Open Journal of Medical Microbiology, 2024, 14, 77-92
https://www.scirp.org/journal/ojmm
ISSN Online: 2165-3380
ISSN Print: 2165-3372

Synthesis of Silver Nanoparticles from Honeybees and Its Antibacterial Potential

Akamu J. Ewunkem[1*], Niore's Johnson[1], A'lyiha F. Beard[1], Ilunga Tshimanga[1], Brittany Justice[1], Jeffery Meixner[2]

[1]Department of Biological Sciences, Winston Salem State University, Winston Salem, NC, USA
[2]Department of Clinical Science and Laboratory Science, Winston Salem State University, Winston Salem, NC, USA
Email: *ewunkemaj@wssu.edu, *judeakamu@gmail.com

How to cite this paper: Ewunkem, A.J., Johnson, N., Beard, A.F., Tshimanga, I., Justice, B. and Meixner, J. (2024) Synthesis of Silver Nanoparticles from Honeybees and Its Antibacterial Potential. *Open Journal of Medical Microbiology*, **14**, 77-92.
https://doi.org/10.4236/ojmm.2024.141007

Received: December 17, 2023
Accepted: March 26, 2024
Published: March 29, 2024

Abstract

Honeybees (*Apis mellifera*) are important pollinators of flowering plants and agricultural crops contributing annually to billions of dollars in revenues to crop production. Honeybees have an average lifespan between 8 weeks to 5 years. Dead honeybees are abundantly available in beehives and can be utilized as an alternative source to synthesize nanoparticles. In recent years, biologically synthesized nanoparticles have been preferred over their chemical counterparts. However, honeybee-based-green synthesis of nanoparticles has not been explored yet. Herein, we report the biosynthesis of silver nanoparticles from honeybees and its antibacterial activity. The synthesis of silver nanoparticles was monitored visually through a gradual change in color. Furthermore, the biosynthesized nanoparticles were confirmed and characterized by UV-visible spectroscopy. Scanning Electron Microscope was utilized to analyze the average size and morphologies of the biosynthesized nanoparticles. Subsequently, the antibacterial potential of the biosynthesized silver nanoparticles was tested against selected Gram-positive and Gram-negative bacterial strains. It was found that a distinct color change from yellow to brown in the reaction solution suggested the formation of silver nanoparticles. The biosynthesized nanoparticles exhibited absorption maxima at 430 nm. The SEM analysis confirmed the spherical and cuboidal shape of the biosynthesized silver nanoparticles with a size range between 10 - 40 nm. Furthermore, the biosynthesized silver nanoparticles exhibited strong antimicrobial potential against tested Gram-positive and Gram-negative bacteria strains by aggregating on the cell surface. This study showcases the biomedical and agricultural applications of biosynthesized silver nanoparticles from honeybee wings.

Keywords

Honeybee, Bacteria, Green Synthesis, Nanoparticles, Antimicrobial

1. Introduction

Honeybees *Apis mellifera* are part of the diversity on which all living organisms on this planet depend for our survival. *A. mellifera* provides high quality valued pollination services for a wide variety of over 90% of global crops [1] [2]. Furthermore, *Apis mellifera* possesses an extraordinary ability to process and store nectar in the form of honey which has traditionally been used as food and medicine products since prehistoric times [3] [4]. Honey contains flavonoids and polyphenols with high antioxidant and anti-inflammatory properties [5] [6]. Additionally, honey is known to possess wound-healing activity as well as antimicrobial properties due to the presence of hydrogen peroxide polyphenolic compounds, methylglyoxal and bee-defensin [6] [7] [8] [9]. Honey has been used for centuries as a method to speed up the treatment of ulcers and bed sores skin infections resulting from burns and wounds. Strong inhibitory activity of honey has been reported against *Staphylococcus aureus*, *Helicobacter pylori.* The antimicrobial activity of honeybees is not limited to honey.

Published research denotes that honeybee wings exhibit antibacterial activity [10]. These wings are studded with a vast array of rough, sharp, and pointed pillars that disrupt bacterial cells and inhibit their growth [10]. The wings of other insects like cicada and dragon flies have also fascinated researchers with their antimicrobial properties attributable to the presence of an array of nanopillars that kill bacteria on contact [11] [12]. This nanostructure-induced lysis of bacterial cells may be the key to developing antimicrobial medical devices to combat multidrug resistant bacteria. This property has also inspired scientists who are in search of new technologies to triumph over drug resistant bacteria. Among many approaches and natural substances used to synthesize nanoparticles, honey seems to be promising because of its ability to act as a reducing and capping/stabilizing agent. Honey-based synthesis of nanoparticles has consistently demonstrated several advantages over the biological mediated methods. Honey mediated synthesis of nanoparticles is a relatively fast process, and does not require cell culture and separation of nanoparticles from microorganism (which is a challenging task). Honey-mediated nanoparticles are shown to exert antimicrobial activity primarily by inhibiting ATP synthase and by inducing the production of reactive oxygen species [4] [5].

Nanotechnology is a promising approach to reduce and combat bacterial resistance [13]. Nanotechnology entails the manipulation of reduction, and fabrication of materials on a nanoscale with sizes ranging from 1 to 100 nm (nm) [14] [15]. Currently there are an increasing number of studies devoted to the synthesis of nanoparticles employing chemical, physical, and green synthesis methods [16] [17] [18] [19]. Herein, we employed green synthesis of nanoparticles because it is cost-effective and improves environmental and human health safety [19] [20]. Green synthesis for the nanoscale metals involves two main steps. First and foremost, preparation of extracts from the organisms at 90˚C for about an hour and pH adjusted, the extract is then added to metal salts or solution and incubated at 28˚C for about 30 minutes. In this reaction the extract serves as a reducing agent, reducing the metal particles [19] [20].

Microorganisms, plants, fungi, spiders, and some insects are for the most part exploited for green synthesis [21] [22] [23] [24]. Insects such as American roaches and termites have been explored as ecofriendly, cost effective and novel biomaterial for the biosynthesis of silver nanoparticles [22] [23]. However, there are no reports on the biosynthesis of nanoparticles from honeybees. The present study for the first time seeks to expand the use of honeybees for green synthesis of silver nanoparticles and to evaluate its antibacterial potential. This novel synthesis approach for nanomaterials is an interesting area in nanoscience and nanotechnology where its finished products can be used as an alternative antimicrobial strategy to reduce the global burden of infectious diseases.

2. Materials and Methods

Collection and preparation of honeybee wings

The dead worker honeybees (*Apis mellifera*) used in the present study were collected in Fall 2023 from Dr. Jeffery Andrew Meixner's beehive, in Winston Salem, North Carolina USA. The wings were gently removed from the body using a sterile scalpel and stored at room temperature in sterile polystyrene Petri dishes (Fisher Scientific) until required.

Green synthesis and characterization of silver nanoparticles from honeybee wings

Green synthesis of silver nanoparticles was carried out as described by previous investigators [23] [25]. Honeybee wings (0.1 g) were hydrolyzed in 0.1 M of NaOH at 90˚C in a water incubator for 60 mins. After cooling the hydrolyzed wing solution was centrifuged at 8000 rpm for 10 minutes. Afterward, the supernatant was collected, and the pH was lowered to 7. Subsequently, 1 ml of the wing extract was transferred to 49 ml of silver nitrate solution for the reduction of silver ions. The reaction was then incubated at 28˚C ± 1˚C for 30 minutes under static conditions for the synthesis of silver nanoparticles via color change.

High Performance Liquid Chromatography (HPLC) System

HPLC was carried out at Chemistry Laboratory in Greensboro North Carolina using Agilent 1100 series with a C18 column (4.6 mm × 250 mm i.d., 5 μm) at 35˚C to identify and quantify compounds present in honeybee wing extracts. The mobile phase consisted of HPLC water (A) and acetonitrile (B) at a flow rate of 1 ml/min. The multi-wavelength detector was monitored at 280 nm.

Characterization of biosynthesized silver nanoparticles honeybee wing extract

The biosynthesized silver nanoparticles from honeybee wings were characterized using various techniques. The absorption spectrum was measured using UV–Visible spectrophotometer (Genesys 10 UV Thermoelectron Corporation, UK) within the ranges of 200 - 1000 nm operated at a resolution of 1 nm at room temperature. Morphological characterization was carried out at the Joint School of Nanoscience and Nanoengineering, Greensboro, North Carolina USA using JEOL's new Field Emission Scanning Electron Microscope, the JSM-IT800.

Antimicrobial activity of biosynthesized nanoparticles from honeybee wings extracts

The antimicrobial activity of the biosynthesized silver nanoparticles from honeybee wing extract was investigated against *Staphylococcus aureus* (ATCC 25923), *Micrococcus luteus* (ATCC 4698), *Escherichia coli* 1946 (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Bacillus megaterium* (ATCC 13639) and *Salmonella typhi* (ATCC 9992V). The antimicrobial activity of the biosynthesized nanoparticles against the tested bacteria was determined by micro dilution method in triplicate using 96-well plates. The tested bacterial strains were grown in Nutrient Broth (NB) medium supplemented with biosynthesized nanoparticles from worker honeybee (10 uM) and incubated at 37˚C for 24 h. The growth was monitored after 24 h by using a 98-well plate format Glomaxmulti plate reader (Promega, USA). Media inoculated with bacterial strain alone served as controls. The experiment was repeated three times.

Morphological characterization of Tested Bacterial Strains

The morphological characterization was carried out at the Joint School of Nanoscience and Nanoengineering, Greensboro, North Carolina USA using JEOL's new Field Emission Scanning Electron Microscope, the JSM-IT800. For morphological characterization of the treated samples, after 24 h of incubation period aliquot of bacterial cells was harvested by centrifugation at 8000 rpm, washed 3 times and resuspended in PBS. Subsequently, the samples were fixed in 2.5% glutaraldehyde solution (configured with PBS) overnight at 4˚C followed by gradient dehydration using different concentrations of ethanol (using 30%, 50%, 70%, 80%, 90%, and twice with 100%). The samples were immediately pre-frozen at −20˚C, freeze dried for 12 hours, and observed with a scanning electron microscope (SEM).

3. Results

Biosynthesis of silver nanoparticles from worker honeybee wings

To synthesize nanoparticles from workers honeybee wings, the wing extract was treated with silver nitrate ($AgNO_3$). During the biosynthesis of silver nanoparticle's reaction, the change of color from light yellow to dark brown (**Figure 1**) of the reaction suggests the formation of silver nanoparticles. As seen in the reaction below:

$AgNO_3 \rightarrow Ag^+ + NO_3$

Ag^+ + honeybee wing extract → Ag^0 (silver nanoparticles)

HPLC analysis

The content of the compound's honeybee wing extract and retention times determined by HPLC analysis are shown in **Figure 2**. The analysis revealed 4 major components.

Characterizations of Silver Nanoparticles from honeybee wings

UV-visible spectrometry is a widely used analytical technique for detecting metal nanoparticles [22] [26] [27] [28]. The absorbance taken by UV-vis spectrometer showed an absorption peak at 440 nm (**Figure 3**) confirming the presence of silver nanoparticles. Also, scanning electron microscopy (SEM) analysis confirmed the presence of dispersed cube-like and spherical shape silver nanoparticles with size range between 10 - 40 nm (**Figure 4**).

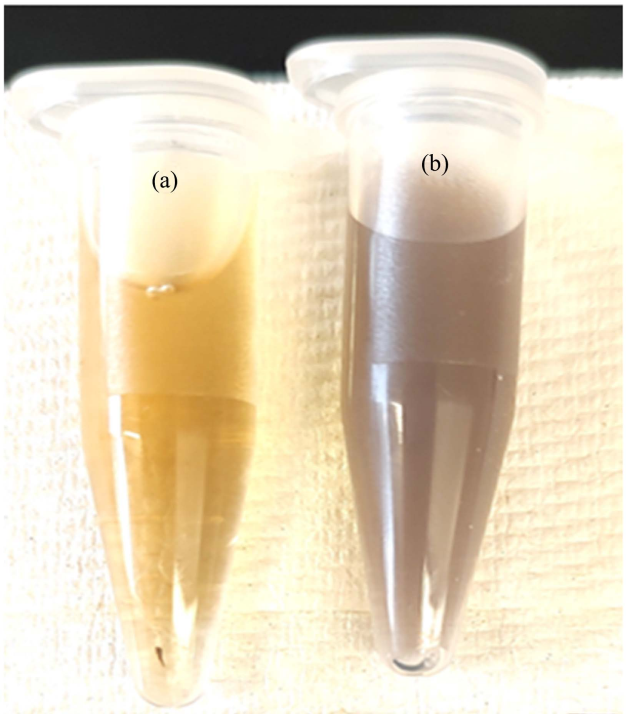


Figure 1. (a) Honeybee wings extract with no silver nitrate. (b) Honeybee wings extract with added silver nitrate.

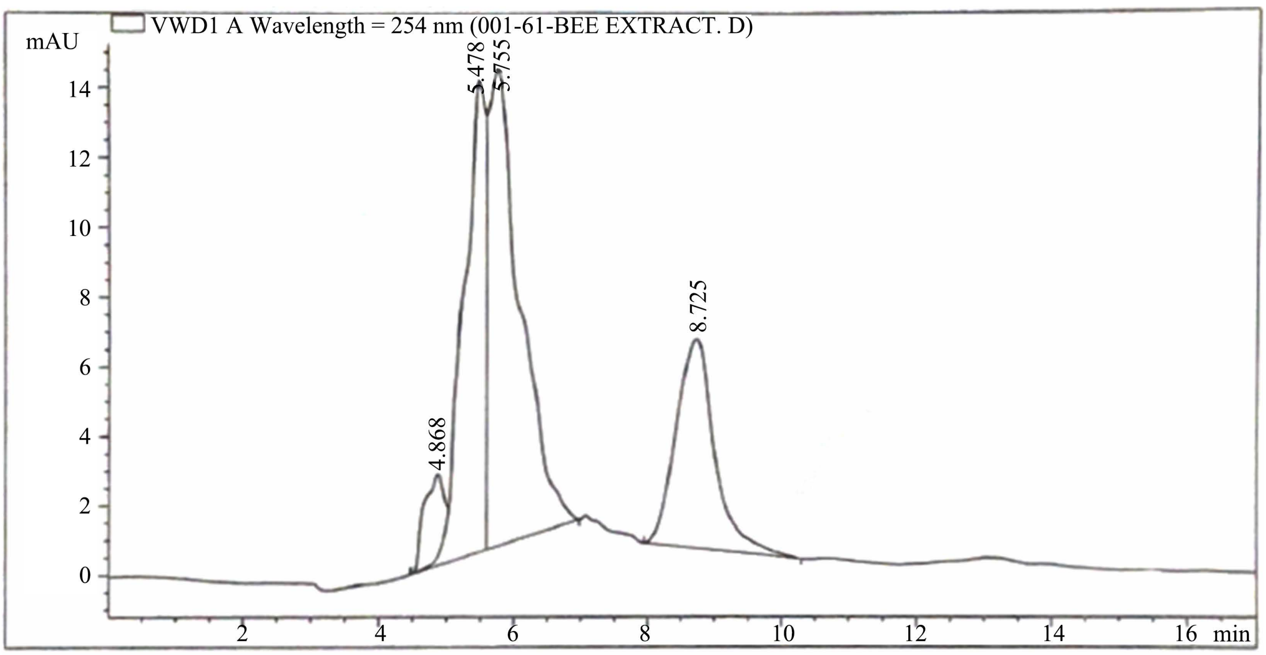


Figure 2. HPLC profile of water extract derived from honeybee wing extracts.

Scan_7_6_2023_6:55:44_AM 1 / 1

06-Jul-2023 06:59 AM Instrument Serial #: 9A3Z183024

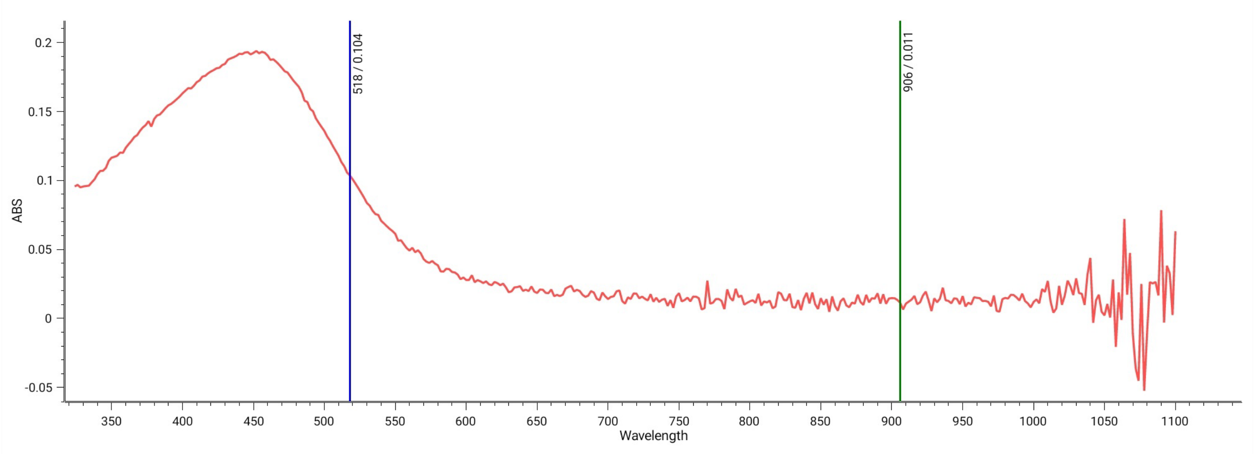
Method name: Scan 06-Jul-2023 Instrument model: GENESYS 50

Method created: 06-Jul-2023 06:52 AM Software Package Version: 2.6 Signature:

Method updated: 06-Jul-2023 06:52 AM

Scan: method parameters

Range	**325 - 1100 nm**
Interval	**2.0 nm**
Speed	**Fast**



Sample	ABS(519)	ABS(906)
Sample 1	0.104	0.011

Figure 3. UV-visible spectra of synthesized silver nanoparticles using worker honeybee wings.

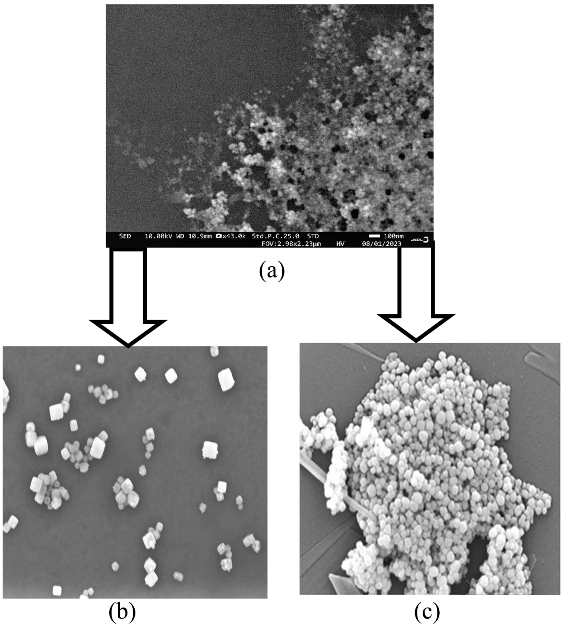


Figure 4. Scanning electron microscopy image of biosynthesized silver nanoparticles from worker honeybee wing extracts (a) biosynthesized silver nanoparticles; (b) cube-like nanoparticle; (c) spherical shape silver nanoparticles.

Antimicrobial Activity of biosynthesized nanoparticles from worker honeybee wings against bacteria

The biosynthesized silver nanoparticles from worker honeybees demonstrated potential antimicrobial activity. Broth microdilution assay revealed that the tested concentration (10 μM) of the biosynthesized silver nanoparticles showed a significant ($P < 0.05$) reduction in the growth of Gram-negative bacteria (Figure 5) and Gram-positive bacteria (Figure 6) compared to the control after 24 hr. The tested concentration almost completely inhibited the growth of all the Gram-negative bacterial strains in this order (*E. coli* < *K. pneumoniae* < *S. typhi* (Figure 6). The growth inhibitory effect of the biosynthesized silver nanoparticles against Gram-negative bacteria can be ranked from the least to the most sensitive as follows (*B. megaterium* < *S. aureus* < *M. luteus*).

To further understand bacterial responses to 10 μM of the biosynthesized nanoparticles after 24 hr, bacterial morphology and nanoparticle-cell associations were examined with scanning electron microscope (SEM). Seen from SEM micrographs, the biosynthesized nanoparticles aggregated and interacted with the treated Gram-negative bacterial cells (Figure 7). A significant morphological alteration was observed in silver nanoparticles treated with *S. typhi*. The cells were corrugated and had some depressions and alterations in length (Figure 7(f)).

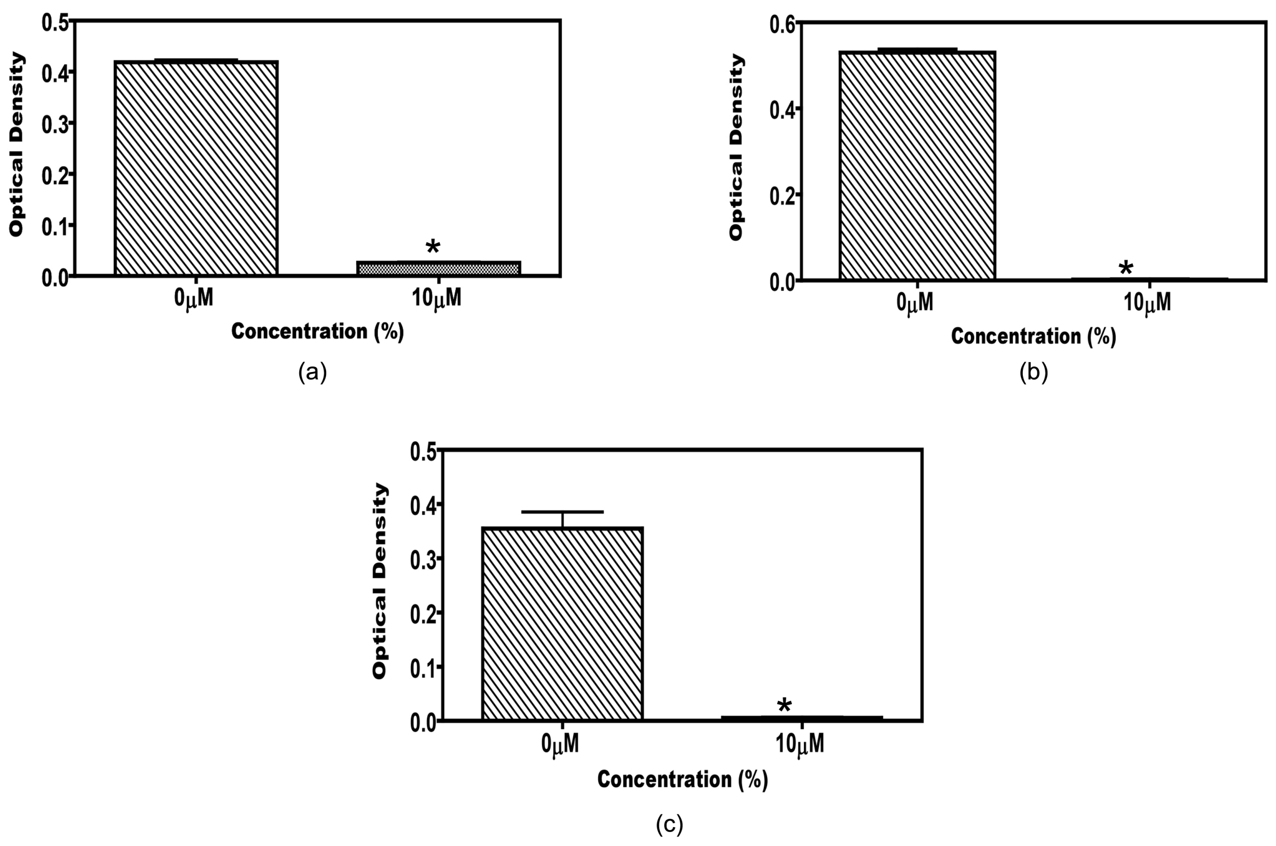


Figure 5. Antimicrobial activity of biosynthesized silver nanoparticles from worker honeybees against Gram-negative bacteria. (a) *Escherichia coli*; (b) *Salmonella typhi* and (c) *Klebsiella pneumoniae*.

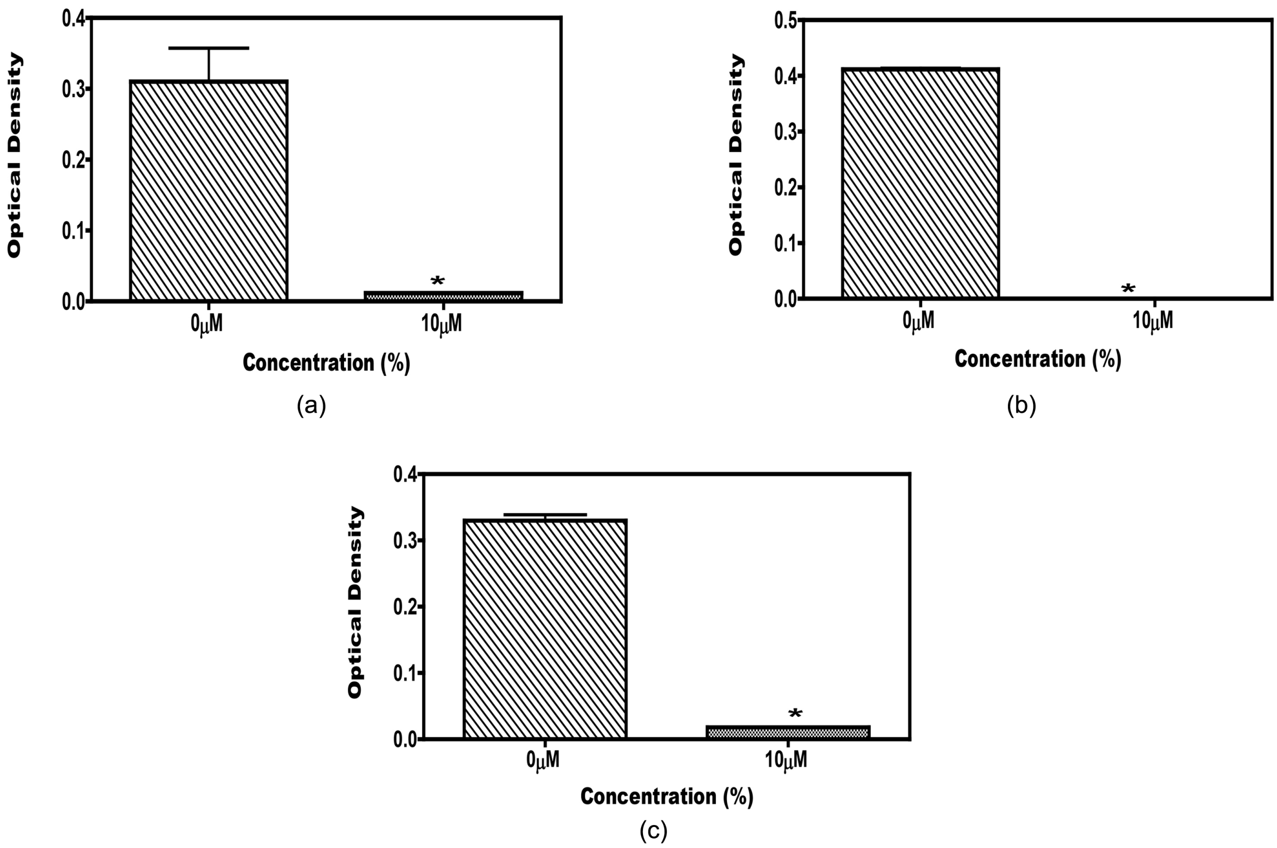


Figure 6. Antimicrobial activity of biosynthesized silver nanoparticles from worker honeybees against Gram-positive bacteria. (a) *Staphylococcus aureus* (b) *Micrococcus luteus* and (c) *Bacillus megaterium*.

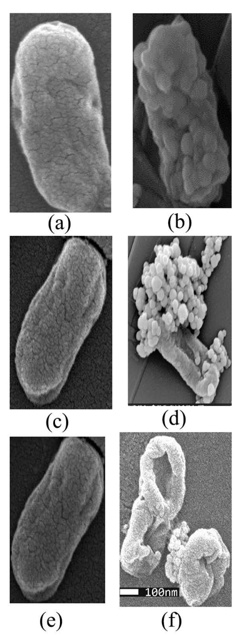


Figure 7. SEM images of Gram-negative bacteria and biosynthesized silver nanoparticles from worker honeybee wings: (a) *E. coli* cell, (b) *E. coli* cell and nanoparticles, (c) *K. pneumonia* cell, (d) *K. pneumonia* cell and nanoparticles, (e) *S. typhi* cell (f) *S. typhi* cell and nanoparticles.

Seen from the SEM, the biosynthesized silver nanoparticles also demonstrated a significant inhibition of the growth of Gram-positive bacteria strains by adhering and interacting on the walls (**Figure 8**). The extensive damage of the bacterial strains treated with the biosynthesized silver nanoparticles was in this order (*M. luteus* > B. *megaterium* > *S. aureus*).

4. Discussion

Honeybees (*Apis mellifera*) are indispensable for life on earth, as they pollinate more than 80% of the flowering plants and agricultural crops and provide high quality products [29]. Honeybees usually live for about 6 weeks. Thereafter most beekeepers usually clear out the dead bees to create room for a new colony. In this study we utilized the wings of dead worker honeybees as an alternative source to synthesize nanoparticles. This technology is known as green synthesis and has gained attraction because it is a benign and cost-effective approach for the synthesis of metallic nanoparticles [30] [31].

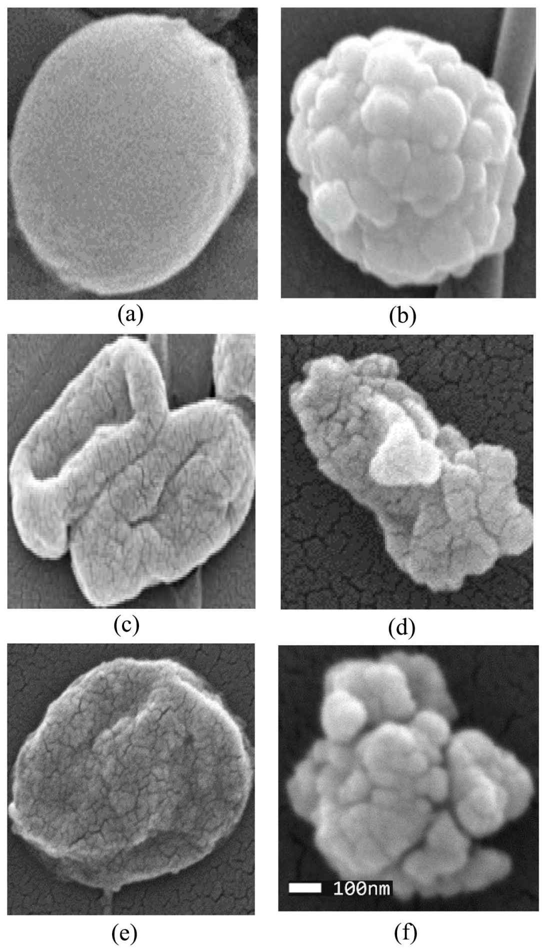


Figure 8. SEM images of Gram-positive bacteria and biosynthesized silver nanoparticles from worker honeybee wings: (a) *S. aureus* cell, (b) *S. aureus* cell and nanoparticles, (c) *B. megaterium cell*, (d) *B. megaterium* and nanoparticles, (e) *M. luteus* cell (f) *M. luteus* cell and nanoparticles.

During the biosynthesis of silver nanoparticles from worker honeybee wing extracts the appearance of the brown color of the reaction solution inarguable indicated the formation of silver nanoparticles due to the excitation of surface plasmon vibrations in metal nanoparticles as reported by other investigators [32] [33]. This change in color is the fundamental barometer for the recovery of silver ions and the formation of silver nanoparticles [22] [34]. The major components found in the wings (**Figure 2**) may be responsible for the recovery of silver ions and the formation of silver nanoparticles [23]. Several studies revealed that insects' wings and mushrooms contain aliphatic hydrocarbons or volatile compounds that may oversee the formation of silver nanoparticles [35] [36] [37] [38]. Further, the biosynthesized silver nanoparticles were confirmed by UV-vis spectroscopy. UV-vis spectroscopy is a tool for detecting metal nanoparticles since localized surface plasmon resonance permits the absorption of photons [39]. The biosynthesized silver nanoparticles by worker honeybee extracts showed a maximum absorbance peak at 440 nm which is specific to silver nanoparticles and most metal nanoparticles as reported in previous studies [22] [40] [41]. The absorbance peak is usually ascribed to the Surface Plasmon Resonance (SPR) due to collective oscillations of elections of metal nanoparticles that depend on the size and shape of the nature and composition of the dispersion medium as well as the size and shape of nanoparticles [42] [43] [44].

The scanning electron microscope (SEM) confirmed the formation of spherical and cubic-shape nanoparticles with an almost uniform size of 20 - 40 nm which agrees with results of [43] [45] who found silver nanoparticles spherical in shape in the range of 10 - 50 nm. Agglomeration of the biosynthesized silver nanoparticles may be due to dehydration during the preparation of the sample for SEM analysis [46]. The morphology of the nanoparticles is known to have profound antimicrobial activity, an important role in exploiting their properties for their use in a variety of emerging technologies such as cellular uptake, drug delivery, intracellular trafficking, optical filters, biosensors, and antimicrobial activity [47] [48].

Silver has been used for centuries as an antimicrobial agent due to its ability to inhibit the enzymatic functions of microbes by interacting with thiol (sulfhydryl) groups and by forming reactive oxygen species [49] [50]. With the emergence of nanotechnology, silver nanoparticles have fascinated researchers owing to their inherent broad-spectrum antimicrobial activity at low concentrations [51]. The antimicrobial activity of the biosynthesized silver nanoparticles from worker honeybees was tested against *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus megaterium* and *Salmonella typhi*. According to the results, the biosynthesized silver nanoparticles exhibited strong antimicrobial activity against the tested bacteria. Therefore, further evaluation in determining the antimicrobial activity of the biosynthesized silver nanoparticles using SEM was needed. Seen from SEM micrographs, the biosynthesized nanoparticles aggregated and interacted on the bacteria surfaces due to electrostatic attraction and affinity of silver ions to SH groups, such as in cysteine [52]. The small size of the nanoparticles can greatly increase the production of ROS causing damage and inactivation of biomolecules including DNA, proteins, and lipids. The potential antimicrobial activity of the biosynthesized silver nanoparticles from worker honeybee wing extract indicates an alternative to conventional antibiotics.

Gram-negative bacteria were generally more sensitive to the biosynthesized silver nanoparticles than Gram negative bacteria. Our data showed that nanoparticles exhibited a relatively strong antimicrobial activity against Gram-negative bacteria (for example *S. Typhi* and *K. pneumoniae*) by destroying the cell wall because of the absence of thick multilayer peptidoglycan. Gram-positive bacteria typically have thick multilayer peptidoglycan that could possibly maintain their cell shape and protect them from extreme environmental conditions [53]. The thicker cell wall renders Gram-positive bacteria comparatively more resistant to silver nanoparticles [54]. Surprisingly, it was clear from the data that the biosynthesized nanoparticles from worker honeybees completely inhibited the growth of tested Gram-positive bacteria *M. luteus*. SEM images revealed that the biosynthesized silver nanoparticles destroyed the cell wall of *M. luteus* resulting in structural changes or loss in membrane integrity which might be attributed to the presence of two polymers, *i.e.*, peptidoglycans and teichuronic [55].

Honeybees are effective pollinators of flowering plants and agriculture crops ensuring the production of food, biofuels, fibers, medicines and building materials [56]. The importance of honeybees is not limited to pollination. Honeybees produce honey that contains antioxidants, which protect the body from inflammation [56]. Honey also has substantial antimicrobial properties due to the production of hydrogen peroxide [7]. The wings of worker bees are known to play an important role in anti-biofouling surfaces and exhibit antimicrobial activity against both Gram negative and Gram-positive bacteria [10]. In this study, we demonstrated the biosynthesis of silver nanoparticles using the wings of worker honeybees, is a cost-effect, and control technology for synthesizing nanoparticles with diverse pharmaceutical and biomedical applications [57].

5. Conclusion

In conclusion, green synthesis of metallic nanoparticles has given close and thoughtful attention among scientists worldwide because it is clean, safe, cost-effective and causes less environmental damage [58]. Herein we focused on the biosynthesis of silver nanoparticles by using worker honeybee wings and evaluated the antimicrobial activity of the biosynthesized silver nanoparticles against Gram-negative and Gram-positive bacteria. The biosynthesized nanoparticles were for the most part cuboidal and specifical with a size range from 10 - 45 nm. Additionally, the silver nanoparticles possessed antimicrobial against a wide variety of Gram-negative and Gram-positive bacteria by aggregating around the bacteria and disrupting important cellular mechanisms.

Acknowledgements

The research leading to these results was Winston Salem State University PDC

Grant (fund number is 211444) Authors are grateful to Department of Biological Sciences at Winston Salem State University and the Joint School of Nanoscience and Nanoengineering for their support in carrying out this research.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Hung, K.L.J., Kingston, J.M., Albrecht, M., Holway, D.A. and Kohn, J.R. (2018) The Worldwide Importance of Honeybees as Pollinators in Natural Habitats. *Proceedings of the Royal Society B*: *Biological Sciences*, **285**, Article ID: 20172140. https://doi.org/10.1098/rspb.2017.2140

[2] Mishra, S., Zhu, M., Bernknopf, R. and Walston, L. (2023) Valuation of Pollination Services from Habitat Management: A Case Study of Utility Scale Solar Energy Facilities in the United States. *Environmental Research Communications*, **5**, Article ID: 065006. https://doi.org/10.1088/2515-7620/acda7f

[3] Saba, Z.H., Suzana, M. and Anum, M.Y. (2013) Honey: Food or Medicine. *Mehealth*, **8**, 3-18.

[4] Faraz, A., Fernando, W.B., Williams, M. and Jayasena, V. (2023) Effects of Different Processing Methods on the Antioxidant and Antimicrobial Properties of Honey: A Review. *International Journal of Food Science & Technology*, **58**, 3489-3501. https://doi.org/10.1111/ijfs.16460

[5] Cianciosi, D., Forbes-Hernández, T.Y., Afrin, S., Gasparrini, M., Reboredo-Rodriguez, P., Manna, P.P., Battino, M., *et al*. (2018) Phenolic Compounds in Honey and Their Associated Health Benefits: A Review. *Molecules*, **23**, Article 2322. https://doi.org/10.3390/molecules23092322

[6] Ranneh, Y., Akim, A.M., Hamid, H.A., Khazaai, H., Fadel, A., Zakaria, Z.A., Bakar, M.F.A., *et al*. (2021) Honey and Its Nutritional and Anti-Inflammatory Value. *BMC Complementary Medicine and Therapies*, **21**, Article No. 30. https://doi.org/10.1186/s12906-020-03170-5

[7] Mandal, M.D. and Mandal, S. (2011) Honey: Its Medicinal Property and Antibacterial Activity. *Asian Pacific Journal of Tropical Biomedicine*, **1**, 154-160. https://doi.org/10.1016/S2221-1691(11)60016-6

[8] Nolan, V.C., Harrison, J. and Cox, J.A. (2019) Dissecting the Antimicrobial Composition of Honey. *Antibiotics*, **8**, Article 251. https://doi.org/10.3390/antibiotics8040251

[9] Tashkandi, H. (2021) Honey in Wound Healing: An Updated Review. *Open Life Sciences*, **16**, 1091-1100. https://doi.org/10.1515/biol-2021-0084

[10] Ewunkem, A.J., A'Lyiha, F.B., Justice, B.L., Peoples, S.L., Meixner, J.A., Kemper, W. and Iloghalu, U.B. (2023) Honeybee Wings Hold Antibiofouling and Antimicrobial Clues for Improved Applications in Health Care and Industries. *AIMS Microbiology*, **9**, 332-345. https://doi.org/10.3934/microbiol.2023018

[11] Van Nieuwenhoven, R.W., Bürger, A. and Gebeshuber, I.C. (2022) Mechanical Bactericide by Biomimetics of the Nanopillars on Insect Wings. Master's Thesis, TU Wien, Vienna.

[12] Linklater, D.P., Le, P.H., Aburto-Medina, A., Crawford, R.J., Maclaughlin, S., Juodkazis, S. and Ivanova, E.P. (2023) Biomimetic Nanopillar Silicon Surfaces Rupture Fungal Spores. *International Journal of Molecular Sciences*, **24**, Article 1298.

https://doi.org/10.3390/ijms24021298

[13] Hetta, H.F., Ramadan, Y.N., Al-Harbi, A.I., Ahmed, E.A., Battah, B., Abd Ellah, N.H., Donadu, M.G., *et al.* (2023) Nanotechnology as a Promising Approach to Combat Multidrug Resistant Bacteria: A Comprehensive Review and Future Perspectives. *Biomedicines*, **11**, Article 413. https://doi.org/10.3390/biomedicines11020413

[14] Horikoshi, S. and Serpone, N. (2013) Microwaves in Nanoparticle Synthesis: Fundamentals and Applications. John Wiley & Sons, New York. https://doi.org/10.1002/9783527648122

[15] Kanwar, R., Rathee, J., Salunke, D.B. and Mehta, S.K. (2019) Green Nanotechnology-Driven Drug Delivery Assemblies. *ACS Omega*, **4**, 8804-8815. https://doi.org/10.1021/acsomega.9b00304

[16] Nam, N.H., Van, N.T.T., Phu, N.D., Hong, T.T., Hai, N.H. and Luong, N.H. (2012) Research Article Magnetic Properties of FePt Nanoparticles Prepared by Sonoelectrodeposition. *Journal of Nanomaterials*, **2021**, Article ID: 801240. https://doi.org/10.1155/2012/801240

[17] Shenava, A. (2013) Synthesis of Silver Nanoparticles by Chemical Reduction Method and Their Antifungal Activity. *International Research Journal of Pharmacy*, **4**, 111-113. https://doi.org/10.7897/2230-8407.041024

[18] Iravani, S., Korbekandi, H., Mirmohammadi, S.V. and Zolfaghari, B. (2014) Synthesis of Silver Nanoparticles: Chemical, Physical and Biological Methods. *Research in Pharmaceutical Sciences*, **9**, 385-406.

[19] Ying, S., Guan, Z., Ofoegbu, P.C., Clubb, P., Rico, C., He, F. and Hong, J. (2022) Green Synthesis of Nanoparticles: Current Developments and Limitations. *Environmental Technology & Innovation*, **26**, Article ID: 102336. https://doi.org/10.1016/j.eti.2022.102336

[20] Tarannum, N. and Gautam, Y.K. (2019) Facile Green Synthesis and Applications of Silver Nanoparticles: A State-of-the-Art Review. *RSC Advances*, **9**, 34926-34948. https://doi.org/10.1039/C9RA04164H

[21] Mohanta, Y.K., Nayak, D., Biswas, K., Singdevsachan, S.K., Abd Allah, E.F., Hashem, A., Mohanta, T.K., *et al.* (2018) Silver Nanoparticles Synthesized Using Wild Mushroom Show Potential Antimicrobial Activities against Food Borne Pathogens. *Molecules*, **23**, Article 655. https://doi.org/10.3390/molecules23030655

[22] Khatami, M., Iravani, S., Varma, R.S., Mosazade, F., Darroudi, M. and Borhani, F. (2019) Cockroach Wings-Promoted Safe and Greener Synthesis of Silver Nanoparticles and Their Insecticidal Activity. *Bioprocess and Biosystems Engineering*, **42**, 2007-2014. https://doi.org/10.1007/s00449-019-02193-8

[23] Jakinala, P., Lingampally, N., Hameeda, B., Sayyed, R.Z., Khan M.Y., Elsayed, E.A. and El Enshasy, H. (2021) Silver Nanoparticles from Insect Wing Extract: Biosynthesis and Evaluation for Antioxidant and Antimicrobial Potential. *PLOS ONE*, **16**, e0241729. https://doi.org/10.1371/journal.pone.0241729

[24] Rajesh, A. and Madhumitha, G. (2023) An Insight into the Insecticidal Activity of Green Synthesized Silver Nanoparticles. *Colloid Journal*, **85**, 854-870.

[25] Lateef, A., Ojo, S.A., Azeez, M.A., Asafa, T.B., Yekeen, T.A., Akinboro, A., Beukes, L.S., *et al.* (2016) Cobweb as Novel Biomaterial for the Green and Eco-Friendly Synthesis of Silver Nanoparticles. *Applied Nanoscience*, **6**, 863-874. https://doi.org/10.1007/s13204-015-0492-9

[26] Vanlalveni, C., Lallianrawna, S., Biswas, A., Selvaraj, M., Changmai, B. and Rokhum, S.L. (2021) Green Synthesis of Silver Nanoparticles Using Plant Extracts and

Their Antimicrobial Activities: A Review of Recent Literature. *RSC Advances*, **11**, 2804-2837. https://doi.org/10.1039/D0RA09941D

[27] Ghasemi, S., Harighi, B. and Ashengroph, M. (2023) Biosynthesis of Silver Nanoparticles Using Pseudomonas Canadensis, and Its Antivirulence Effects against Pseudomonas Tolaasii, Mushroom Brown Blotch Agent. *Scientific Reports*, **13**, Article No. 3668. https://doi.org/10.1038/s41598-023-30863-x

[28] Razi, S.S., Nautiyal, V.K. and Hitkari, G. (2023) UV-Visible Spectroscopy in Biomedical Nanotechnology. In: Kaushik, A., Srinivasan, S.S. and Mishra, Y.K., Eds., *Analytical Techniques for Biomedical Nanotechnology*, IOP Publishing, Bristol, 3-1 to 3-12. https://doi.org/10.1088/978-0-7503-3379-5ch3

[29] Gorlova, L. and Pirogova, E. (2023) Influence of the Insect Pollinators on Formation of Winter Rapeseed Yield in the Central Zone of the Krasnodar Region. *AIP Conference Proceedings*, **2777**, Article ID: 020048. https://doi.org/10.1063/5.0140339

[30] Shekhar, S., Singh, S., Gandhi, N., Gautam, S. and Sharma, B. (2023) Green Chemistry Based Benign Approach for the Synthesis of Titanium Oxide Nanoparticles Using Extracts of *Azadirachta Indica*. *Cleaner Engineering and Technology*, **13**, Article ID: 100607. https://doi.org/10.1016/j.clet.2023.100607

[31] Devi, D., Julkapli, N.M., Sagadevan, S. and Johan, M.R. (2023) Eco-Friendly Green Synthesis Approach and Evaluation of Environmental and Biological Applications of Iron Oxide Nanoparticles. *Inorganic Chemistry Communications*, **152**, Article ID: 110700. https://doi.org/10.1016/j.inoche.2023.110700

[32] Veerasamy, R., Xin, T.Z., Gunasagaran, S., Xiang, T.F.W., Yang, E.F.C., Jeyakumar, N. and Dhanaraj, S.A. (2011) Biosynthesis of Silver Nanoparticles Using Mangosteen Leaf Extract and Evaluation of Their Antimicrobial Activities. *Journal of Saudi Chemical Society*, **15**, 113-120. https://doi.org/10.1016/j.jscs.2010.06.004

[33] Liaqat, N., Jahan, N., Anwar, T. and Qureshi, H. (2022) Green Synthesized Silver Nanoparticles: Optimization, Characterization, Antimicrobial Activity, and Cytotoxicity Study by Hemolysis Assay. *Frontiers in Chemistry*, **10**, Article 952006. https://doi.org/10.3389/fchem.2022.952006

[34] Saif, S., Tahir, A. and Chen, Y. (2016) Green Synthesis of Iron Nanoparticles and Their Environmental Applications and Implications. *Nanomaterials*, **6**, Article 209. https://doi.org/10.3390/nano6110209

[35] Bhat, R., Deshpande, R., Ganachari, S.V., Huh, D.S. and Venkataraman, A. (2011) Photo-Irradiated Biosynthesis of Silver Nanoparticles Using Edible Mushroom Pleurotus Florida and Their Antibacterial Activity Studies. *Bioinorganic Chemistry and Applications*, **2011**, Article ID: 650979. https://doi.org/10.1155/2011/650979

[36] Ivanova, E.P., Nguyen, S.H., Webb, H.K., Hasan, J., Truong, V.K., Lamb, R.N., Crawford, R.J., *et al.* (2013) Molecular Organization of the Nanoscale Surface Structures of the Dragonfly *Hemianax papuensis* Wing Epicuticle. *PLOS ONE*, **8**, e67893. https://doi.org/10.1371/journal.pone.0067893

[37] Nguyen, S.H.T., Webb, H.K., Hasan, J., Tobin, M.J., Crawford, R.J. and Ivanova, E.P. (2013) Dual Role of Outer Epicuticular Lipids in Determining the Wettability of Dragonfly Wings. *Colloids and Surfaces B*: *Biointerfaces*, **106**, 126-134. https://doi.org/10.1016/j.colsurfb.2013.01.042

[38] Mohanta, Y.K., Singdevsachan, S.K., Parida, U.K., Panda, S.K., Mohanta, T.K. and Bae, H. (2016) Green Synthesis and Antimicrobial Activity of Silver Nanoparticles Using Wild Medicinal Mushroom *Ganoderma applanatum* (Pers.) Pat. from Similipal Biosphere Reserve, Odisha, India. *IET Nanobiotechnology*, **10**, 184-189. https://doi.org/10.1049/iet-nbt.2015.0059

[39] Tomaszewska, E., Soliwoda, K., Kadziola, K., Tkacz-Szczesna, B., Celichowski, G., Cichomski, M., Grobelny, J., *et al.* (2013) Detection Limits of DLS and UV-Vis Spectroscopy in Characterization of Polydisperse Nanoparticles Colloids. *Journal of Nanomaterials*, **2013**, Article ID: 313081. https://doi.org/10.1155/2013/313081

[40] Singh, S., Bharti, A. and Meena, V.K. (2015) Green Synthesis of Multi-Shaped Silver Nanoparticles: Optical, Morphological and Antibacterial Properties. *Journal of Materials Science: Materials in Electronics*, **26**, 3638-3648. https://doi.org/10.1007/s10854-015-2881-y

[41] Agustina, T.E., Handayani, W. and Imawan, C. (2021) The UV-VIS Spectrum Analysis from Silver Nanoparticles Synthesized Using *Diospyros maritima* Blume. Leaves Extract. In: Ma, W. and Prastowo, S., Eds., *Proceedings of the* 3*rd KOBI Congress, International and National Conferences* (*KOBICINC* 2020), Atlantis Press, Dordrecht, 411-419. https://doi.org/10.2991/absr.k.210621.070

[42] Jana, J., Ganguly, M. and Pal, T. (2016) Enlightening Surface Plasmon Resonance Effect of Metal Nanoparticles for Practical Spectroscopic Application. *RSC Advances*, **6**, 86174-86211. https://doi.org/10.1039/C6RA14173K

[43] Asif, M., Yasmin, R., Asif, R., Ambreen, A., Mustafa, M. and Umbreen, S. (2022) Green Synthesis of Silver Nanoparticles (AgNPs), Structural Characterization, and Their Antibacterial Potential. *Dose-Response*, **20**, Article 15593258221088709. https://doi.org/10.1177/15593258221088709

[44] Moosavy, M.H., De La Guardia, M., Mokhtarzadeh, A., Khatibi, S.A., Hosseinzadeh, N. and Hajipour, N. (2023) Green Synthesis, Characterization, and Biological Evaluation of Gold and Silver Nanoparticles Using *Mentha spicata* Essential Oil. *Scientific Reports*, **13**, Article No. 7230. https://doi.org/10.1038/s41598-023-33632-y

[45] Hemlata Meena, P.R., Singh, A.P. and Tejavath, K.K. (2020) Biosynthesis of Silver Nanoparticles Using *Cucumis prophetarum* Aqueous Leaf Extract and Their Antibacterial and Antiproliferative Activity against Cancer Cell Lines. *ACS Omega*, **5**, 5520-5528. https://doi.org/10.1021/acsomega.0c00155

[46] Sigamoney, M., Shaik, S., Govender, P. and Krishna, S.B.N. (2016) African Leafy Vegetables as Bio-Factories for Silver Nanoparticles: A Case Study on *Amaranthus dubius* C Mart. Ex Thell. *South African Journal of Botany*, **103**, 230-240. https://doi.org/10.1016/j.sajb.2015.08.022

[47] Ankamwar, B. (2012) Size and Shape Effect on Biomedical Applications of Nanomaterials. In: Hudak, R., Penhaker, M. and Majernik, J., Eds., *Biomedical Engineering-Technical Applications in Medicine*, IntechOpen, Pune, 93-114. https://doi.org/10.5772/46121

[48] Choo, P., Liu, T. and Odom, T.W. (2021) Nanoparticle Shape Determines Dynamics of Targeting Nanoconstructs on Cell Membranes. *Journal of the American Chemical Society*, **143**, 4550-4555. https://doi.org/10.1021/jacs.1c00850

[49] Fu, S., Zhang, Y., Yang, Y., Liu, X., Zhang, X., Yang, L., Zhang, E., *et al.* (2022) An Antibacterial Mechanism of Titanium Alloy Based on Micro-Area Potential Difference Induced Reactive Oxygen Species. *Journal of Materials Science & Technology*, **119**, 75-86. https://doi.org/10.1016/j.jmst.2021.12.031

[50] Shahed, C.A., Ahmad, F., Günister, E., Foudzi, F.M., Ali, S., Malik, K. and Harun, W.S.W. (2023) Antibacterial Mechanism with Consequent Cytotoxicity of Different Reinforcements in Biodegradable Magnesium and Zinc Alloys: A Review. *Journal of Magnesium and Alloys*, **11**, 3038-3058. https://doi.org/10.1016/j.jma.2023.08.018

[51] Xu, L., Wang, Y.Y., Huang, J., Chen, C.Y., Wang, Z.X. and Xie, H. (2020) Silver Nanoparticles: Synthesis, Medical Applications and Biosafety. *Theranostics*, **10**,

8996-9031. https://doi.org/10.7150/thno.45413

[52] Slavin, Y.N., Asnis, J., Hńfeli, U.O. and Bach, H. (2017) Metal Nanoparticles: Understanding the Mechanisms behind Antibacterial Activity. *Journal of Nanobiotechnology*, **15**, Article No. 65. https://doi.org/10.1186/s12951-017-0308-z

[53] Rajagopal, M. and Walker, S. (2017) Envelope Structures of Gram-Positive Bacteria. In: Bagnoli, F. and Rappuoli, R., Eds., *Protein and Sugar Export and Assembly in Gram-Positive Bacteria*, Springer, Cham, 1-44. https://doi.org/10.1007/82_2015_5021

[54] Munir, M.U. and Ahmad, M.M. (2022) Nanomaterials Aiming to Tackle Antibiotic-Resistant Bacteria. *Pharmaceutics*, **14**, Article 582. https://doi.org/10.3390/pharmaceutics14030582

[55] Yang, S., Sugawara, S., Monodane, T., Nishijima, M., Adachi, Y., Akashi, S., Takada, H., *et al.* (2001) *Micrococcus luteus* Teichuronic Acids Activate Human and Murine Monocytic Cells in a CD14- and Toll-Like Receptor 4-Dependent Manner. *Infection and Immunity*, **69**, 2025-2030. https://doi.org/10.1128/IAI.69.4.2025-2030.2001

[56] Khalifa, S.A., Elshafiey, E.H., Shetaia, A.A., El-Wahed, A.A.A., Algethami, A.F., Musharraf, S.G., El-Seedi, H.R., *et al.* (2021) Overview of Bee Pollination and Its Economic Value for Crop Production. *Insects*, **12**, Article 688. https://doi.org/10.3390/insects12080688

[57] Singh, H., Desimone, M.F., Pandya, S., Jasani, S., George, N., Adnan, M., Alderhami, S.A., *et al.* (2023) Revisiting the Green Synthesis of Nanoparticles: Uncovering Influences of Plant Extracts as Reducing Agents for Enhanced Synthesis Efficiency and Its Biomedical Applications. *International Journal of Nanomedicine*, **18**, 4727-4750. https://doi.org/10.2147/IJN.S419369

[58] Ewunkem, A.J., Williams, Z.J., Johnson, N.S., Brittany, J.L., Maselugbo, A. and Nowlin, K. (2023) Exploring the "Carpenter" as a Substrate for Green Synthesis: Biosynthesis and Antimicrobial Potential. *Gene & Protein in Disease*, **2**, Article 2155. https://doi.org/10.36922/gpd.2155