticles and selecting appropriate amounts for each condition. Studies of the long-term exposure effects and the impact of nanosilver on various human systems, as well as its environmental effects, should also be conducted. This will lead to the development of safer materials with improved physical and mechanical properties.

The current research employs a green method throughout the process, from the synthesis of AgNPs to the activation of charcoal and the assembly of composite materials. This green method is becoming increasingly popular as it represents sustainable and environmentally friendly development, reducing the drawbacks of high-cost and potentially toxic chemical methods. The bamboo composite material with AgNPs in the current research exhibits special antimicrobial properties, making it suitable for use in various treatment systems and medical devices for environmental safety and public health enhancement.

4. Conclusion

This research produced a composite material consisting of AgNPs coated on the surfaces of activated carbon from bamboo. The activated carbon was prepared by physical activation of bamboo under high-temperature steam, with an optimal activation time of 30 min. Antibacterial activity tests using the agar diffusion method showed that the AgNPs-AC composite significantly inhibited the growth of pathogenic bacteria. AgNPs-AC30 exhibited superior antibacterial activity against both Gram-positive and Gram-negative bacteria compared to other tested substances. The inhibition or clear zones of AgNPs-AC30 measured 12.00±1.00 mm and 10.67±0.29 mm for *E. coli* and *S. aureus*, respectively. MIC values of 1.56 µg/mL resulted in MBC values of 25 µg/mL. The study results suggest that AgNPs-AC composites are effective in inhibiting both Gram-positive and Gram-negative pathogenic bacteria. Their high antimicrobial activity makes them a satisfactory option for use in various therapeutic systems or medical devices. This study highlights the potential of combining AgNPs with activated carbon derived from agricultural wastes to develop commercially viable antimicrobial products, leveraging advancements in nanotechnology and biomedical science.

5. Suggestions

The study scope can be expanded to industrial-level experiments, possibly incorporating other types of materials to enhance the overall performance of the composite. Additionally, testing should be conducted with other pathogenic strains that have antibiotic resistance issues.

6. Acknowledgement

This research was financially supported by the Routine to Research (R2R) funding program of Khon Kaen University in 2024. We extend our sincere gratitude to the Faculty of Interdisciplinary Studies, Khon Kaen University, for their invaluable support, including access to BSL-2 laboratories and the provision of essential tools, equipment, and chemicals.

7. References

[1] A.H. Virgili, D.C. Laranja, P.S. Malheiros, M.B. Pereira, T.M.H. Costa, E.W. de Menezes, Nanocomposite film with antimicrobial activity based on gold nanoparticles, chitosan and aminopropylsilane, Surf. Coat. Technol. 415 (2021) 127086. 10.1016/j.surfcoat.2021.127086.

[2] M. Sajid, J. Plotka-Wasylka, Nanoparticles: synthesis, characteristics, and applications in analytical and other sciences, Microchem. J. 154 (2020) 104623. 10.1016/j.microc.2020.104623.

[3] V. Harish, M.M. Ansari, D. Tewari, A. B. Yadav, N. Sharma, S. Bawarig, M.L. García-Betancourt, A. Karatutlu, M. Bechelany, A. Barhoum, Cutting-edge advances in tailoring size, shape, and functionality of nanoparticles and nanostructures: A review, J. Taiwan Inst. Chem. Eng. 149 (2023) 105010. 10.1016/j.jtice.2023.105010.

[4] A.Ahmed, M. Usman, Z. Ji, M. Rafiq, B. Yu, Y. Shen, H. Cong, Nature-inspired biogenic synthesis of silver nanoparticles

for antibacterial applications, Mater. Today Chem. 27 (2023) 101339. 10.1016/j.mtchem.2022.101339.

[5] A.A. Yaqoob, K. Umar, M.N.M. Ibrahim, Silver nanoparticles: Various methods of synthesis, size affecting factors and their potential applications—A review, Appl. Nanosci. 10 (2020) 1369–1378. 10.1007/s13204-020-01318-w.

[6] K. Karnpimon, R. Phatramanon, Plant extract synthesized silver nanoparticles and their antimicrobial activity, Khon Kaen Sci. J. 45(1) (2017) 34–52.

[7] K.X. Ge, C. Y., W.Y. Lam, C.H. Chu, O.Y. Yu, A novel glass ionomer cement with silver zeolite for restorative dentistry, J. Dent. 133 (2023) 104524. 10.1016/j.jdent.2023.104524.

[8] M. Alavi, M.R. Hamblin, Antibacterial silver nanoparticles: effects on bacterial nucleic acids, Cell. Mol. Biomed. Rep. 3(1) (2023) 35–40. 10.55705/cmbr.2022.361677.1065.

[9] T.R. Walsh, A.C. Gales, R. Laxminarayan, P.C. Dodd, Antimicrobial Resistance: Addressing a Global Threat to Humanity, PLoS Med. 20(7) (2023) e1004264. 10.1371/journal.pmed.1004264.

[10] S. Tiengrim, V. Thamlikitkul, Inhibitory Activity of Fermented Milk with Lactobacillus Casei Strain Shirota against Common Multidrug-Resistant Bacteria Causing Hospital-Acquired Infections, J. Med. Assoc. Thailand. 95(2) (2012) S1-S5. https://www.thaiscience.info/journals/Article/JMAT/10971373.pdf.

[11] A. Menichetti, A. Mavridi-Printezi, D. Mordini, M. Montal, Effect of size, shape and surface functionalization on the antibacterial activity of silver nanoparticles, J. Funct. Biomater. 14(5) (2023) 244. 10.3390/jfb14050244.

[12] D. Zou, X. Zheng, Y. Ye, D. Yan, H. Xu, S. Si, X. Li, Effect of different amounts of bamboo charcoal on properties of biodegradable bamboo charcoal/polylactic acid composites, Int. J. Biol. Macromol. 216 (2022) 456–464. 10.1016/j.ijbiomac.2022.06.209.

[13] M.S. Reza, C.S. Yun, S. Afroze, N. Radenahmad, M.S.A. Bakar, R. Saidur, J. Taweekun, A.K. Azad, Preparation of activated carbon from biomass and its applications in water and gas purification, a review, Arab J. Basic Appl. Sci. 27(1) (2020) 208–238. 10.1080/25765299.2020.1766799.

[14] S. Audtarat, P. Hongsachart, T. Dasri, S. Chio Srichan, S. Soontaranon, W. Wongsinlatam, S. Sompech, Green synthesis of silver nanoparticles loaded into bacterial cellulose for antimicrobial application, Nanocomposites. 8(1) (2022) 34–46. 10.1080/20550324.2022.2055375.

[15] CLSI, Performance Standards for Antimicrobial Susceptibility Testing, 30th ed., Clinical and Laboratory Standards Institute, Wayne (PA), 2020.

[16] C. Liu, S. Zhang, S. Yan, M. Pan, H. Huang, Mechanical and antibacterial properties of bamboo charcoal/ZnO-modified bamboo fiber/polylactic acid composites, Forests. 15(2) (2024) 371. 10.3390/f15020371.

[17] P..Nie, Y. Zhao, H. Xu, Synthesis, applications, toxicity and toxicity mechanisms of silver nanoparticles: A review. Ecotoxicol. Environ. Saf. 253 (2023) 114636. https://doi.org/10.1016/j.ecoenv.2023.114636.

[18] M. Noga, J. Milan, A. Frydrych, K. Jurowski, Toxicological aspects, safety assessment, and green toxicology of silver nanoparticles (AgNPs)—Critical review: State of the art, Int. J. Mol. Sci. 24 (2023) 5133.https://doi.org/10.3390/ijms24065133.

[19] K. Kraśniewska, S. Galus, M. Gniewosz, Biopolymers-based materials containing silver nanoparticles as active packaging for food applications – A review, Int. J. Mol. Sci. 21(3) (2020) 698. 10.3390/ijms21030698.