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ANTIBACTERIAL ACTIVITY OF BIOSYNTHESIZED GRAPHITIC CARBON NITRIDE NANOHYBRID

ANTIBAKTERIAALNE AKTIIVSUS BIOSÜNTEESITUD GRAFIITSE SÜSINIKNITRIIDI NANOHÜBRIIDIL

Final Thesis
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Antimicrobial resistance (AMR) is a rapidly escalating global health threat driven by the excessive and improper use of antibiotics in both human and veterinary medicine. The shared use of antimicrobial agents across these sectors has accelerated the emergence of resistant bacteria, leading to treatment failures, increased mortality, and major economic losses. Due to the declining effectiveness of conventional antibiotics and the limited development of new antimicrobial drugs, nanotechnology has emerged as a promising complementary strategy for AMR control. The aim of this thesis was to evaluate the antibacterial activity of a zinc oxide-doped graphitic carbon nitride nanohybrid (ZnO/GCN-NH) using a green synthesis approach. Antibacterial activity was evaluated using disk diffusion and well diffusion methods against two Gram-positive bacteria, *Staphylococcus aureus* and *Streptococcus dysgalactiae*, and two Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*. The ZnO/GCN-NH demonstrated antibacterial activity against all tested bacterial strains. In the disk diffusion method, inhibition zones ranged from 14 to 17.2 mm, while in the well diffusion method they ranged from 17.5 to 18.5 mm. In comparison, the pure plant extract, pure graphitic carbon nitride nanoparticles (GCN-NP) and pure zinc oxide nanoparticles (ZnO-NP) showed only limited antibacterial activity. These results suggest that the formation of the ZnO/GCN-NH improved antibacterial performance compared with the individual components.

Keywords: carbon-based nanoparticles, antibacterial activity, green synthesis, reactive oxygen species

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Antimikroobne resistentsus (AMR) on kiiresti süvenev ülemaailmne oht, mida põhjustab antibiootikumide kergekäeline ja ebaotstarbeline kasutamine nii humaan- kui ka veterinaarmeditsiinis. Antimikroobsete ravimite suurenenud kasutamine mõlemas sektoris on kiirendanud resistentsete bakterite teket ja levikut. See on muutnud ravi ebaefektiivseks, suurenenud on suremus ning seeläbi tekitanud märkimisväärset majanduslikku kahju. Olemasolevate antibiootikumide efektiivsus on vähenemas ja uute antimikroobsete ravimite arendamine on piiratud, seega on hakatud lähemalt uurima nanomaterjale, kuna on tõestatud nende antimikrobiaalne toime ja see on kujunenud täiendavaks strateegiaks AMR-i kontrollimisel. Käesoleva lõputöö eesmärk oli hinnata rohelise sünteesi meetodil valmistatud tsinkoksiidiga dopeeritud grafiitse süsiniknitriidi nanohübriidi (ZnO/GCN-NH) antibakteriaalset aktiivsust. Antibakteriaalset aktiivsust hinnati kasutades disk difusiooni ja kaevu difusiooni meetodi kahe Gram-positiivse bakteri, *Staphylococcus aureus* ja *Streptococcus dysgalactiae* ning kahe Gram-negatiivse bakteri, *Escherichia coli* ja *Pseudomonas aeruginosa* suhtes. ZnO/GCN-NH näitas antibakteriaalset aktiivsust kõigi testitud bakteritüvede suhtes. Disk difusiooni meetodil jäid inhibeeritud alad vahemikku 14–17.2 mm ning kaevu difusiooni meetodil vahemikku 17.5–18.5 mm. Võrdluseks avaldasid puhas taimne ekstrakt, puhtad tsinkoksiidi nanoosakesed (ZnO-NP) ja puhtad grafiitse süsiniknitriidi nanoosakesed (GCN-NP) vaid minimaalset antibakteriaalset toimet. Tulemused viitavad sellele, et ZnO/GCN-NH parandas antibakteriaalset aktiivsust võrreldes aktiivusega, mida need nanoosakesed eraldi avaldasid.			
Märksõnad: süsiniku nanoosakesed, antibakteriaalne aktiivsus, roheline süntees, reaktiivsed hapnikuühendid			

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LIST OF ABBREVIATIONS

AgNP – silver nanoparticles

AMR – antimicrobial resistance

CNP – carbon-based nanoparticles

DNA – deoxyribonucleic acid

EUCAST – European Committee on Antimicrobial Susceptibility Testing

GCN – graphitic carbon nitride

GCN-NP - graphitic carbon nitride nanoparticles

MBN – metal-based nanoparticles

ROS – reactive oxygen species

SEM – scanning electron microscopy

ZnO/GCN-NH – zinc oxide-doped graphitic carbon nitride hybrid

ZnO-NP – zinc oxide nanoparticles

UV-Vis – ultraviolet visible

XRD – X-ray diffraction

INTRODUCTION

Nanotechnology has emerged as a rapidly developing field in modern science, offering innovative solutions in medicine, environmental protection, and veterinary practice. Due to their nanoscale size, high surface area, and enhanced reactivity, nanomaterials possess unique physicochemical properties that make them suitable for biomedical and antimicrobial applications (Murthy, 2007; Fortune *et al*., 2025). In recent years, nanoparticles have gained attention as complementary antimicrobial agents because of their broad-spectrum activity and multi-target mechanisms of action (Sánchez-López *et al*., 2020). This interest is closely related to the global escalation of antimicrobial resistance (AMR), which threatens both human and veterinary medicine. The excessive and inappropriate use of antibiotics in humans, animals, and agriculture has accelerated the emergence and spread of resistant microorganisms, resulting in reduced treatment efficacy, prolonged illness, increased mortality, and major economic losses (Palma *et al*., 2020; Ahmed *et al*., 2024). From a One Health perspective, AMR involves interconnected transmission between humans, animals, and the environment, with livestock production, manure, wastewater, and agricultural runoff serving as important reservoirs for resistance dissemination (Ahmed *et al*., 2024; Al-Khalaifah *et al*., 2025). Therefore, safe, effective, and environmentally sustainable antimicrobial strategies are urgently needed. Among carbon-based nanoparticles, graphitic carbon nitride (GCN) has gained scientific interest due to its metal-free composition, chemical stability, layered structure, and favourable biocompatibility (Alaghmandfard and Ghandi, 2022; Panchal *et al*., 2025). Recent studies have shown that biosynthesized zinc oxide-doped graphitic carbon nitride nanohybrids (ZnO/GCN-NH) exhibit promising antibacterial activity and good biocompatibility, making them relevant candidates for sustainable antimicrobial applications (Panchal *et al*., 2025). However, the antibacterial potential of green-synthesized GCN-based nanohybrids remains insufficiently studied in medicine. In particular, limited comparative data are available on the antibacterial activity of plant extract, pure zinc oxide nanoparticles (ZnO-NP), pure graphitic carbon nitride nanoparticles (GCN-NP), and ZnO/GCN-NH against both Gram-positive and Gram-negative bacterial pathogens using disk and well diffusion methods. Therefore, further experimental evaluation of biosynthesized ZnO/GCN-NH is needed. The aim of this thesis is to evaluate the antibacterial activity of a biosynthesized ZnO/GCN-NH.

1. LITERATURE REVIEW

1.1. Antimicrobial resistance

AMR is the ability of bacteria to survive antimicrobial drugs designed to kill them or inhibit their growth. It is a critical global health concern resulting from the excessive and inappropriate use of antimicrobial agents. It poses a major threat to both human and animal health. Most antimicrobial classes used in human medicine are also applied in veterinary practice, leading to the development of shared resistance (Palma *et al*., 2020; Ahmed *et al*., 2024). As a result, standard treatments become less effective, making infections in humans and animals difficult to treat, resulting in prolonged illness, extended hospitalization, and significant economic losses (Ahmed *et al*., 2024).

1.1.1. Different mechanisms of antimicrobial resistance in bacteria

Bacteria that survive an incomplete antibiotic course may acquire resistance (Ahmed *et al*., 2024). Over evolutionary time acquired AMR has contributed to the diversification of both innate and acquired resistance mechanisms. Bacteria have evolved a wide range of strategies to survive antimicrobial exposure. Innate resistance represents an ancient biological adaptation that allows bacteria to withstand toxic compounds long before the introduction of modern antibiotics. These natural mechanisms, including efflux pumps and reduced cell permeability, are now complemented by acquired resistance transmitted through plasmids and integrons (Palma *et al*., 2020). Typical AMR mechanisms (Figure 1) include enzymatic inactivation or degradation of antibiotics, reduced intracellular antibiotic accumulation due to decreased membrane permeability, and alterations of metabolic pathways. Bacteria may also modify antibiotic target sites, such as ribosomal structures, thereby lowering drug-binding efficiency, or enhance the activity of efflux pumps that actively expel antibiotics from the cell before therapeutic concentrations are reached. In addition, many bacteria form biofilms, surface-attached microbial communities characterized by nutrient gradients and restricted antibiotic penetration, which further contribute to reduced antimicrobial effectiveness (Ahmed *et al*., 2024). Together, these processes explain how AMR continues to evolve in both natural and clinical environments, intensifying under selective pressure from antibiotic misuse in agriculture and veterinary medicine (Palma *et al*., 2020).

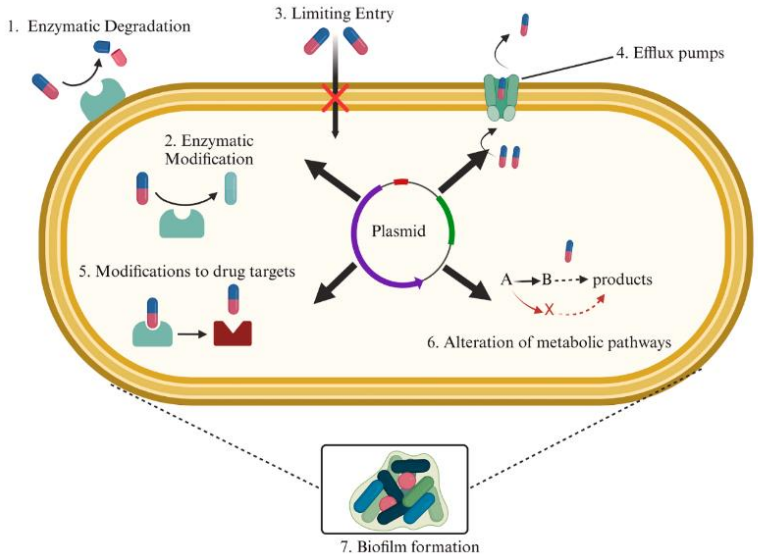

Figure 1. Diagram representing different mechanisms of antimicrobial resistance in bacteria (Ahmed *et al*., 2024).

1.1.2. Global impact of antibiotic overuse and antimicrobial resistance

Until recently, antibiotics were used not only to treat infectious diseases but also at subtherapeutic doses in farm animals to prevent illness and promote growth. However, such practices greatly increased the risk of developing AMR. To address this issue, the European Union banned the use of antibiotics as growth-promoting agents in 2006 as a preventive measure against AMR (Palma *et al*., 2020). In 2019, AMR was linked to approximately five million deaths worldwide, and if current trends continue, it could lead to up to ten million deaths annually by 2050 (Al-Khalaifah *et al*., 2025). Consequently, common diseases and minor injuries may once again become lethal and challenging to cure. In human medicine, inappropriate prescribing, self-medication, and incomplete antibiotic courses also contribute to the selection and spread of resistant bacteria (Ahmed *et al*., 2024). At the same time, the development of new antibiotics has slowed, while research interest in alternative antimicrobial strategies, including nanoparticles, has increased. Nanoparticles have been identified as promising agents against bacteria resistant to traditional antibiotics (Sánchez-López *et al*., 2020).

In addition to its broader global impact, antibiotic overuse in livestock has created significant reservoirs of resistance (Ahmed *et al*., 2024; Al-Khalaifah *et al*., 2025). Limited treatment options for resistant animal infections can contribute to outbreaks among cattle, poultry, and sheep, necessitating culling and generating substantial economic losses while threatening food

security. Estimates indicate that AMR may impose a financial burden of approximately three to four billion USD on the livestock sector alone in the coming decades (Ahmed *et al*., 2024). The prevalence of AMR has been further exacerbated by the indiscriminate use of antibiotics in agriculture, contributing to the spread of resistance genes. These developments illustrate a self-reinforcing cycle in which increased antibiotic use accelerates resistance development, thereby driving continued demand for novel antimicrobial strategies. Rational and regulated use of antibiotics in agriculture and veterinary medicine is therefore essential to mitigate excessive antimicrobial consumption and slow down the spread of resistance (Ahmed *et al*., 2024).

1.1.3. One Health concept

One Health is an integrated approach that aims to optimize the health of humans, animals and ecosystems in a sustainable way. It is based on the understanding that humans, animals, plants and environmental health are closely connected. Through cross-sectoral collaboration, the One Health approach helps to address major global health challenges such as emerging infectious diseases, AMR and food safety. By linking these sectors, One Health supports effective disease prevention, detection, preparedness and response at all levels (WHO, 2025). From a One Health perspective, AMR is driven by the interconnected use and dissemination of antibiotics across human healthcare, livestock production and the environment. Food-producing animals act as important reservoirs of resistant bacteria, which can be transmitted to humans through direct contact, the food chain, and environmental exposure, contributing to zoonotic spread of resistance. Transmission of resistant bacteria and resistance genes can occur in multiple directions between humans, animals and the environment, which supports the need for a unified One Health approach (Ahmed *et al*., 2024; Al-Khalaifah *et al*., 2025). Environmental reservoirs such as agricultural runoff, manure, slurry and wastewater act as major AMR hotspots that facilitate the persistence and horizontal transfer of resistance genes (Al-Khalaifah *et al*., 2025). Therefore, controlling AMR requires coordinated veterinary antimicrobial stewardship, environmental protection, and cross-sector collaboration under the One Health framework (Palma *et al*., 2020; Ahmed *et al*., 2024).

1.2. Nanoparticles

Nanoparticles are chemical substances or materials with at least one dimension in the range of 1 to 100 nm in size (Murthy, 2007; Fortune *et al*., 2025). Their small dimensions and large

surface area enhance reactivity, energy absorption, and biological mobility, enabling applications that conventional materials cannot achieve. Nanoparticles are widely used in modern medicine for targeted drug delivery, diagnostic imaging, and controlled release of therapeutic agents, where they improve drug solubility, bioavailability, and treatment efficiency (Murthy, 2007). Nanoparticles have become essential tools in modern medicine due to their nanoscale size and unique physicochemical properties that differ from bulk materials. In clinical practice, nanoparticles are increasingly incorporated into wound dressings, coatings, and sprays to prevent surgical site infections and promote faster healing (Fortune *et al*., 2025). At the same time, continued research emphasizes that nanoparticles may present toxicity risks, including oxidative stress, organ accumulation, inflammation, and immune system activation, which require thorough biological safety evaluation prior to clinical application (Fortune *et al*., 2025).

1.3. *In vitro* toxicity of nanoparticles

1.3.1. Oxidative stress

Reactive oxygen species (ROS) are reactive oxygen-derived molecules that include both radical and non-radical forms. Examples of ROS include superoxide anions ($O_2 \cdot^-$), hydroxyl radicals ($\cdot OH$) and hydrogen peroxide (H_2O_2). ROS are naturally formed during aerobic cellular metabolism. Under normal conditions inside the cell, ROS levels are regulated by antioxidant defence and repair systems. Oxidative stress develops when ROS production exceeds the cell's ability to neutralize or remove them. This imbalance can damage lipids, proteins, deoxyribonucleic acid (DNA), and ribonucleic acid, and may contribute to inflammation, cellular dysfunction or cell death (Alfei *et al*., 2024).

The role of ROS is relevant in antibacterial research because excessive ROS accumulation can overwhelm bacterial defence mechanism. In bacterial cells, oxidative stress can damage the cell membrane, oxidize proteins, disrupt metabolism, and damage genetic material. These combined effects can inhibit bacterial growth and may ultimately lead to cell death. For this reason, ROS-mediated mechanisms are frequently discussed in relation to antimicrobial approaches, including nanoparticle-based systems. However, ROS are not completely selective and may also damage mammalian cells. Therefore, the safety and biocompatibility of ROS-generating antimicrobial systems must be carefully evaluated (Alfei *et al*., 2024; Sánchez-López *et al*., 2020).

1.3.2. DNA damage

DNA damage is one possible consequence of oxidative stress and nanoparticle exposure. It can include oxidative changes in DNA bases and breaks in DNA strands. ROS can damage bacterial DNA and interfere with normal cellular processes, including replication and cell division. In bacteria, this contributes to antibacterial activity because damage to genetic material can inhibit growth and lead to cell death. From a safety perspective, DNA damage is also important when evaluating nanoparticle-based antimicrobial systems. Nanoparticles or released ions may enter cells, induce ROS formation, and interact with cellular structures such as proteins, membranes and DNA. If these effects occur in mammalian cells, they may contribute to genotoxicity or cytotoxicity. Therefore, DNA damage should be considered when assessing the biological safety of nanoparticles (Sánchez-López *et al*., 2020; Alfei *et al*., 2024).

1.4. Nanotechnology uses in antibacterial activity

1.4.1. Metal-based nanoparticles

Metal-based nanoparticles (MBN) are among the most widely studied inorganic nanoparticles for antimicrobial applications. They are considered promising in the context of AMR because several metallic and metal-oxide nanoparticles have shown broad activity against pathogenic microorganisms, while AMR continues to reduce the effectiveness of antimicrobial treatments (Correa *et al*., 2020; Sánchez-López *et al*., 2020; Ahmed *et al*., 2024). MBN show broad-spectrum antibacterial activity through multiple non-specific mechanisms. Their main characteristic is to release metal ions. These released metal ions induce ROS through different chemical reactions and damage DNA which lead to oxidative stress, metabolic dysfunction and ultimately bacterial cell death (Sánchez-López *et al*., 2020). Silver nanoparticles (AgNP) are among the most studied MBN and are known to have antibacterial efficacy against both Gram-positive and Gram-negative bacteria (Sánchez-López *et al*., 2020). AgNP exhibit strong antibacterial properties but may present toxicity concerns due to bioaccumulation, which has encouraged researchers to explore safer and more sustainable alternatives such as zinc or carbon nanomaterials (Panchal *et al*., 2025). These materials maintain high antibacterial potential while minimizing environmental and cytotoxic risks (Sánchez-López *et al*., 2020; Panchal *et al*., 2025).

1.4.2. Carbon-based nanoparticles

Carbon-based nanoparticles (CNP) are a diverse group of nanomaterials mainly composed of carbon. This group includes carbon dots, fullerenes, carbon nanotubes, graphene, graphene oxide, graphene quantum dots and nanodiamonds (Figure 2; Holmannova *et al*., 2022; Hussen *et al*., 2024). These materials have attracted attention because of their high surface area, structural stability, and tunable surface chemistry (Dizaj *et al*., 2015; Al-Jumaili *et al*., 2017). CNP differ from MBN mainly in their chemical composition. CNP are based on carbon allotropes or carbon-rich structures, whereas MBN are composed of metals or metal oxides (Correa *et al*., 2020). This compositional difference affects their antibacterial mechanisms. While MBN often act through metal ion release, CNP mainly act through direct contact with bacterial cells, membrane damage, oxidative stress, and electron-transfer processes (Dizaj *et al*., 2015; Al-Jumaili *et al*., 2017; Correa *et al*., 2020).

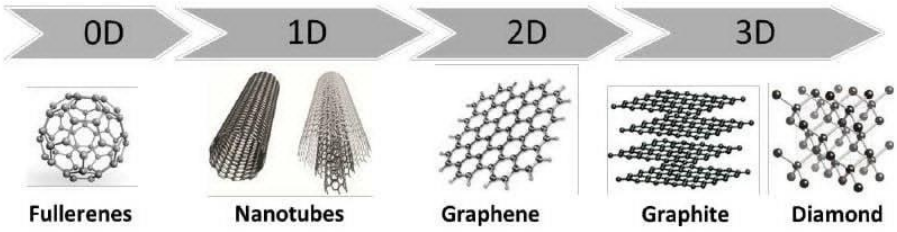

Figure 2. Different types of carbon materials according to the arrangement of carbon atoms. Fullerenes are zero-dimensional (0D) small cage balls. Nanotubes are tube-shaped one-dimensional (1D) structures. Graphene is a two-dimensional (2D) single flat sheet. Graphite and diamond are three-dimensional (3D) and extend in all three directions (Srivatsa, 2017).

CNP are considered promising for biomedical use because their surface can be modified for different biological purposes. Surface functionalization can improve water solubility, reduce aggregation, and support more controlled interactions with cells or drugs (Dizaj *et al*., 2015; Hussen *et al*., 2024). This is important because many carbon nanomaterials have poor solubility in physiological media without further modification (Dizaj *et al*., 2015). Functionalized CNP may also be used as carriers for antimicrobial drugs. This could improve drug delivery, increase bioavailability and support more targeted treatment (Dizaj *et al*., 2015; Holmannova *et al*., 2022). Carbon dots are especially relevant in this context, as they are described as water-dispersible, easily functionalized, and biocompatible (Hussen *et al*., 2024).

The antibacterial effect of CNP depends on several material-related factors. These include particle size, surface charge, functional groups, concentration, aggregation state, and the bacterial species tested (Dizaj *et al*., 2015; Al-Jumaili *et al*., 2017). Smaller particles may interact more efficiently with bacteria because they provide a larger active surface area (Dizaj *et al*., 2015). However, safety remains a key limitation. CNP should not be described as completely non-toxic, because their effects on mammalian cells depend on their physicochemical properties and exposure conditions (Al-Jumaili *et al*., 2017; Holmannova *et al*., 2022). Current studies suggest that some CNP have good biocompatibility, but long-term toxicity, accumulation, and immune responses still need further investigation before wider biomedical or veterinary use (Holmannova *et al*., 2022; Hussen *et al*., 2024).

1.4.3. Nanohybrids

Nanohybrids are materials formed by combining two or more nanoscale components into one integrated structure. The purpose of forming nanohybrids is to combine the beneficial properties of different materials and create a stronger overall effect than individual components alone are capable of. In antibacterial applications, this is important because hybrid materials may show improved stability, increased surface area, better contact with bacterial cells, and enhanced multi-target antibacterial activity (Yah and Simate, 2015; Dahiya *et al*., 2023; Panchal *et al*., 2025). The improved antibacterial performance of nanohybrids is often explained by synergistic effects between the combined materials. One nanoparticle may improve bacterial membrane interaction, while another may enhance ROS formation or release antibacterial ions. Together, these mechanisms can increase oxidative stress, damage bacterial membranes, disrupt proteins and DNA, and inhibit normal cellular functions. (Yah and Simate, 2015; Sánchez-López *et al*., 2020).

Carbon-based materials are often used in nanohybrids because they provide a stable support structure and a large active surface area. When combined with metal or metal oxide nanoparticles, they can improve particle distribution and reduce aggregation. Carbon nanotube/silver nanoparticle hybrids have been reported to show stronger antimicrobial properties than the individual components alone (Yah and Simate, 2015). Similarly, ZnO/GCN-NH are relevant to antibacterial research because ZnO contributes antibacterial activity through Zn^{2+} ion release, ROS-related oxidative stress, and membrane interaction, while GCN provides a stable carbon-based structure with favourable biocompatibility. Recent

studies have reported antibacterial activity of green-synthesized ZnO/GCN-NH against both Gram-positive and Gram-negative bacteria, supporting their potential as sustainable antimicrobial materials (Dahiya *et al*., 2023; Panchal *et al*., 2025).

1.4.4. Green synthesis

Green synthesis is an environmentally friendly approach for producing nanomaterials using biological sources such as plant extracts, microorganisms, algae, fungi, or enzymes. Compared with many physical and chemical synthesis methods, green synthesis can reduce the use of hazardous reducing agents, toxic solvents, high temperatures, and expensive equipment. Therefore, this approach is considered safer, more cost-effective, biocompatible, and more suitable for sustainable large-scale nanoparticle production (Correa *et al*., 2020; Fortune *et al*., 2025). Green-synthesized nanomaterials also align with the One Health approach, because they aim to reduce environmental burden while supporting the development of alternative antimicrobial strategies relevant to human, animal, and environmental health (Palma *et al*., 2020; Ahmed *et al*., 2024).

Plant extracts are commonly used in green synthesis because they contain bioactive compounds that can act as reducing, stabilizing, and capping agents during nanoparticle formation. Several plant-based extracts have been used in previous studies for nanoparticle or nanohybrid synthesis. Holy basil (*Ocimum tenuiflorum*) leaf extract has been used to synthesize ZnO/GCN-NH, GCN-NP and ZnO-NP, which showed antibacterial activity against both Gram-positive and Gram-negative bacteria and acceptable biocompatibility in reported cell-based studies (Panchal *et al*., 2025). Weeping fig (*Ficus benjamina)* leaf extract has been used for ZnO/GCN nanocomposites synthesis, which showed enhanced photocatalytic activity and antibacterial activity against *Escherichia coli* (Dahiya *et al*., 2023). Neem (*Azadirachta indica*) leaf extract has also been used for the green synthesis of ZnO and Ag-doped ZnO-NP, where Ag-doped ZnO-NP showed antibacterial activity against cocci and *E. coli*, as well as potential wound-healing applications (Iqbal *et al*., 2021).

1.4.5. Graphitic carbon nitride

GCN is a metal-free, polymeric semiconductor that has attracted attention in antibacterial research due to its low cost, relatively simple synthesis, layered structure, and good chemical and thermal stability (Paul *et al*., 2020; Alaghmandfard and Ghandi, 2022). Its structure is based

on triazine and heptazine units, which form a layered carbon-nitrogen framework. This structure gives GCN important optical and electronic properties (Alaghmandfard and Ghandi, 2022). The band gap is important because it represents the amount of energy needed to activate the material. When GCN absorbs light energy equal to or greater than its band gap, electrons can move from the valence band to the conduction band. This process creates electron-hole pairs where one electron becomes excited to a higher energy level, while a positively charged hole remains in its original position. These excited electrons and holes can react with oxygen and water molecules and contribute to the formation of ROS, that can damage bacterial cell walls, membranes, and intracellular components, which may lead to bacterial cell death. This explains why GCN-based antibacterial activity is often associated with light activation. (Panchal *et al*., 2025).

However, pure GCN may have limited antibacterial efficiency because the excited electrons and holes can quickly recombine. When recombination occurs, less ROS is produced, which can reduce antibacterial activity. For this reason, GCN is often combined with metal oxides such as ZnO to form nanohybrids. In ZnO/GCN-NH, the interaction between the two materials can improve charge separation and reduce electron-hole recombination (Alaghmandfard and Ghandi, 2022; Dahiya *et al*., 2023). A recent study reported that ZnO/GCN nanocomposites showed antibacterial activity against *E. coli* and linked this effect to reduced band gap energy, improved electron-hole separation and slower recombination (Dahiya *et al*., 2023). Similarly, studies have also reported antibacterial activity of green-synthesized ZnO/GCN-NH against both Gram-positive and Gram-negative bacteria (Panchal *et al*., 2025). These findings support the potential of ZnO/GCN-NH as sustainable antibacterial nanomaterials.

1.4.6. Limitations and challenges of nanoparticle-based antimicrobials

Despite their promising antimicrobial potential, nanoparticle-based antimicrobials still face several limitations that restrict their use in real biomedical applications. The main challenges are related to safety, toxicity, delivery, reproducibility, environmental impact, and regulation. Although nanoparticles can improve antimicrobial activity, their nanoscale properties may also create risks, including cytotoxicity, oxidative stress, inflammation, organ accumulation, and uncertain long-term effects (Holmannova *et al*., 2022; Fortune *et al*., 2025). Another important limitation is that many nanoparticle-based antimicrobials are still mainly studied under laboratory conditions. Their effectiveness and safety may vary depending on particle size,

surface properties, concentration, formulation, and the type of target bacteria. In addition, some nanoparticle systems may have limited clinical data, possible toxicity, and delivery-related challenges, which makes their practical use more difficult than conventional antibiotics (Ahmed *et al*., 2024; Fortune *et al*., 2025). For these reasons, nanoparticles cannot yet fully replace antibiotics. Antibiotics remain essential because their clinical use, dosing, and safety requirements are more established. In contrast, nanoparticle-based antimicrobials still require standardized testing methods, reproducible large-scale production, long-term safety studies, and clearer regulatory guidelines before wider application. Therefore, they should currently be considered complementary antimicrobial tools rather than complete replacements for antibiotics, especially in veterinary medicine and AMR control strategies (Ahmed *et al*., 2024; Saeed *et al*., 2024; Fortune *et al*., 2025).

1.5. Antimicrobial susceptibility testing

1.5.1. Agar disk diffusion method

The agar disk diffusion method is one of the most widely used methods for antimicrobial susceptibility testing in clinical and research laboratories. In this method, sterile filter paper disks impregnated with a defined concentration of the test substance are placed on the surface of an agar plate previously inoculated with a standardized microbial suspension. During incubation, the antimicrobial agent diffuses into the agar and inhibits microbial growth, producing a clear circular zone of inhibition around the disk (Figure 3). The diameter of this zone is measured in millimetres and used to evaluate bacterial susceptibility to the tested substance (Balouiri *et al*., 2016; Correa *et al*., 2020; Hossain *et al*., 2022).

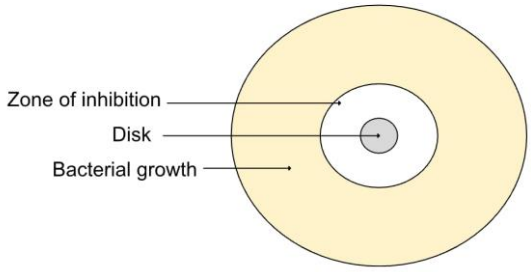

Figure 3. Schematic representation of agar disk diffusion method (Author: Mari-Liis Oberg).

In Europe, disk diffusion antimicrobial susceptibility testing is standardized according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). These guidelines provide harmonized recommendations for inoculum preparation, disk content,

incubation conditions, and interpretation of inhibition zone diameters. Following EUCAST standards helps ensure the reproducibility and comparability of antimicrobial susceptibility results across laboratories (EUCAST, 2025).

1.5.2. Agar well diffusion method

The agar well diffusion method is similar to the agar disk diffusion method, but instead of applying the test substance to a paper disk, a measured volume of the sample is introduced directly into a well created in the agar medium. After inoculation of the agar surface with the test microorganism, sterile wells are aseptically punched into the agar and filled with the test solution or extract. During incubation, the antimicrobial agent diffuses into the surrounding medium and inhibits microbial growth, producing a measurable zone of inhibition (Figure 4). This method is particularly suitable for testing liquid extracts, essential oils, crude extracts, and other compounds that are not easily absorbed by paper disks or require larger sample volumes. As in disk diffusion, the size of the inhibition zone provides a comparative indication of antimicrobial activity, although it may also be influenced by diffusion rate and solubility of the tested substance (Balouiri *et al*., 2016; Correa *et al*., 2020; Hossain *et al*., 2022).

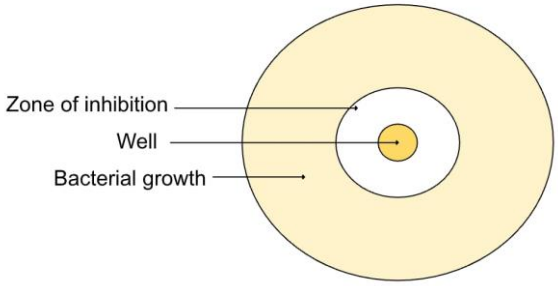

Figure 4. Schematic representation of agar well diffusion method (Author: Mari-Liis Oberg).

Both disk diffusion and well diffusion methods are widely used for preliminary antimicrobial screening, but well diffusion is often more suitable for liquid or compounds that are not easily absorbed by paper disks because the sample is applied directly into the agar well in a defined volume. Both methods provide rapid, visual, and reproducible data on the inhibitory potential of compounds against bacteria. Their simplicity, low equipment demand, and ability to test various antimicrobial substances make these methods essential techniques in both microbiological and pharmaceutical research (Balouiri *et al*., 2016; Correa *et al*., 2020).

1.6. Biosynthesis of nanoparticles and their characterization

Traditional physical and chemical methods for nanoparticle synthesis often require high temperatures, toxic solvents and expensive equipment, which may limit their environmental sustainability and large-scale application. To overcome these disadvantages, biosynthesis, also referred to as green synthesis, has emerged as an environmentally friendly alternative for nanoparticle production (Correa *et al*., 2020; Dahiya *et al*., 2023; Panchal *et al*., 2025). In this approach, biological materials such as plant extracts, bacteria, fungi, or algae are used during nanoparticle formation. Plant extracts are especially useful because they are easily available, cost-effective and can replace hazardous chemical reagents (Correa *et al*., 2020). They contain phytochemicals, such as polyphenols, flavonoids, alkaloids, and proteins, which act as reducing and stabilizing agents by facilitating the reduction of metal precursors and preventing particle aggregation (Correa *et al*., 2020; Iqbal *et al*., 2021; Dahiya *et al*., 2023).

Recent studies have shown that plant-based synthesis can be successfully applied to GCN-NP and ZnO-NP, including ZnO/GCN-NH, with promising antibacterial properties. However, after synthesis, it is necessary to confirm that the desired material has formed and to determine its relevant physicochemical properties. For this purpose, various characterization techniques are employed to analyse optical behaviour, crystalline structure, phase composition, and surface morphology of the synthesized nanomaterials (Dahiya *et al*., 2023; Panchal *et al*., 2025).

1.6.1. Ultraviolet-Visible analysis

Ultraviolet-visible (UV-Vis) spectroscopy is commonly used for nanomaterial characterization because it allows fast and non-invasive evaluation of nanomaterial size, concentration and aggregation state. The method requires minimal sample preparation and is considered simple and inexpensive, which makes it widely applicable in laboratory settings (Quevedo *et al*., 2021). UV-Vis spectroscopy helps us understand basic properties of nanoparticles like their light absorption and band gap. These properties are related to how the material is structured at the nanoscale. In antimicrobial studies, UV-Vis is useful because it confirms that the nanoparticles are formed properly and allows comparison between different samples. Differences seen in UV-Vis results can help explain why one material may behave differently from another in test, based on their physical properties (Dahiya *et al*., 2023; Panchal *et al*., 2025).

1.6.2. X-ray diffraction

X-ray diffraction (XRD) is a common characterization technique for nanoscale materials and provides information that complements microscopic and spectroscopic methods, including phase identification, sample purity, crystallite size, and, in some cases, morphology (Holder and Schaak, 2019; Burlec *et al*., 2023). The principle of XRD was further developed by William Henry Bragg and William Lawrence Bragg, who demonstrated that diffraction could be described as reflection from parallel crystal planes. This relationship is known as Bragg's law, and for this work they were awarded the Nobel Prize in Physics in 1915 (Krastev and Exner, 2013).

In XRD analysis, each diffraction peak appears due to constructive interference of X-rays reflected by specific crystal planes in the material. Therefore, the position of each peak is related to the interplanar spacing of a particular set of lattice planes and serves as a fingerprint of the crystal structure (Holder and Schaak, 2019; Leonardi and Bish, 2020). Each crystal structure produces a unique diffraction pattern, allowing researchers to confirm whether the desired compound has formed. In general, broader diffraction peaks are associated with smaller crystallite size, whereas narrower peaks indicate larger crystallites, although peak broadening may also be influenced by lattice strain or structural defects (Holder and Schaak, 2019; Burlec *et al*., 2023). XRD analysis of nanoparticles that are synthesized using plant extracts is a relatively new application of the technique and is increasingly used to evaluate the structure and crystalline size of green-synthesized nanoparticles (Kumar *et al*., 2022; Burlec *et al*., 2023; Panchal *et al.,* 2025).

1.6.3. Scanning electron microscopy

Scanning electron microscopy (SEM) is a characterization technique that provides visual confirmation of the nanomaterial's surface morphology and particle distribution. Nanoparticles can have different surface morphologies, which vary between crystalline or amorphous, smooth or rough, and spherical, flat, cylindrical, tubular, conical or irregular shapes (Burlec *et al*., 2023). Morphological characteristics are important because they can influence the surface area, agglomeration behaviour, and functional properties of nanomaterials, including their antibacterial activity. Previous studies have shown that ZnO-NP appear as agglomerated structure with irregular shaped grains and GCN-NP exhibit layered, graphite-like structure with uniformly separated plate like sheets at a microscale (Panchal *et al*., 2025).

2. AIM OF THE THESIS

The aim of this thesis is to evaluate the antibacterial activity of a zinc oxide-doped graphitic carbon nitride nanohybrid using a green synthesis approach.

3. MATERIALS AND METHODS

3.1. Materials and bacterial isolates

The materials used in this study included ZnO/GCN-NH, pure plant extract (basil leaves), pure GCN-NP and pure ZnO-NP. The bacterial isolates used in this study were two Gram-positive bacteria, *Staphylococcus aureus* and *Streptococcus dysgalactiae*, and two Gram-negative bacteria, *E. coli* and *Pseudomonas aeruginosa*.

3.2. Biosynthesis and characterization of nanoparticles

Nanoparticles were synthesized using a green synthesis method. The biosynthesis of nanoparticles and plant extract were performed as described in the study of Panchal *et al*. (2025). The synthesized nanomaterials were characterized using UV-Vis, XRD and SEM (Panchal *et al*., 2025). Biosynthesis and characterization for this work was done by Priyanka Panchal.

3.3. Antibacterial activity testing

For antibacterial activity testing four bacterial isolates, Nutrient Agar (OXOID) plates were used. Those tests were performed in Microbiology Laboratory of Estonian University of Life Sciences. The antibacterial activity was evaluated using the disk diffusion and well diffusion methods. The bacterial suspension was adjusted to approximately 1×10^8 CFU/mL, corresponding to 0.5 McFarland standard.

3.3.1. Disk diffusion method

Sterile Petri dishes were filled with 30 mL of sterile nutrient agar and allowed to solidify. The agar surface was inoculated with 100 µL of bacterial suspension adjusted to 0.5 McFarland standard using a sterile spreader. Each plate was divided into four quadrants. Sterile paper disks (6 mm diameter) were soaked with 50 µL of each sample suspension corresponding to 5 mg of material, including the biosynthesized ZnO/GCN-NH, pure plant extract, pure GCN-NP, and pure ZnO-NP, and then placed onto the inoculated agar surface. The plates were incubated at 37 °C for 16 h. After incubation, the inhibition zones were measured in millimetres. All experiments were performed in duplicate.

3.3.2. Well diffusion method

Sterile Petri dishes were filled with 30 mL of sterile nutrient agar and allowed to solidify. The agar surface was inoculated with 100 µL of bacterial suspension adjusted to 0.5 McFarland standard using a sterile spreader. Each plate was divided into four quadrants. Wells (small holes approximately 5 mm) were created in the agar using a sterile cork borer. Subsequently, each well was loaded directly with 50 µL (5 mg) of the tested samples: biosynthesized ZnO/GCN-NH, pure plant extract, pure GCN-NP, and pure ZnO-NP. The plates were incubated at 37 °C for 16 h. After incubation, the plates were examined visually, and the zones of inhibition were measured in millimetres.

4. RESULTS

4.1. Evaluation of antibacterial activity

4.1.1. Antibacterial activity by disk diffusion

In the disk diffusion method, the inhibition zones of ZnO/GCN-NH were 17 mm for *S. aureus*, 14 mm for *S. dysgalactiae*, 17.2 mm for *E. coli* and 16 mm for *P. aeruginosa* (Table 1). Pure plant extract, pure GCN-NP and pure ZnO-NP showed inhibition zones of approximately 1–2 mm (Figure 5).

Table 1. Inhibition zones of biosynthesized zinc oxide-doped graphitic carbon nitride nanohybrid using disk and well diffusion methods

Bacterial isolates	Zone of inhibition (mm)	
	Disk diffusion	Well diffusion
Staphylococcus aureus	17	17.5
Streptococcus dysgalactiae	14	18.5
Escherichia coli	17.2	18.1
Pseudomonas aeruginosa	16	18

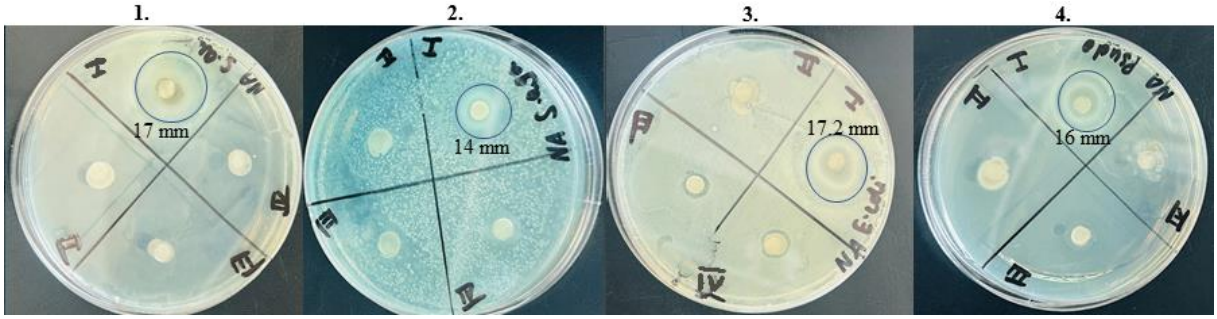

Figure 5. Disk diffusion method inhibition zones (mm) of *Staphylococcus aureus* (1), *Streptococcus dysgalactiae* (2), *Escherichia coli* (3), *Pseudomonas aeruginosa* (4) with zinc oxide doped graphitic carbon nitride nanohybrid (I), pure plant extract (II), pure graphitic carbon nitride nanoparticles (III) and pure zinc oxide nanoparticles (IV).

4.1.2. Antibacterial activity by well diffusion

In the well diffusion method, the inhibition zones of ZnO/GCN-NH were 17.5 mm for *S. aureus*, 18.5 mm for *S. dysgalactiae*, 18.1 mm for *E. coli*, 18 mm for *P. aeruginosa* (Table 1). Pure plant extract, pure GCN-NP, and pure ZnO-NP again showed inhibition zones of approximately 1–2 mm (Figure 6).

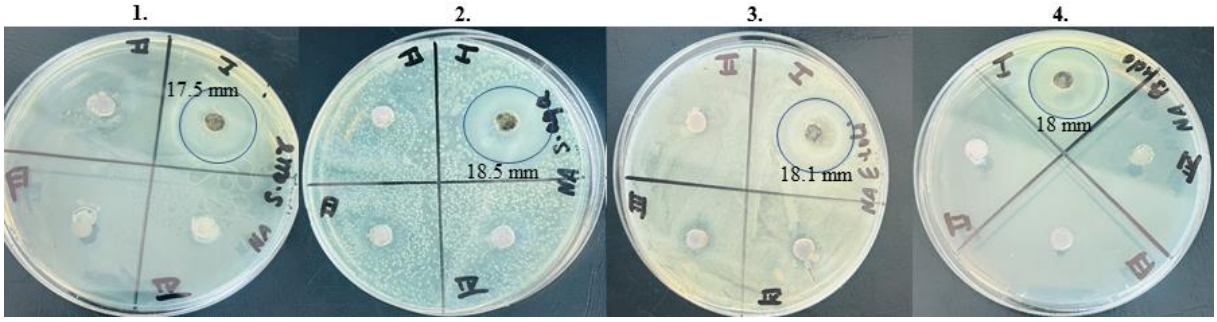

Figure 6. Well diffusion method inhibition zones (mm) of *Staphylococcus aureus* (1), *Streptococcus dysgalactiae* (2), *Escherichia coli* (3), *Pseudomonas aeruginosa* (4) with zinc oxide doped graphitic carbon nitride nanohybrid (I), pure plant extract (II), pure graphitic carbon nitride nanoparticles (III) and pure zinc oxide nanoparticles (IV).

5. DISCUSSION

According to the literature, different nanoparticles have shown promising results in the context of antibacterial activity including zinc and carbon nanoparticles (Dahiya *et al*., 2023; Panchal *et al*., 2025). This study demonstrates the antibacterial activity of ZnO/GCN-NH, pure plant extract, pure GCN-NP and pure ZnO-NP using disk and well diffusion methods. Both methods support that ZnO/GCN-NH showed antibacterial activity against the tested bacterial strains. In contrast, pure plant extract, pure GCN-NP and ZnO-NP showed little to no inhibitory effect against the tested Gram-positive and Gram-negative bacteria. However, ZnO/GCN-NH produced clear and consistent zones of inhibition. This indicates that the formation of the ZnO/GCN-NH structure substantially improves their antimicrobial performance.

The improved antibacterial activity of ZnO/GCN-NH can be explained by synergistic interactions between ZnO and GCN. One key factor is the enhancement of charge separation within the hybrid system. In pure GCN-NP, rapid recombination of photogenerated electron-hole pairs limits its photocatalytic and antibacterial efficiency. However, when combined with ZnO-NP, a heterojunction is formed that facilitates electron transfer between the two materials (Paul *et al*., 2020; Dahiya *et al*., 2023; Panchal *et al*., 2025). This reduces recombination and prolongs the lifetime of charge carriers, thereby increasing the generation of ROS. Increased ROS production is strongly associated with bacterial cell damage, including membrane disruption, protein oxidation, and DNA damage, ultimately leading to cell death (Correa *et al*., 2020; Alfei *et al*., 2024; Panchal *et al*., 2025).

The results obtained using agar disk diffusion and well diffusion methods were generally consistent, confirming the antibacterial potential of the ZnO/GCN-NH. However, slightly larger inhibition zones were observed in the well diffusion method. This difference can be attributed to the direct application of the liquid sample into the agar, which may allow better diffusion and interaction with bacterial cells compared to disk diffusion delivery. Despite this variation, both methods support that ZnO/GCN-NH showed antibacterial activity against the tested bacterial strains. Minor differences in susceptibility between bacterial strains may be related to differences in cell wall structure. Gram-positive bacteria have a thicker but more porous peptidoglycan layer, whereas Gram-negative bacteria possess an additional outer membrane that can act as a protective barrier (Al-Jumaili *et al*., 2017; Correa *et al*., 2020; Panchal *et al*., 2025).

The findings of this study are in agreement with previous research reporting antibacterial activity of ZnO/GCN nanomaterials. Panchal *et al*. (2025) reported that ZnO/GCN-NH showed inhibition zones against *S. aureus*, *S. dysgalactiae*, *E. coli* and *P. aeruginosa*, using disk and well diffusion methods. In that study, pure GCN-NP showed no antibacterial activity, while pure ZnO-NP showed only minimal inhibition. The stronger antibacterial effect of ZnO/GCN-NH was linked to increased ROS generation, improved separation of electron-hole pairs and the synergistic interaction between ZnO-NP and GCN-NP (Panchal *et al*., 2025). Therefore, the results of the present study support previous findings that the formation of ZnO/GCN-NH improves antibacterial activity compared with the individual components.

Despite these promising findings, certain aspects of this study provide opportunities for further investigation. The current work utilized inhibition zone measurements, which offer a reliable initial assessment of antibacterial activity. Future studies could complement these findings with more quantitative approaches, such as minimum inhibitory concentration and minimum bactericidal concentration assays, to provide a more comprehensive evaluation of antimicrobial potency. In addition, while the present study focused on antibacterial efficacy, further research into the interaction of ZnO/GCN-NH with mammalian cells would be valuable to confirm their biocompatibility and support their potential biomedical or veterinary applications. Given that ROS play a role in antimicrobial activity, understanding their effects in biological systems will help optimize both efficacy and safety. Another important area for future development is the standardization of testing methods for nanoparticle-based antimicrobials. Currently, the absence of widely accepted frameworks comparable to established systems such as EUCAST can make direct comparison between studies challenging. The development of such standardized approaches would greatly enhance reproducibility and facilitate broader application of these materials.

Overall, the findings of this study demonstrate that green-synthesized ZnO/GCN-NH exhibit promising antibacterial activity against both Gram-positive and Gram-negative bacteria. Their improved performance compared to individual components highlights the potential of nanohybrid design as an effective strategy for enhancing antimicrobial activity. With further investigation into their mechanisms, safety, and standardized evaluation, these materials show strong potential for future applications in antimicrobial research.

CONCLUSIONS

The aim of this thesis was to evaluate the antibacterial activity of a biosynthesized ZnO/GCN-NH. The aim was achieved, as the ZnO/GCN-NH produced measurable inhibition zones against both Gram-positive and Gram-negative bacterial strains in disk diffusion and well diffusion assays. In comparison, pure plant extract, pure GCN-NP and pure ZnO-NP showed only limited inhibition under the same experimental conditions. These results indicate that the formation of the ZnO/GCN-NH improved antibacterial activity compared with the individual components. Therefore, biosynthesized ZnO/GCN-NH may represent a promising complementary antimicrobial material. However, further studies are needed to evaluate their cytotoxicity, clarify their antibacterial mechanisms, and confirm their activity using standardized quantitative methods such as minimum inhibitory concentration and minimum bactericidal concentration testing.

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I would like to thank my closest classmates, with whom I have shared the past six years. Through both challenges and achievements, our mutual support, understanding and encouragement have been invaluable.

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ÜLDKOKKUVÕTE

Antibakteriaalne aktiivsus biosünteesitud grafiitse süsiniknitriidi nanohübriidil

Antimikroobne resistentsus on bakterite võime ellu jääda neile loodud ebasoodsates tingimustes kasutades ravimeid, mis on mõeldud nende kasvu pidurdamiseks või nende täielikuks hävitamiseks. Tegemist on globaalse probleemiga, mis on tingitud liigsest ja sihiotstarbetust antibiootikumide kasutamisest nii humaani kui ka veterinaaria sektoris (Palma jt, 2020; Ahmed jt, 2024). Bakteritel on erinevaid ellujäämise strateegiaid. Näiteks on neil võime moodustada biokile tänu millele ei saa ravimid toimida ja see vähendab nende antibakteriaalset aktiivsust (Ahmed jt, 2024). Antud probleemi lahendamiseks Euroopa Liit 2006 aastal võttis vastu otsuse, et keelustada antibiootikumide kasutamine loomadel kasvu soodustamise eesmärgil (Palma jt, 2020). Aastal 2019 seostati antimikroobse resistentsusega ligikaudu viis miljonit surmaga lõppenud juhtumit. Kui miski ei muutu, siis aastaks 2050 on eeldatavasti juhtumite arv kahekordistunud. Tulevikus võivad levinud haigused ja väikesed vigastused olla keeruliselt ravitavad või isegi muutuda surmavaks (Ahmed jt, 2024). Paljud farmaatsiaettevõtted on suunanud oma väljavaated alternatiivsete meetodide suunas. Nanoosakesed on ühed populaarsemad alternatiivid millel on tuvastatud antibakteriaalsed omadused ja mida uuritakse järjest rohkem (Sánchez-López jt, 2020; Correa jt, 2020). Nanoosakeseks defineeritakse osakest, millel on vähemalt üks mõõde vahemikus 1-100 nanomeetrit (Murthy, 2007; Fortune jt, 2025). Nanoosakeste omadused sõltuvad muu hulgas nende suurusest, morfoloogiast, koostisest, pinnamodifikatsioonist ja eripinnast (Correa jt, 2020). Nanoskaalas materjalidel võivad esineda omadused, mis erinevad sama materjali suurematest vormidest, näiteks muutunud keemiline reaktiivsus, energia neeldumine ja bioloogiline liikuvus (Murthy, 2007). Tänu nanoosakeste antibakteriaalsetele toimetele on need juba kasutuses kliinilises praktikas. Näiteks lisatakse neid haavageelidesse, plaastritesse ja spreidesse, et vältida bakterite kasvu ja soodustada vigastuste kiiremat paranemist (Fortune jt, 2025). Samal ajal tuleb jätkata uuringutega, et välja selgitada nende täpsem toime rakkudele. Nanoosakesi võib sünteesida erinevatest materjalidest, sealhulgas metallidest, metallioksiididest, polümeeridest ja süsinikupõhistest materjalidest (Correa jt, 2020; Holmannova jt, 2022). Metalseid nanoosakesi on laialdaselt uuritud, sest mitmed neist on näidanud antibakteriaalset aktiivsust erinevate bakteriliikide suhtes (Sánchez-López jt, 2020; Correa jt, 2020). Metalsed nanoosakesed avaldavad laiaulatuslikku antibakteriaalset toimet mitme mittespetsiifilise mehhanismi kaudu. Võivad toimida otse bakteri raku pinnaga, kahjustada membraani, tekitada reaktiivseid hapnikuühendeid, vabastada metalliioone ja kahjustada raku sisemisi struktuure nagu DNA ja

proteiinid. Mitmete metalsete nanoosakeste probleemiks on nende pikaajalise kasutamise toksilised efektid teistele keharakkudele. Seega on hakatud uurima rohelise sünteesi meetode ja mittemetalseid nanoosakesi. Antud töö eesmärgiks oli uurida süsiniku nanoosakesi kasutades biosünteesi, mis on keskkonna sõbralikum ja vähem kulukam meetod osakeste omaduste uurimiseks. Süsinikul baseeruvad nanoosakesed koosnevad suuresti süsiniku aatomitest. Need on teadlastele huvi pakkunud oma väikeste mõõtmete, suure eripinna, struktuurse stabiilsuse ja kohandatava pinnakeemia omaduste poolest (Dizaj jt, 2015; Al-Jumaili jt, 2017). Antud töö eesmärgiks oli tuvastada ZnO/GCN-NH antibakteriaalsed omadused ja neid võrrelda taimse ekstrakti, puhta GCN-NP ja puhta ZnO-NP-ga. Nanoosakeste sünteesiks kasutati rohelist sünteesi ja nende iseloomustamiseks UV-Vis, XRD ja SEM meetodeid, mis olid eelnevalt läbi viidud nagu Panchal jt (2025) töös kirjeldati. Selleks, et hinnata nanoosakeste antibakteriaalset aktiivsust kasutati disk diffusion ja kaevu difusiooni meetodi. Nutrient Agar plaatidele peale kultiveeriti Gram-positiivsed bakterid *Staphylococcus aureus* ja *Streptococcus dysgalactiae* ning Gram-negatiivsed bakterid *Escherichia coli* ja *Pseudomonas aeruginosa*. Tulemused näitasid, et Gram-positiivsete ja Gram-negatiivsete bakterite vahel ei esinenud olulist erinevust, kuna mõlema rühma bakteritel olid inhibeerimistsoonid selgelt väljendunud ja sarnased (joonised 5 ja 6). Siiski kaevu diffusion meetodi puhul olid inhibeeritud alad ZnO/GCN-NH puhul suuremad kui disk diffusioni puhul (tabel 1). See võib olla tingitud sellest, et kaevu diffusion meetodil on proov otsekontaktis agariga. Võrdluseks olid puhta taimse ekstrakti, puhta GCN-NP ja puhta ZnO-NP inhibeeritud alad olid väga tagasihoidlikud. Tulemused viitavad sellele, et ZnO-NP ja GCN-NP kombineerimisel on nende antibakteriaalsed omadused tugevamad. Edasised uuringud ZnO/GCN-NH on vajalikud, kuna nende ohutust imetajate rakkudele pole tõestatud, seega vajaks antud teema põhjalikumat uurimist.

USE OF ARTIFICIAL INTELLIGENCE (AI)

In this work ChatGPT- 5.1 was used to support literature searching, article analysis, language refinement, and paraphrasing during the writing process. It was also used to produce the initial draft content for the abstract, introduction and conclusion part of the thesis.
Grammarly was used to improve the use of English language.

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