ARTIKEL ILMIAH

ANTIBACTERIAL ACTIVITY OF SYNTHETIC ZnO/PECTIN ON *STAPHYLOCOCCUS AUREUS*

Disusun oleh:
Putri Nur Angelina
NPI. 202602200103122001

Departemen Kimia
Fakultas Matematika dan Ilmu Pengetahuan Alam

Artikel Ilmiah Belum Dipublikasikan
Disusun sebagai dokumen karya ilmiah yang disimpan pada Perpustakaan IPB University dan digunakan untuk keperluan dokumentasi akademik serta pemenuhan administrasi Beban Kerja Dosen (BKD).

INSTITUT PERTANIAN BOGOR
BOGOR
2026

LEMBAR PERSETUJUAN

Nomor: 1816/IT3.F6.3/TU.00/M/B/2026

Yang bertanda tangan di bawah ini:

Nama : Prof. Dr. Dra. Dyah Iswanitini Pradono, M.Sc.Agr.
NIP : 196707301991032001
Jabatan : Ketua Departemen Kimia
Fakultas : Matematika dan Ilmu Pengetahuan Alam

Dengan ini menyatakan bahwa naskah artikel ilmiah yang berjudul:

"*Antibacterial Activity of Synthetic ZnO/Pectin on* Staphylococcus aureus"

yang disusun oleh:

Nama : Putri Nur Angelina
NPI : 202602200103122001
Departemen : Kimia
Fakultas : Matematika dan Ilmu Pengetahuan Alam

telah diperiksa dan diketahui oleh Departemen Kimia serta disetujui untuk disimpan pada Perpustakaan IPB sebagai karya ilmiah yang belum dipublikasikan. Naskah tersebut merupakan hasil karya ilmiah penulis dan dapat digunakan untuk keperluan dokumentasi akademik, pelaporan kinerja, serta pengelolaan koleksi ilmiah di lingkungan IPB University sesuai dengan ketentuan yang berlaku.

Demikian lembar persetujuan ini dibuat dengan sebenar-benarnya untuk digunakan sebagaimana mestinya.

Bogor, 17 Juni 2026
Menyetujui,
Ketua Departemen Kimia

Prof. Dr. Dra. Dyah Iswantini Pradono, M.Sc.Agr.
NIP. 196707301991032001

Antibacterial Activity of Synthetic ZnO/Pectin on *Staphylococcus aureus*

Putri Nur Angelina[a], Zaenal Abidin[a]

[a] Department of Chemistry, Faculty of Sciences and Mathematics, IPB University, Bogor, Indonesia

Abstract. The hydrothermal method is commonly used to synthesize ZnO nanoparticles because it produces products with high crystallinity and purity. However, this method is relatively time-consuming. This research combines hydrothermal and precipitation methods to produce ZnO in a shorter time. ZnO was also synthesized using a capping agent to control the smaller particle size as confirmed by the aging time needed to precipitate the particles. The synthesized ZnO nanoparticles can be added to food and beverage products as zinc additives and antibacterial agents which are beneficial to the body. Furthermore, the ZnO nanoparticles were tested for their antibacterial activity using *Staphylococcus aureus* as a model for gram-positive bacteria.

Keywords: antibacterial; *Staphylococcus aureus*; pectin; ZnO

1. Introduction

Nanotechnology is a branch of technology related to the synthesis, characterization, and exploration of various nanomaterials, specifically nanometer-scale materials (1–100 nm). These nanomaterials have better characteristics than larger materials [1]. One of the commonly developed nanomaterials is metal oxide, such as zinc(II) oxide (ZnO) because of its wide applications, environmental friendliness, and diverse physicochemical characteristics [2].

ZnO nanoparticles can be formed naturally in the form of the mineral zincite with relatively low abundance in nature, so ZnO nanoparticles are widely produced synthetically [3]. ZnO nanoparticles can be synthesized by solution-based and vapor-phase methods. The solution-based method is simpler as compared to the vapor phase-based method. It is also easier to control the morphology and size of ZnO nanoparticles with this approach by adjusting experimental factors such as the type of solvent, type of precursor, and reaction conditions. Solution-based ZnO nanoparticle synthesis methods consist of hydrothermal, solvothermal, precipitation, sol-gel, microwave, microemulsion, electrospinning, flux, wet chemical, electrochemical deposition, and polyols [4].

A combination of precipitation and hydrothermal methods was used in this research. The growth of nanoparticle crystals using the hydrothermal method utilizes high temperature and pressure [5]. The hydrothermal method produces ZnO nanoparticles with high purity and crystallinity, as well as uniform size, morphology, and crystal phase [6]. However, the hydrothermal method requires relatively long reaction times, to be specific 4 hours [7], 10

hours [8], and 17–19 hours [9]. Therefore, in this research, the time required for the hydrothermal process is shortened by using precursors in the form of crystals previously formed through a precipitation process, so that the ZnO nanoparticles still have high crystallinity and uniform size with a shorter synthesis time.

The ZnO nanoparticles have high surface energy, so they tend to aggregate and agglomerate with other particles. Aggregation and agglomeration of particles occurred during the synthesis process leading to bigger size particles so that there is probability that the particles are out of nanometer range. The aggregation and agglomeration of nanoparticles can be circumvented using capping agents such as pectin. These capping agents act as stabilizers that inhibit the over-growth of nanoparticles [10]. Pectin molecules interact with the surface of ZnO nanoparticles through electrostatic interaction so that ZnO surface energy will decrease. In addition, pectin molecules also surround ZnO nanoparticles so that interactions between nanoparticles can be prevented, minimizing the aggregation and agglomeration of ZnO nanoparticles during the synthesis process.

ZnO nanoparticles have high antibacterial activity with high effectiveness at low concentrations, that is 0.16–5 mmol/L, against Gram-positive and Gram-negative bacteria [11]. The smaller ZnO nanoparticles will exhibit higher antibacterial activity due to the larger specific surface area so it will have higher particle surface reactivity [1]. The mechanism of ZnO nanoparticles as an antibacterial involves direct interaction between the ZnO surface and the bacterial cell wall. Therefore, the presence of pectin as a capping agent around the surface of ZnO nanoparticles potentially affects the antibacterial properties of these nanoparticles. In this study, the antibacterial activity of complex ZnO/pectin will be compared to ZnO nanoparticles synthesized without pectin as the capping agent.

2. Methodology

2.1. Tools and Materials

The tools used in this study were glassware, analytical balance (Kern ADB 200-4), 1 mL and 5 mL micropipette (Nichipet EX K08716091 and H07Z02841), hotplate (Ika HS-7), oven (Memmert UN- 55), centrifuge (HETTICH EBA-200), centrifuge tube, 35 mL plastic bottle, test tube, test tube rack, gloves, and brush. The materials used in this study were pectin, 1 M NaOH (Merck), zinc(II) acetate dihydrate ($Zn(CH3COO)2{\cdot}2H2O$) 0.1 M (Merck), distilled water, universal pH indicator, and KBr.

2.2. Experimental

2.2.1. Synthesis of ZnO using Pectin as a Capping Agent

The ZnO synthesis method is referred to [2] with modifications. A total of 50 mL of 0.25% (w/v) pectin was mixed with 12.5 mL of water and then stirred with a magnetic stirrer. The solution was then added with 12.5 mL of 0.1 M Zn(CH3COO)2·2H2O and stirred for 5–10 minutes and the pH of the mixture was measured. After that, 5 mL of 1 M NaOH was added to the solution dropwise (dropwise) until the pH of the solution reached 13 and the changes were observed. The solution was stirred at 250 rpm, then heated for 10 minutes using a heating plate at 80 °C and the bottle was closed. The hot solution was reacted hydrothermally by placing it in the oven for 2 hours at a temperature of 80 °C. The solution that has been heated in the oven is then cooled and the pH of the solution is measured again. The solution was cooled and left at room temperature until it settled. Part of the sediment sample was separated from the supernatant then washed using distilled water until the pH was neutral and then dried. Other samples were encapsulated without separating the precipitate and supernatant and drying first.

2.2.2. Material Characterization

The morphology and particle size of ZnO/pectin were characterized using SEM at a magnification value of 10,000–25,000 times. The material is prepared by coating using gold metal.

2.2.3. Antibacterial Activity of Synthesized ZnO/Pectin Analysis

The media used in this study was Muller Hinton Agar (MHA). MHA media was dissolved in an Erlenmeyer flask with distilled water. The dissolved media was then heated and mixed until homogeneous. The media was sterilized using an autoclave at 121 °C for 15 minutes. Around 15 mL of the media was poured into a petri dish and left to solidify. After that, suspension of Staphylococcus aureus test colonies was made by taking one loop of colonies from solid NA media into a test tube containing physiological NaCl. Turbidity in the test colony suspension was standardized to the 0.5 McFarland standard (approximately 1.5 x 108 CFU/mL).

Antibacterial activity analysis of each sample was carried out using 0.1 mL suspension of test bacteria inoculated in MHA media. The suspension was then spread evenly with a hockey stick and left to dry. Then, 50 µL of each ZnO nanoparticle sample was added to the disc paper and placed on the surface of the media aseptically. After that, the media containing bacteria suspension and ZnO nanoparticle sample was incubated for 24 hours at a temperature

of 37 °C. The inhibition zone formed around the paper disc was then measured using a caliper, representing the antibacterial activity of the analyzed samples.

3. Results and Discussion

3.1. Synthesized ZnO/Pectin

ZnO was synthesized through the combination of precipitation and hydrothermal methods. $Zn(CH_3COO)_2{\cdot}_2H_2O$ was used as the zinc source, NaOH as the precipitate to form ZnO crystals, and pectin as the capping agent. Pectin was first mixed with $Zn(CH_3COO)_2{\cdot}_2H_2O$ solution. The pectin used in this study is high methoxyl pectin (HMP) so the addition of $Zn(CH_3COO)_2{\cdot}_2H_2O$ to the pectin does not cause a gelation reaction. Gelation of the pectin mixture with Zn^{2+} ions occurs due to the interactions between cations and the oxygen atoms of the carboxylate groups of pectin molecules. These cations are cross-linked to two carboxylic groups from different pectin chains to form a three-dimensional structure [13]. The degree of esterification of HMP is higher compared to low methoxyl pectin (LMP). The amount of available oxygen atoms in HMP to interact with cations is relatively small so that the cross-linking of two different pectin chains is also relatively small. Therefore, there is no gelation occurred during the synthesis process.

NaOH was then added dropwisely to the previously prepared mixture until ZnO crystals were obtained. The precipitation process produced a white solution which was then reacted hydrothermally in the oven at a temperature of 80 °C. The white color of the solution became more intense after the hydrothermal reaction, which indicated an increase in the number of ZnO crystals formed during the hydrothermal reaction. The ZnO formation reaction occured is as follows [14].

$$Zn(CH_3COO)_2{\cdot}2H_2O + 2NaOH \rightarrow Zn(OH)_2 + 2CH_3COONa + 2H_2O$$

$$Zn(OH)_2 \rightarrow ZnO + H_2O$$

The synthesized sample had a pH of 13 due to the remaining CH_3COONa and OH^- ions. After the synthesis process, the sample was settled, then two layers of the solution were formed due to the separation of the precipitate and supernatant. The sample was then washed using distilled water 3 times to remove the CH_3COONa content and OH^- ions that were still present in the sample until the sample pH became neutral. The sample was then dried in an oven to remove the water content, resulting in a white powder-like solid with a yield of more than 90% (Figure 1).

Figure 1. Synthesized ZnO/pectin powder

Pectin as a capping agent controlled the size of ZnO particles. ZnO samples synthesized with the addition of pectin as a capping agent took a longer time to be precipitated compared to samples synthesized without the capping agent addition. This showed the successful role of pectin in controlling the size of ZnO particles. Pectin molecules surround the ZnO particles to minimize aggregation and agglomeration of the ZnO particles [10]. Therefore, ZnO particles were smaller and formed a more stable colloid so that it took a longer time to be precipitated.

These results are validated as can be seen in the scanning electron microscope (SEM) images of the synthesized materials. ZnO particles synthesized without capping agent are 100–1000 nm in size with a flower-like morphology. This morphology is in accordance with the result reported by [15] that ZnO particles synthesized with a pH close to or more than 11 will have flower-like morphology. Meanwhile, particles synthesized using capping agents tend to be smaller with higher particle size uniformity in the range of 100–200 nm. The exact size of the ZnO particles is difficult to determine due to the presence of pectin on the surface of the particles. The calcination process can be carried out to remove the pectin content in the sample so that the exact ZnO particle size can be determined.

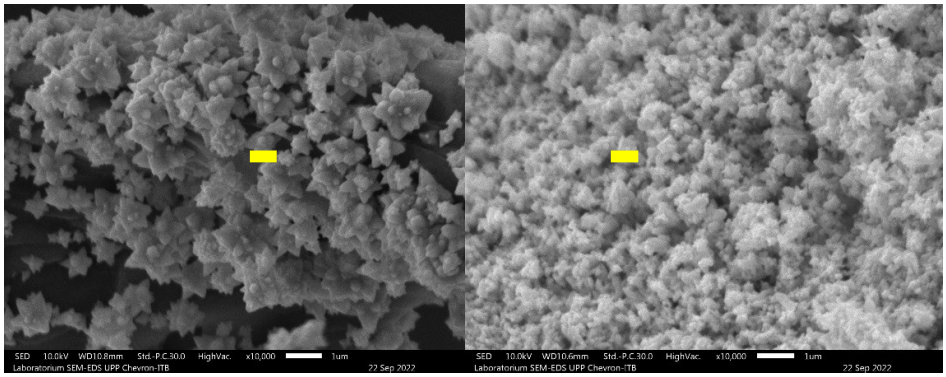

Figure 2. SEM images of ZnO synthesized without pectin (left) and with pectin (right)

3.2. Antibacterial Activity of Synthesized ZnO/Pectin

Numerous studies have shown that ZnO nanoparticles exhibit remarkable antibacterial activity against both Gram-positive and Gram-negative bacteria. The antibacterial activity of ZnO nanoparticles depends on their size related to the mechanism of their action [16]. The antibacterial activity of the samples in this study was analyzed using the disc zone method. The inhibition zone of the sample represents the antibacterial activity of the sample. A wider inhibition zone indicates higher antibacterial activity. The experimental results in **Table 1** and **Figure 3** show that the ZnO/pectin sample exhibits the highest antibacterial activity compared to commercial ZnO and synthesized ZnO. ZnO sample synthesized using pectin as a capping agent, in this case, ZnO/pectin, has a smaller size than commercial ZnO sample and ZnO synthesized without pectin as mentioned in the previous sub-chapter. These findings are similar to El-Masry *et al.* [17] who reported that the smaller size of ZnO nanoparticles showed a more effective antibacterial growth inhibition. This phenomenon could be explained through the ZnO nanoparticles' mechanism of action as the antibacterial agent that is related to the interaction between nanoparticle's surfaces with the bacteria.

Table 1. Antibacterial activity of ZnO and ZnO/pectin against *Staphylococcus aureus*

Samples	*S. aureus ATCC* 6538 (mm)
Commercial ZnO	14.53 ± 2.58
ZnO/pectin	17.52 ± 0.23
ZnO without pectin	12.20 ± 0.24

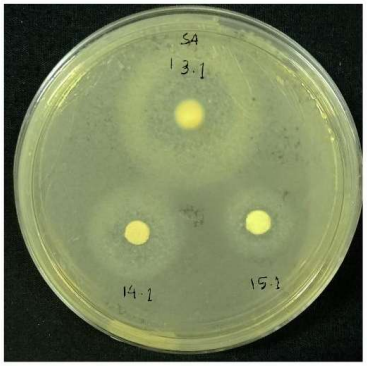

Figure 3. Antibacterial activity of ZnO/pectin (13.1), commercial ZnO (14.1) and ZnO without pectin (15.1) against *Staphylococcus aureus*

There are 2 proposed mechanisms of action of ZnO nanoparticles as antibacterial in this study. The first mechanism is the generation of reactive oxygen species (ROS) induced by ZnO nanoparticles that inhibit the growth of the bacteria and even cause the death of the

bacteria. ZnO nanoparticles are able to induce the production of ROS, such as hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), and superoxide anion (O_2^-), with the help of ultraviolet (UV) light by converting water and oxygen from the air to its reactive species or ROS [18]. Electrons in the valence band of ZnO nanoparticles leave the positively charged holes in the presence of UV light and undergo numerous redox reactions with water and oxygen to produce ROS [19]. ROS could cause membrane damage that would lead to further disruptions in bacteria cells. Furthermore, ROS could also disrupt the DNA, lipids, and proteins that are important in supporting bacteria's life [20]. So, the disruptions inhibit the growth of the bacteria, or worse, cause the death of bacteria as the mechanism shown in **Figure 4**.

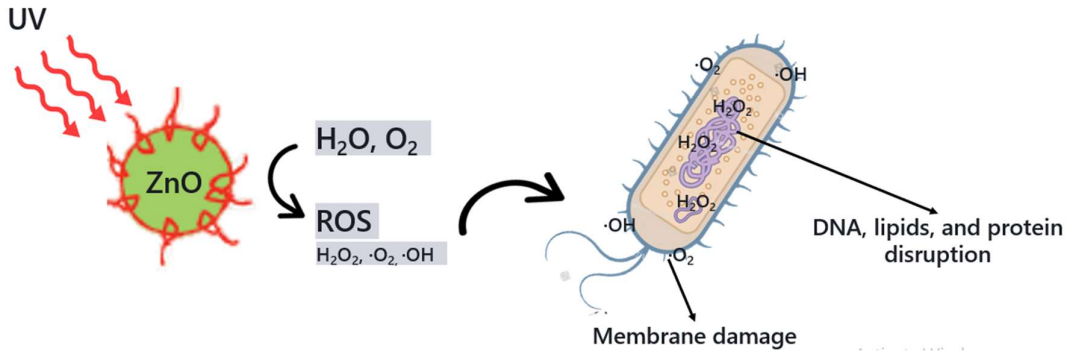

Figure 4. Antibacterial activity of synthesized ZnO mechanism through the generation of ROS [18]

The second mechanism is the deposition of ZnO nanoparticles on the surface of bacteria and the accumulation of ZnO nanoparticles in the cytoplasm or periplasmic region. This process could cause cellular dysfunction or disruption and disorganization of the membranes. The deposition of ZnO nanoparticles produces Zn^{2+}. These ions will be attracted to the negatively charged bacterial membrane causing the imbalance charges on the membrane that will lead to membrane deformation [21]. However, there is a possibility that small particles such as ZnO nanoparticles get through the plasma membrane of bacteria and are transported to the cytoplasm. This process leads to the inhibition of some metabolic exchange of substances and energy production by the ZnO nanoparticles causing the death of bacteria [22]. These mechanisms correlate to the results in this study that interaction between the smaller particles and bacteria surface will be more massive due to the larger particle's surfaces. Furthermore, the smaller particles will be easier to get into the cell wall so the transportation of the particles into the cells will be easier resulting in a more massive accumulation of particles in the cytoplasm.

4. Conclusion

ZnO nanoparticles can be synthesized through the combination of precipitation and hydrothermal methods using pectin as a capping agent to control the particle size. Samples synthesized using the capping agent are smaller in size than samples synthesized without the capping agent, proven by the time needed for the ZnO particles to be precipitated. ZnO/pectin also produces higher antibacterial activity than commercial ZnO and synthesized ZnO due to the smaller size of particles. The mechanism of ZnO/pectin and ZnO as antibacterial can be explained through several mechanisms including the generation of reactive oxygen species (ROS) induced by ZnO nanoparticles, deposition of ZnO nanoparticles on the surface of bacteria, and the accumulation of ZnO nanoparticles in the cytoplasm or periplasmic region.

Acknowledgement

The authors acknowledge financial support from PTUPT under the Directorate General of Higher Education, Ministry of Education of Indonesia with contract No. 15896/IT3.D10/PT.01.02/P/T/2023.

References

[1] Amna Sirelkhatim, Shahrom Mahmud, Azman Seeni, Noor Haida Mohamad Kaus, Ling Chuo Ann, Siti Khadijah Mohd Bakhori, Habsah Hasan, Dasmawati Mohamad, Review on zinc oxide nanoparticles: antibacterial activity and toxicity mechanism, *Nano-Micro Letters*, 7, 3, (2015), 219-242, https://doi.org/10.1007/s40820-0150040-x

[2] Attarad Ali, Abdul-Rehman Phull, Muhammad Zia, Elemental zinc to zinc nanoparticles: is ZnO NPs crucial for life? synthesis, toxicological, and environmental concerns, *Nanotechnology* Review, 7, 5, (2018), 413-441 https://doi.org/10.1515/ntrev-2018-0067

[3] Divyapriya S, Sowmia C, Sasikala S, Synthesis of zinc oxide nanoparticles and antimicrobial activity of *Murraya koeiniggi*, *World Journal of Pharmacy and Pharmaceutical Sciences*, 3, 12, (2014), 1635-1645

[4] Chin Boon Ong, Law Yong Ng, Abdul Wahab Mohammad, A review of ZnO nanoparticles as solar photocatalysts: synthesis, mechanisms, and applications, *Renewable and Sustainable Energy*, 81, (2018), 536-551, https://doi.org/10.1016/j.rser.2017.08.020

[5] RL Manjunatha, KV Usharani, D Naik, Synthesis and characterization of ZnO nanoparticles: a review, *Journal of Pharmacognosy and Phytochemistry*, 8, 3, (2019), 1095–1101

[6] Agnieszka Kolodziejczak-Radzimska, Teofil Jesionowski, Zinc oxide-from synthesis to application: a review, *Material*, 7, 4, (2014), 2833-2881, https://doi.org/10.3390/ma7042833

[7] Ali Moulahi, F Sediri, ZnO nanoswords and nanopills: hydrothermal synthesis, characterization and optical properties, *Ceramics International*, 40, 1, (2014), 943-950, https://doi.org/10.1016/j.ceramint.2013.06.090

[8] Junwei Ding, Shiying Zhu, Tau Zhu, Wei Sun, Qing Li, Gang Wei, Zhiqiang Su, 2015, Hydrothermal synthesis of zinc oxide-reduced graphene oxide nanocomposites for an electrochemical hydrazine sensor, *RSC Advances*, 5, 29, (2015), 22935-22942, https://doi.org/10.1039/c5ra00884k

[9] Sonalika Agarwal, Prabhakar Rai, Eric Navarrete Gatell, Eduard Llobet, Frank Guell, Manoj Kumar, Kamlendra Awasthi, Gas sensing properties of ZnO nanostructures (flowers/rods) synthesized by hydrothermal method, *Sensors and Actuators B: Chemical*, 292, (2019), 24-31 https://doi.org/10.1016/j.snb.2019.04.083

[10] Rabia Javed, Muhammad Zia, Sania Naz, Samson O. Aisida, Noor ul Ain, Qiang Ao, Role of capping agents in the application of nanoparticles in biomedicine and environmental remediation: recent trends and future prospects, *Journal of* Nanobiotechnology, 18, 172, (2020), 1-15, https://doi.org/10.1186/s12951-020-00704-4

[11] Sergey V. Gudkov, Dmitriy E. Burmistrov, Dmitriy A. Serov, Maxim B. Rebezov, Anastasia A. Semenova, Andrey B. Lisitsyn, A mini review of antibacterial properties of ZnO nanoparticles, *Frontiers in Physics*, 9, 641481, (2021), 1-12. https://doi.org/10.3389/fphy.2021.641481

[12] Ai Jun Wang, Qi Chen Liao, Jiu Ju Feng, Pei Pei Zhang, Ao Qi Li, Jian Ji Wang, Apple pectin-mediated green synthesis of hollow double-caged peanut-like ZnO hierarchical superstructures and photocatalytic applications, *CrystEngComm*, 14, (2012), 256-263, https://doi.org/10.1039/c1ce05830d

[13] Miete Celus, Clare Kyomugasho, Ann M. Van Loey, Tara Grauwet, Marc E. Hendrickx, Influence of pectin structural properties on interactions with divalent cations and its associated functionalities, *Comprehensive Reviews in Food Science and Food Safety*, 0, (2018), 1-19 https://doi.org/10.1111/1541-4337.12394

[14] TT Ha, TD Canh, NV Tuyen, A quick process for synthesis of ZnO nanoparticles with the aid of microwave irradiation, *ISRN* Nanotechnology, 2013, (2013) 1-8, https://doi.org/10.1155/2013/497873

[15] Kaoruho Sakata, Katerina Minhova Macounova, Roman Nebel, Petr Krtil, pH dependent ZnO nanostructures synthesized by hydrothermal approach and surface sensitivity of their photoelectrochemical behavior, *SN Applied Sciences*, 2, 203, (2020), 1-8, https://doi.org/10.1007/s42452-020-1975-1

[16] Nataliya Babayevska, Lucja Przysiecka, Igor Iatsunskyi, Grzegorz Nowaczyk, Marcin Jarek, Ewa Janiszewska, Stefan Jurga, ZnO size and shape effect on antibacterial activity and cytotoxicity profile, *Scientific Reports*, 12, 8148, (2022), 1-12, https://doi.org/10.1038/s41598-022-12134-3

[17] Reham M. El-Masry, Dalia Talat, Shahira A. Hassoubah, Nidal M. Zabermawi, Nesreen Z, Eleiwa, Rasha M. Sherif, Mohammed A.S. Abourehab, Randa M. Abdel-Sattar, Mohammed Gamal, Madiha S. Ibrahim, Ahmeed Elbestawy, Evaluation of the antimicrobial activity of ZnO nanoparticles against enterotoxigenic *Staphylococcus aureus*, *Life*, 12, 1662, (2022), 1-13, https://doi.org/10.3390/life12101662

[18] Shengjie Jiang, Kaili Lin, Ming Cai, ZnO nanomaterials: current advancements in antibacterial mechanisms and applications, *Frontiers in Chemistry*, 8, 580, 1-5, https://doi.org/10.3389/fchem.2020.00580

[19] Leigang Miao, Biming Shi, Nawrat Stainslaw, Chaomin Mu, Kezhen Qi, Facile synthesis of hierarchical ZnO microstructures with enhanced photocatalytic activity, *Materials Science-Poland*, 35, 1, (2017), 45-49, https://doi.org/10.1515/msp-2017-0007

[20] Shaoheng Tang, Jie Zheng, Antibacterial activity of silver nanoparticles: structural effects, *Advanced Healthcare Materials*, 7, 13, (2018), e1701503, https://doi.org/10.1002/adhm.201701503

[21] Yan-Wen Wang, Aoneng Cao, Yu Jiang, Xin Zhang, Jia-Hui Liu, Yuanfang Liu, Haifang Wang, Superior antibacterial activity of zinc oxide/graphene oxide composites originating from high zinc concentration localized around bacteria, *ACS Applied Materials & Interfaces*, 6, 4, (2014), 2791-2798, https://doi.org/10.1021/am4053317

[22] Shengjie Jiang, Kaili Lin, Ming Cai, ZnO nanomaterials: current advancements in antibacterial mechanisms and applications, *Frontiers in Chemistry*, 8, 580, (2020), 1-5, https://doi.org/10.3389/chem.2020.00580