

T.R.
BOLU ABANT İZZET BAYSAL UNIVERSITY
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**THE BIOLOGICAL PREPARATION OF NANOPARTICLES
FROM PLANT EXTRACT AND INVESTIGATION OF THEIR
ANTIBACTERIAL ACTIVITIES ON PATHOGENIC
BACTERIA ISOLATED FROM PATHOGENIC SOURCES**

MASTER OF SCIENCE

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BOLU, TEMMUZ - 2023

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THE BIOLOGICAL PREPARATION OF NANOPARTICLES FROM PLANT EXTRACT AND INVESTIGATION OF THEIR ANTIBACTERIAL ACTIVITIES ON PATHOGENIC BACTERIA ISOLATED FROM PATHOGENIC SOURCES submitted by **Muhja SATTAR HAFEDH AL-KARKHĪ** and defended before the Examining Committee Members listed below in partial fulfillment of the requirements for the degree of **Master of Science in Department of Biology, Institute of Graduate Studies of Bolu Abant İzzet Baysal University in 28.07.2023** by

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ABSTRACT

THE BIOLOGICAL PREPARATION OF NANOPARTICLES FROM PLANT EXTRACT AND INVESTIGATION OF THEIR ANTIBACTERIAL ACTIVITIES ON PATHOGENIC BACTERIA ISOLATED FROM PATHOGENIC SOURCES

MSC THESIS

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Green synthesis is known as the process of combining nanoparticles using plants or their metabolites and focuses on environmentally friendly applications. In these green strategies, plant metabolites and natural substances are used to facilitate the formation of nanoparticles, and they may have applications in the pharmaceutical industry and other fields. Allium plants are known for their interesting biological properties and as producers of abundant secondary metabolites. In this thesis, we explained the basic green synthesis techniques with a focus on metal oxide (MgO) nanoparticles. MgO nanoparticles were extracted using the leaves of the leek plant. In the microscopic characterization, the average grain size of MgO NPs was determined as 44.34 nm and their shape was found to be cylindrical. Trials were conducted to determine the antibacterial effects of MgO nanoparticles at different concentrations (100, 50 and 25 µg/ml) on *Klebsiella pneumoniae* gram (-) bacteria. The highest MgO nanoparticle effect was obtained at a concentration of 100 µg/ml and the inhibition zone diameter was measured as 17 mm. As the MgO nanoparticle concentration decreased (25 µg/mL), the inhibition zone diameter (13.56 mm) also decreased. In addition, when MgO nanoparticles were examined for antibacterial activity against a gram-negative bacteria, *Klebsiella pneumoniae*, they were shown to be effective at minimum inhibitory concentration (MIC). The antibacterial effect of MgO nanoparticles was the highest at minimum inhibition concentrations (0.8 µg/ml) and the inhibition zone diameter was measured as 36 mm. When examined in terms of antioxidants, green synthesized MgO nanoparticles were found to have antioxidant properties and the highest antioxidant activity (88.7%) was obtained at the highest MgO nanoparticle concentration (100 g/mL). The antibacterial effects of green synthesized MgO nanoparticles from leek leaf extract were stronger when compared to antibiotics.

KEYWORDS: Green synthesis, Nanotechnology, Antibacterial, MgO, Plant Extract.

ÖZET

**BİTKİ ÖZÜTLERİ KULLANILARAK NANOPARTİKÜLLERİN
BİYOLOJİK OLARAK HAZIRLANMASI VE PATOJENİK
KAYNAKLARDAN İZOLE EDİLEN PATOJENİK BAKTERİLER
ÜZERİNDEKİ ANTİBAKTERİYEL AKTİVİTELERİNİN
ARAŞTIRILMASI
YÜKSEK LİSANS TEZİ
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Yeşil sentez, bitkileri veya metabolitlerini kullanarak nanopartiküllerin birleştirilme süreci olarak bilinirler ve çevre dostu uygulamalara odaklanır. Bu yeşil stratejilerde, bitki metabolitleri ve doğal maddeler, nanopartiküllerin oluşumunu kolaylaştırmak için kullanılır ve bunlar farmasötik endüstrisi ve diğer alanlarda uygulamalara sahip olabilir. *Allium* bitkileri ilgi çeken biyolojik özellikleri ile ve bol miktarda sekonder metabolit üreticisi olarak bilinmektedir. Bu tezde, temel yeşil sentez tekniklerini, metal oksit (MgO) nanopartiküllere odaklanarak açıkladık. MgO nanopartikülleri pırasa bitkisinin yaprakları kullanılarak ekstrakte edilmiştir. Mikroskopik olarak yapılan karakterizasyonda MgO NPs ortalama tane büyüklüğü 44.34 nm olarak belirlenmiş ve şekillerinin ise silindirik olduğu görülmüştür. Farklı konsantrasyonlardaki (100, 50 ve 25 µg/ml) MgO nanopartiküllerinin *Klebsiella pneumoniae* gram (-) bakterileri üzerine antibakteriyel etkilerini belirlemek amacıyla denemeler yapılmıştır. En yüksek MgO nanopartikül etkisi 100 µg/ml konsantrasyonunda elde edilmiş ve inhibisyon zon çapı 17 mm olarak ölçülmüştür. MgO nanopartikül konsantrasyonu azaldıkça (25 µg/ml) inhibisyon zon çapı (13.56 mm) da azalmıştır. Ayrıca MgO nanoparçacıkları gram-negatif bir bakteri olan *Klebsiella pneumoniae*'ye karşı antibakteriyel etkinlik bakımından incelendiğinde, minimum inhibitör konsantrasyonda (MIC) etkili oldukları gösterilmiştir. MgO nanopartiküllerin minimum inhibisyon konsantrasyonlarında (0.8 µg/ml) antibakteriyel etkisi en yüksek olmuş ve inhibisyon zon çapı 36 mm olarak ölçülmüştür. Yeşil sentezlenen MgO nanopartiküllerin antioksidan özelliğe sahip oldukları görülmüş ve en yüksek antioksidat aktivite (88,7 %) en yüksek MgO nanopartikül konsantrasyonunda (100 g/ml) elde edilmiştir. Pırasa yaprağı ekstraktından yeşil sentezlenmiş MgO nanopartiküllerinin antibakteriyel etkileri antibiyotiklerle karşılaştırıldığında daha güçlü olmuştur.

ANAHTAR KELİMELELER: Yeşil Sentez, Nanopartikül, Antibakteriyel, MgO, Bitki Özütü

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LIST OF ABBREVIATIONS AND SYMBOLS

NP	: Nanoparticle
MgO	: Magnesium oxide
AFM	: Aromatic force microscopy
ROS	: Reactive oxygen species
FE-SEM	: Field Emission Scanning Electron Microscopes
ESBL	: Extended-spectrum lactamases
ICU	: Critical care units
CRKP	: Carbapenem-resistance <i>K.pneumoniae</i>
LLE	: Leek leaf extract
UTI	: Urinary tract infection
DDW	: Deionized –distilled water
BHI	: Brain heart infusion
MDR	: Multi drug resistance
MIC	: Minimal inhibitory concentration
%	: Percent
µg/L	: Microgram/liter
gr	: Gram
L	: Liter
Mol	: Mole
°C	: Celsius degrees
DNA	: Deoxyribonucleic acid
DPPH	: 1,1-Diphenyl-2-picryl-hydrazyl
FESEM	: Field emission scanning electron microscopy
<i>K. pneumoniae</i>	: <i>Klebsiella pneumoniae</i>
ROS	: Reactive oxygen species
AFM	: Aromatic force microscopy

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1. INTRODUCTION

1.1 Nanotechnology and Green Synthesis

Green technology is being developed at the fastest possible pace by science and technology. Nanotechnology is one of the most important areas used to form and utilize materials and their structural properties. In the twenty-first century, nanotechnology developed as a scientific innovation (Abe et al., 2018a). NP is a zero-dimensional nanoparticle; in contrast to one-dimensional and two-dimensional nanomaterials, nanoparticles may be distinguished from microparticles, and with this tiny size, they are used in a variety of branches of science (physical, chemical, and biological). They differ from bulk equivalents in terms of size, chemical reactivity, movement, energy absorption, and so on (Janthima et al., 2018).

Figure 1.1 compares nanosize to other sizes to help you comprehend the size range. Although Richard Zsigmondy coined the word "nanometer" in 1925, Richard Feynman is widely regarded as the undisputed founder of nanotechnology (Bukhari et al., 2021).

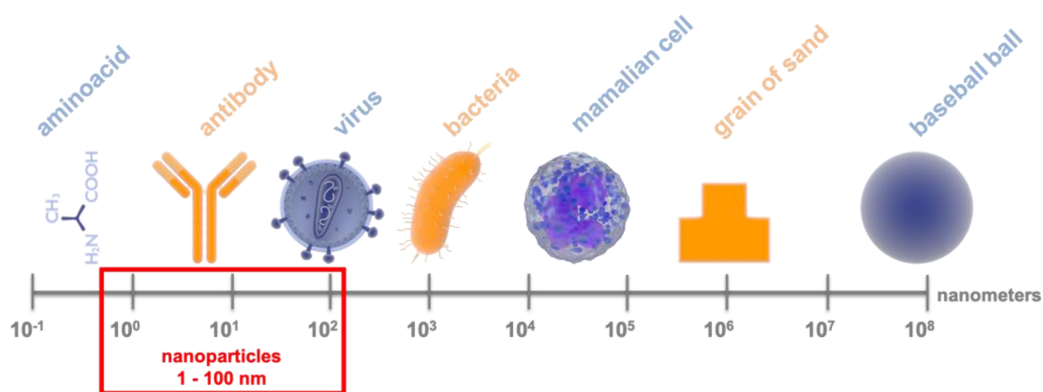


Figure 1.1. The sizes of nanoparticles compared to other objects are presented on a logarithmic scale for comparison (Bukhari et al., 2021).

The size, shape, and surface area of the nanoparticles are all affected by the synthesis process as well as the experimental situations. Manipulation of the synthesis conditions can result in shape- and size- controlled NPs (Abdallah et al., 2019a). Certain NPs have optical, magnetic, or antibacterial properties that are unique to them, but not all types of nanoparticles do, and such nanoparticles have demonstrated excellent uses in a variety of sectors (Mohammadi Ziarani et al., 2019).

Modern studies have demonstrated that metal oxides and metal nanomaterials have shown significant positive effects in many areas (Shah et al., 2015). Metal oxide NPs have attracted attention because of their increasing applications in some fields such as cosmetics, electronics, materials science, catalysis, the environment, energy, and medicinal usage (Hussein et al., 2021). Nowadays, nanotechnology has become a widespread and fundamental technological area.

Nanotechnology is the scientific study of the forming, manipulation, and use of materials with nanometer-scale dimensions. NPs are commonly used in a wide range of applications, ranging from the removal of heavy metals, dyes, and antibiotics from water sources to biomedical fields such as site-specific medicinal delivery and tumor cell damage (Bhuiyan et al., 2020).

There is a growing demand to develop eco-friendly NP production without harmful chemicals (Sahoo, 2010). Green synthesis offers several advantages over chemical and physical synthesis methods, including cost-efficiency, environmental friendliness, and the absence of high pressure, energy, heat, or harmful compounds (Rasmussen et al., 2010). Plants are known as chemical factories in nature that are cheaper and environmentally friendly. Plants have shown a remarkable ability to detoxify and remove heavy metals, which may help reduce pollution and related problems (Rasmussen et al., 2010), since trace amounts of these heavy metals are also dangerous. The synthesis of NPs from plant extracts has several advantages compared with other biosynthetic methods such as bacteria, fungi, actinomycetes, and algae. In the production of green NPs,

plant extracts are used as reducing and capping agents, eliminating the requirement for hazardous reducing chemicals (Abe et al., 2018b) . The utilization of organic compounds derived from plant extracts enhances the stability of green synthetic nanoparticles. Plant-mediated nanoparticles are a cost-effective alternative to bacteria-mediated nanomaterials (Pal Singh et al., 2020).

Relevant materials in this technology are those whose nanoscale architectures display novel phenomena and functions (Mirzaei & Davoodnia, 2012). When nanoparticles are reduced in size, it can lead to significant changes in their chemical, mechanical, electrical, structural, morphological, and optical properties at the nanoscale. These altered features enable the NPs to interact with cell molecules in a unique manner; as a result, the NPs are able to physically penetrate the inner structures of cells (Pillai et al., 2020) . Nanoparticles exhibit a wide range of morphologies and demonstrate significant antibacterial activity against various bacterial species. This area of research has been extensively investigated by numerous scientists (Pillai et al., 2020) and (D. Zhang et al., 2020). In recent years, silver, gold, copper, copper oxide, zinc oxide, magnesium, and magnetite-based metal and metal oxide nanoparticles have also been used in medicine, dentistry, pharmacy, and biology (Salgado et al., 2011). MgO (magnesium oxide) is recognized as safe for both humans and animals. There are several methods available for producing nano-sized MgO particles, including Laser Deposition, Sol-Gel Synthesis, Hydrothermal Synthesis, Aerosol Synthesis, and Chemical Gas Deposition (Ankri & Mirelman, 1999) .

1.2 Strategies for Nanoparticle Synthesis

The production of nanoparticles can be achieved through two main methods: the top-down technique and the bottom-up approach, as shown in Figures (1.2) and (1.3). The bottom-up technique starts at the molecular level and carefully controls the molecule structure to create larger systems. Top-down formation of NPs employs bigger particles to control their assembly (Stover, 2004) .

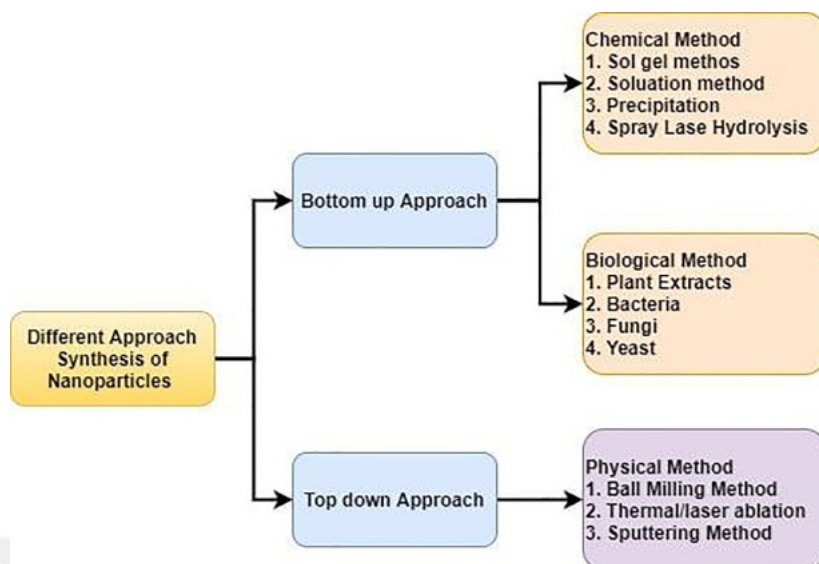


Figure 1.2. Synthesis of nanoparticle approaches (Stover, 2004).

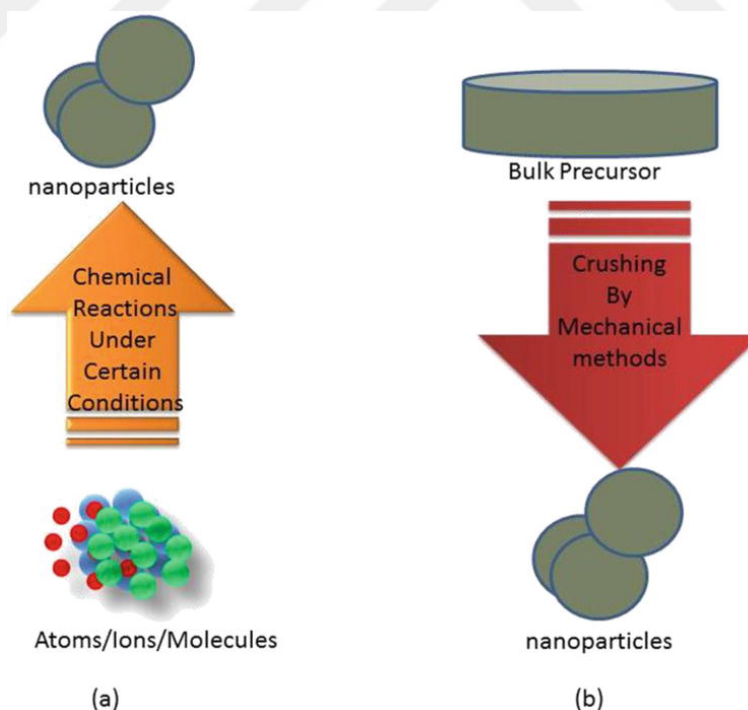


Figure 1.3. Two primary methods for producing nanoparticles: (a) bottom-up; (b) top-down approaches (Ijaz et al., 2020)

Green synthesis, which adopts a bottom-up approach, shares similarities with chemical reduction processes. However, in green synthesis, expensive

chemical reducing agents are replaced with extracts obtained from natural sources like tree leaves, crops, or fruits. These natural extracts are employed for the synthesis of metal or metal oxide nanoparticles. (Shyam et al., 2020).

The manufacture of nanoparticles using biological agents including bacteria, algae, fungi, and plant extracts has drawn a lot of interest in the field of nanotechnology during the past several decades. A "bottom-up" approach is used to create green NPs, with most of the processing methods being chemical and biological. The biological method offers an environmentally benign way to create nanoparticles and is referred to as an alternative to chemical and physical procedures. Additionally, no costly, dangerous, or harmful materials are needed for this procedure. Green technology has a variety of features that are highly stable and have acceptable measurements as a consequence of their one-step synthesis. The production of metal NPs from the appropriate metal ions is schematically shown in Figure 1.4. Biological approaches have been increasingly popular in recent years (Pourali et al., 2013). Proteins, sugars, terpenoids, polyphenols, alkaloids, phenolic acids, and other compounds may also help to reduce metal ions into NPs and preserve their stability after they have done (Gopinath et al., 2012).

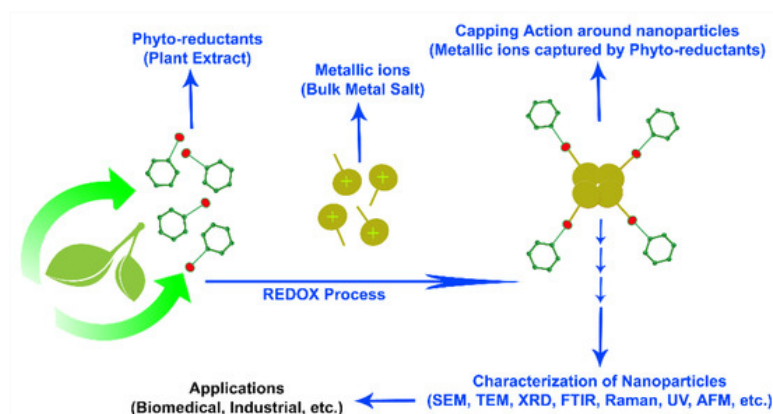


Figure 1.4. A diagram outlining the process of green in vitro nanoparticle manufacturing (Na et al., 2011)

Plant extracts play an important role in the synthesis of metal NPs. In this synthesis, polyphenols found, for example, in red grape pomace, tea waste, and leek leaf, are assumed to be the primary active ingredient. Green NP synthesis looks superior to common approaches because it is rapid, inexpensive, readily

repeatable, and produces more stable compounds. The benefits and drawbacks of the green synthesis process are shown in Table 1.1.

Table 1.1. The advantages and disadvantages of the green synthesis of NPs

Advantage	Disadvantage
Clean, nontoxic, compatible, and eco-friendly method for synthesis nanoparticles	Culturing of micro-organisms is time consuming
Cost effective, safe and sustainable	Achieving precise control over the size, shape, and crystallinity of nanoparticles can be challenging.
Bacteria easy to handle and can be easy manipulated	The particles exhibit a lack of uniformity in size distribution, and the production rate is relatively slow.

Although microorganisms are capable of producing nanoparticles, their rate of synthesis is low, and they are more limited in terms of suitable shapes and sizes than plant-based processes. Plants are famous across the world as factories that produce nanoparticles in a sustainable manner (Mastuli et al., 2014) .

The capacity of some bacteria, fungi, and yeast to create non-toxic noble nanoparticles is widely recognized. Due to the medium cost and strict aseptic requirements, the microbially mediated production of NPs is not commercially viable. Thus, research into plants as potential bio-factories has focused more on the biological synthesis of NPs. As a result, the possibility of using plant systems as a bioreactor for the production of metal NPs without the use of dangerous chemicals has been considered (Xu et al., 2017).

In order to lessen the hazardous character of the product, organisms such as plants, bacteria, fungi, and algae are often substituted for chemical solving and stabilizing agents (Khalid et al., 2019).

Large amounts of antioxidants, such as methylxanthines, phenolic acids, flavonoids, and saponins, are thought to be present in plant extracts. Despite the

awareness, plant extracts have far higher concentrations of the active chemical components than do the phytochemical properties of antioxidants (Aldalbahi et al., 2020).

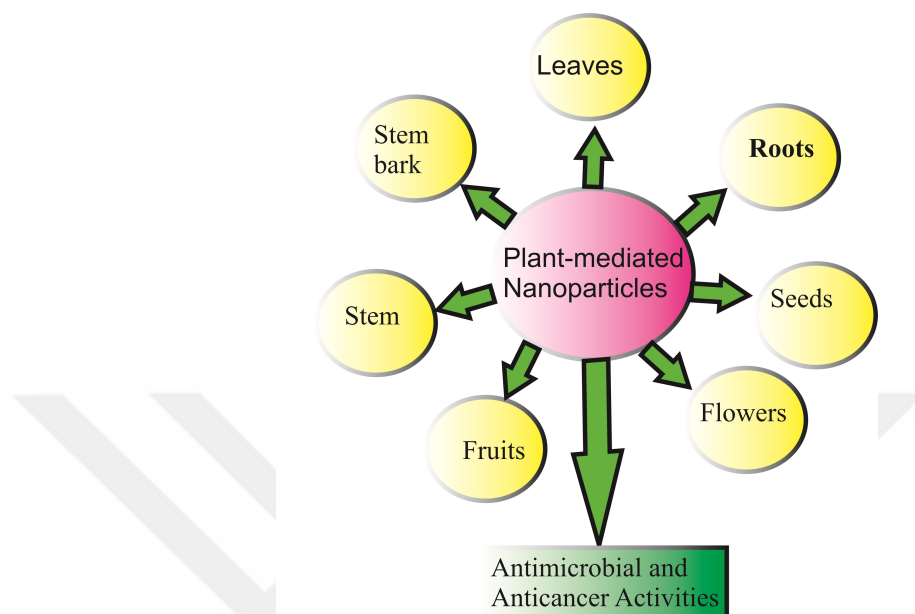


Figure 1.5. Antimicrobial and anticancer activities of plant-based NPs.

1.3 Magnesium Oxide:

Nanoparticles of MgO possess a large band gap, physicochemical features, and a high specific area. Due to these characteristics, it is stated that they exhibit antibacterial capabilities. Moreover, when MgO nanoparticles interact with oxygen in the bacterial cell wall, they tend to generate superoxide. The highly reactive superoxides produced quickly break down the phospholipids and cell wall of the bacterium. MgO nanoparticles can also be found in ceramics, batteries, supercapacitors, electronic parts, catalysts, and fuel cells (Kim et al., 2007).

MgO has several morphological forms, including platelets, spheres, and rods. They are ideal for showcasing novel nanomaterial properties since they have the shapes of flowers, stars, cubes, and needles (Figure 1.6) (Athreya et al., 2020).

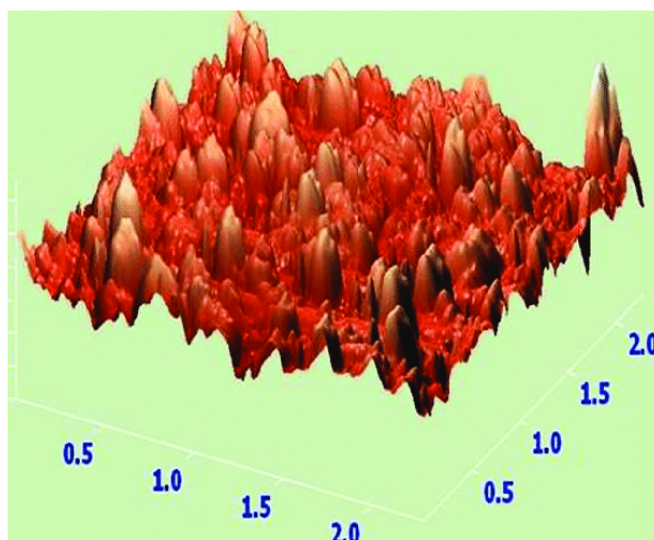


Figure 1.6. AFM images for MgO NPs

An increasing number of studies have focused on MgO nanoparticles. Energy cells, photocatalysis, antibacterial agents, sensors, and many more products have all employed MgO. Another new area of investigation is the novel use of MgO NPs in biomedical engineering. MgO is used to create novel cancer therapies, regenerate tissue, cover implants, perform bioimaging, and heal wounds. According to research, the biomolecules included in the plant extract are what induce the decrease of Mg^{2+} ions to the MgO valence state. The precise concentration of phytochemicals in the biological substrate derived from plant sources and the bioprocess used to decrease metallic NPs are therefore difficult to quantify. Theoretically, MgO NPs are synthesized through the interaction of a biological substrate with Mg^{2+} salt, resulting in the production of reduced Mg metal or a complex with the metal (M. et al., 2021). They found that the physiologically active chemicals contained in the leaf extract are what change MgO NPs made from the appropriate precursor (Altemimi et al., 2017).

1.4 Magnesium Oxide Nanoparticle Applications

1.4.1 Utilization of Energy

Recent fossil fuel depletion has led to climate change, an increase in air pollution, and an ensuing rise in sea level. Therefore, it is crucial to find a substitute. Finally, there is a place for fuel, solar cells (Rotti et al., 2023a), and batteries as fossil fuel substitutes. Additionally, they will produce hydrogen that is

a fantastic fuel and an improved replacement for carbon-based byproducts. Magnesium performs the function of hydrogen storage more effectively than other metals (Das et al., 2018).

1.4.2 Applications in the Environment

Xanthomonas oryzae, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Salmonella enterica*, *Candida albicans*, and *Aspergillus niger* are just a few examples of the microbes that MgO NPs effectively inhibit. (Saied et al., 2020) (Shao et al., 2018) (Masoud et al., 2018). Against the human leukemia cell lines HL-60 and HeLa, MgO NPs exhibit potent anticancer and antioxidant activities (Wang et al., 2019) (Abdallah et al., 2019b). Because of their huge active surface area, strong reactivity, and specificity, MgO NPs are effective at removing organic dyes like congo red, methyl orange, reactive black dye, methylene blue, and methyl red from calcareous stone artifacts (Hussain et al., 2016) (Cai et al., 2018). MgO NPs have been successfully employed by Srivastava as an adsorbent to remove heavy metals from contaminated water. Additionally, MgO NPs have demonstrated exceptional seed germination abilities for the species of chickpea, tomato, mung bean, green peach, maize, peanut, and wheat (Makhluf et al., 2005) (Das et al., 2018).

1.4.3 MgO as a Catalyst

MgO is commonly employed in heterogeneous catalysis for various chemical processes, including the benzylation of aromatics (Umaralikhani & Jamal Mohamed Jaffar, 2018), dehydrohalogenation of halogenated hydrocarbons (Dobrucka, 2016), pyranopyrazole synthesis and the synthesis of its derivatives, oxidative coupling of methane, and epoxidation of alkenesemic and electronic properties.

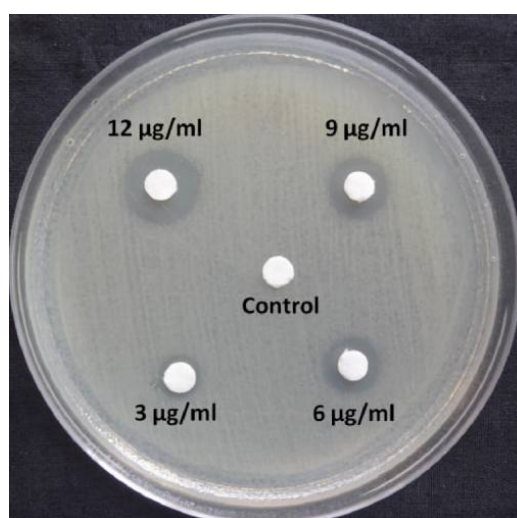
1.4.4 Mechanism of MgO Nanoparticles in Antibacterial Activity

The precise antibacterial mechanism of MgO NPs remains uncertain. Several mechanisms have been proposed to elucidate their antibacterial effects, including the generation of reactive oxygen species (ROS), the interaction

between NPs and bacteria leading to cellular damage, and an alkaline effect (Haldorai & Shim, 2014) . It has been noted that Magnesium NPs exhibit effective antibacterial properties owing to their huge surface area, which allows for more interaction with microorganisms. Depending on their size, the nanoparticles can pass through the bacterial cell membrane or stick to its surface (Moorthy et al., 2015).

The antibacterial activity of all MgO NPs was shown to decline as particle size decreased, and they were all determined to be especially damaging to the bacterial strains (Figure 1.8). When NP size approached the sub-10 nanometer range, this effect was dramatically amplified, with MgO NPs with a 5 nm size exhibiting the quickest bactericidal action compared to those with a 7 nm and 10 nm size at their respective minimum bactericidal concentrations. NPs interact with sulfur-containing proteins found in the bacterial membrane as well as molecules like DNA that contain phosphorus. Magnesium has a greater tendency to attach to biomolecules in the cell that contain phosphorus and sulfur (Suresh et al., 2014). Figure 1.7 shows FE-SEM scans of Mg-NPs-treated *E. coli*.

Picture 1.1 After being exposed to 6 g/mL Mg NPs, *E. coli* showed alterations in its morphology. A detailed description of the cell membrane rupture that results in bacterial cell death is displayed.



Picture 1.2. The susceptibility of *E. coli* to MgO nanoparticles is evaluated using the disc diffusion test on Muller-Hinton agar medium (Rahman et al., 2019).

The antimicrobial efficacy of MgO nanoparticles against *E. coli* was observed to exhibit a direct correlation with the concentration of MgO NPs with increasing concentrations (up to 60), there was a corresponding increase in bacterial inactivation, indicating that higher concentrations of MgO NPs resulted in more significant antimicrobial activity against *E. Coli* (Rathore & Tarafdar, 2015).

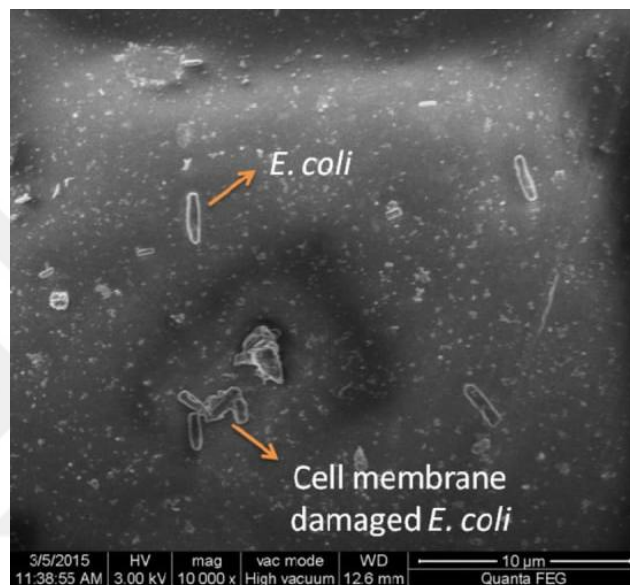


Figure 1.7. FE-SEM images of *E. coli* treated with MgO NPs (6 g/mL)(Jhansi et al., 2017) .

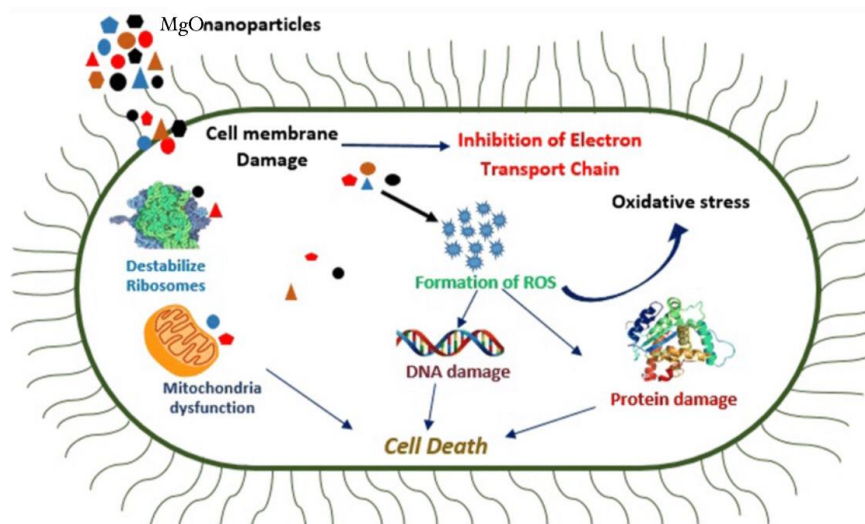


Figure 1.8. Proposed mechanism of action of MgO nanoparticles against bacterial growth/proliferation (Rathore & Tarafdar, 2015) .

1.5 Resistance in Bacteria, Especially *Klebsiella pneumoniae*

The most significant member of the Enterobacteriaceae genus *Klebsiella* is the rod-shaped, gram-negative bacterium known as *Klebsiella pneumoniae*. Infections acquired in the community and hospitals are significantly impacted by it (Aramendía et al., 2003). When *K. pneumoniae* was discovered to be resistant to a number of lactam antibiotics in 1983, it was discovered that this resistance was caused by the bacterium's production of extended-spectrum lactamases (ESBLs) (Sajadi et al., 2012). Humanity's battle against infectious illnesses is well known. Antibiotics have inspired confidence that illnesses may be managed and avoided. In the underdeveloped world, infections remain the biggest cause of death (Jeevanandam et al., 2015). This is related to the advent of new illnesses, the reemergence of illnesses that had been suppressed earlier, and, more specifically, the development of antibiotic resistance. Antimicrobial resistance is widely recognized as a significant challenge in the management of microbial infections both within healthcare facilities and in the general community, and it seems inevitable for nearly every new antibiotic (Babaie & Sheibani, 2011). The purpose of this study is to obtain new ways to reduce the resistance of bacteria by applying MgO NPs. For this purpose, it is necessary to understand the fundamental architecture of a bacterial cell (Rai et al., 2009).

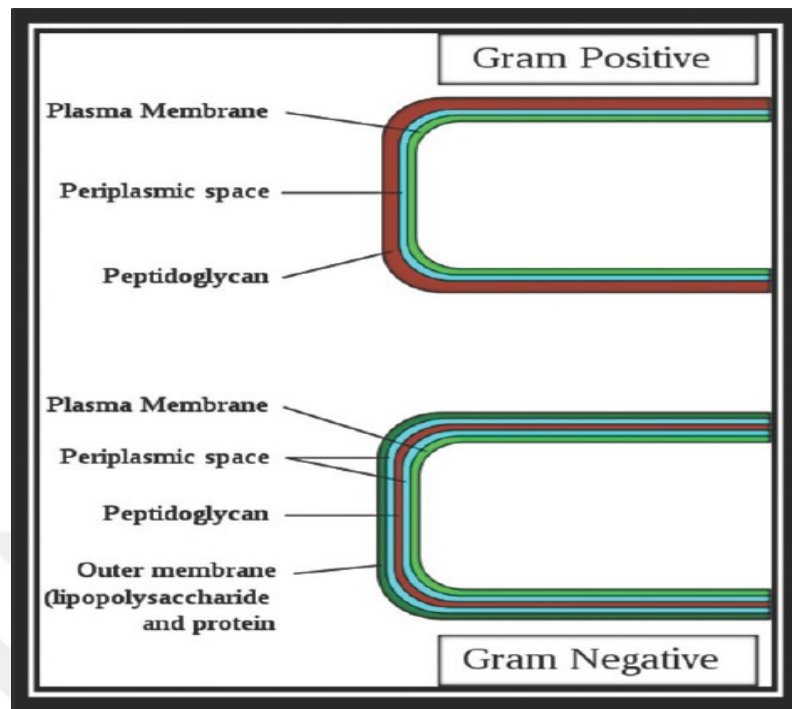


Figure 1.9. Structure of the bacterial cell envelope (Agnihotri et al., 2014).

Bacterial cell walls are made of long sugar polymers and peptidoglycans as seen in Figure 1.10. The activity of transglycosidases results in the cross-linking of the glycan strands. In the peptidoglycan, the peptide chains extend from the polymer's sugars to create cross-linkages between the peptides (Park, 2014). In the presence of penicillin-binding proteins, glycine residues (PBPs) cross-link the D-alanyl-alanine portion of peptide chains. The PBP is ineffective in the production of peptidoglycans due to its interaction with the lactamase ring. When the peptidoglycan layer is damaged, bacteria lyse (Feng et al., 2000). Glycopeptides The glycopeptide linkages are located in the D-alanyl-D-alanine region of the peptide side chain of the precursor peptidoglycan subunit. This Dalanyl component's inability to attach to the PBP is prevented by the large pharmaceutical molecule vancomycin, which prevents the development of cell walls (Sondi & Salopek-Sondi, 2004).

In critical care units (ICUs), *Klebsiella spp.* are among the most frequently isolated pathogens, and *Klebsiella pneumoniae* is the most often found Enterobacteriaceae that produces carbapenemase (Morones et al., 2005). Rising antibiotic resistance, particularly in carbapenem-resistant *K. pneumoniae* (CRKP),

is largely responsible for substantial increases in disease and mortality. For infections caused by CRKP, there are few antimicrobial treatment options (Juan et al., 2010). The resistome is defined as the collection of all antibiotic-resistant genes, as seen in Figure 1.10 (Hanan et al., 2018). The use of metal-based antibacterials may be associated with a broader spectrum of activity and less susceptibility to developing bacterial resistance than antibiotics alone, as researchers have been developing and emerging new technologies and effective antibacterial agents in response to the prevalence and increase of microbial resistance to multiple antibiotics. (LLF) and determine whether there are any synergistic effects between antibiotics and gram-positive and gram-negative bacterial strains (Yang et al., 2020) (Dibah et al., 2014) (Santos et al., 2023) (Kapoor et al., 2017) .

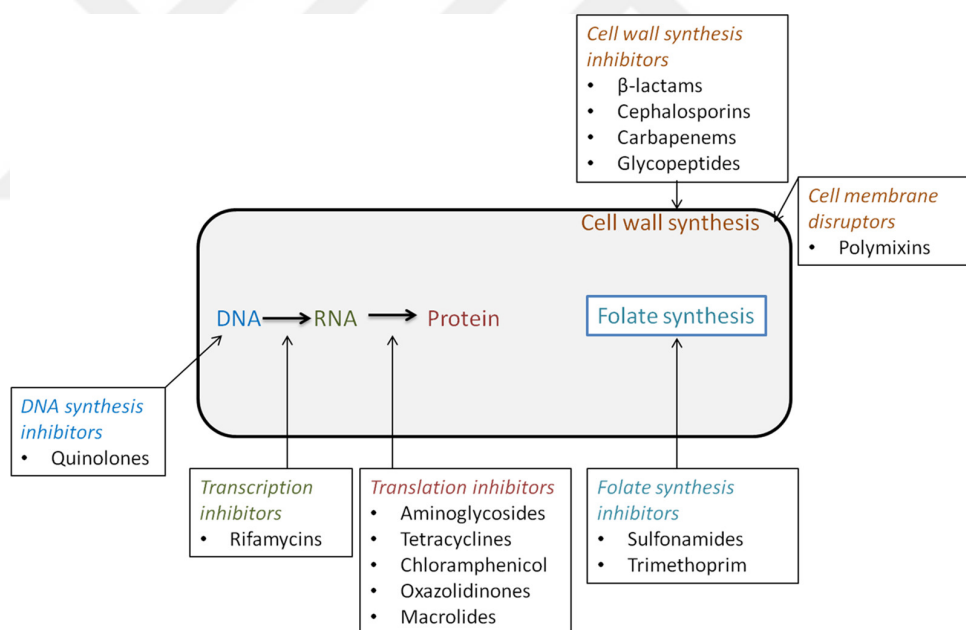


Figure 1.11. Mechanisms of Antibiotic Resistance Bacteria can develop resistance in one of three ways: (a) by altering the efflux of antibiotics; (b) by altering the antibiotics; or (c) by altering their targets (Kahne et al., 2005).

1.6 *Allium porrum*

We present the simple, quick, and one-pot aqueous manufacturing of these nanoparticles using the leaf extract of *Allium porrum* (of the Fabaceae family), often known as leek, in our ongoing efforts to synthesize Magnesium NPs in a

green manner (Picture 1.2). They are abundant in a number of beneficial substances, including flavonoids and water-soluble antioxidant polyphenols, according to research that is still ongoing (Reynolds, 1989). It has been shown that plant-derived NPs have biological characteristics including antibacterial, antioxidant, and anticancer activities (Peterson & Kaur, 2018).



Picture 1.3. *Allium porrum* plant.

1.6.1 Synonym

Table 1.2. *Allium ampeloprasum* var. *porrum*, Leek botanical classification

Clade	Angiosperms
Clade	Monocots
Order	Asparagales
Family	Amaryllidaceae
Sunfamily	Allioideae
Genus	<i>Allium</i>
Species	<i>Ampeloprasum</i>

1.6.2 Distribution

Allium porrum, known as leek, is a native of temperate climates and is cultivated in southern America, Australia, Europe, temperate Asia, and Africa (Saleh et al., 1990).

1.6.3 Traditional Use

Leeks are commonly consumed either raw or cooked and are often added to salads for their distinct flavor. Additionally, the entire plant has been traditionally utilized for its expectorant properties. Moths and other insects were repelled by the plant's juice (Saleh et al., 1990) (Gupta et al., 2004).

1.6.4 Chemical Compounds

Because *Allium porrum* contains an essential oil in which Allyl Sulfide is the main component, its components all have an unpleasant, pungent smell and taste (Lu et al., 2002). *Allium porrum*, like other *Allium* species, creates Alkylcysteine sulfoxides, which are nonprotein sulfur aminoacids made from cystein. These amino acids interact with the enzyme alliinase that is created following the rupture of plant tissue cells, resulting in the formation of sulfur volatiles, primarily in the form of thiosulfinates (Pan et al., 2018), which then decompose and rearrange into disulfides and trisulfides. As a result, these amino acids are considered precursors of sulfur volatiles. Alliinase thiosulfinates and di- or trisulfides make up this complex. an increase in sulfur compound synthesis, including in precursor and volatile forms, that only happens in reaction to extreme *Allium porrum* (raw bulb) contained: water 86 g, energy kcal 35, protein 1.9 g, total fat 0.4 g, carbohydrate available 5.9 g, dietary fiber 3.3 g, ash 1 g, sodium 9 mg, phosphorus 43 mg, potassium 310 mg, calcium 63 mg, iron 1.1 mg, beta-carotene equivalents 4 g, total vitamin A equivalents 8 g, thiamin 0.1 mg, riboflavin 0.05 mg, niacin 0.6 mg, vitamin C 18 mg, cholesterol 0 mg, total saturated fatty acids: 0. *Allium porrum*'s phenol content ranges from 41.6 to 88.2 mg/100 g fresh weight (Upadhyay, 2016) (Bernaert et al., 2012). The green leaves of *Allium porrum* mostly contain kaempferol glycoside and 0.10 mg/100 g quercetin-3-glucoside (Mathan et al., 2017).

1.6.5 Pharmacological Effects

Alliums were renowned for their anti-bacterial and anti-fungal properties, as well as the presence of potent antioxidants, sulfur, and various phenolic chemicals that piqued people's curiosity (Bernaert et al., 2012) (Sabry et al., 2018). According to the Dictionary of Chinese Herbal Medicines (Jin et al., 2017), Chinese leek seeds, which have the capacity to tonify the kidney, have been used in traditional Chinese medicine for the treatment of impotence and nocturnal emissions, which are excessive ejaculations and orgasms that occur while a person is sleeping, since antiquity. It has been used to treat headaches, hemoptysis, asthma, obesity, constipation, hemorrhoids (Russo et al., 2018), and goats. In the food industry, ultrasonic extracts of leek sections can be used for their diverse biological and pharmacological benefits in addition to protecting goods from oxidation (Borkowska et al., 2012). Leek leaf extract exhibits significant anthelmintic action and may be employed in the killing and inactivation of metacercarial parasite infection in fish. They discovered that injecting catfish with a modest dosage of leek extract may help protect fish from the detrimental effects of benzo[a]pyrene by enhancing biotransformation and immune systems. It has been shown that leek juice treatment reduced the degree of oxidative damage associated with dimethoate toxicity and had hepatoprotective effects (Hajra & Liu, 2004), claimed success in utilizing leeks as a preservative to extend the storage life of cheese, and the leeks imparted a new taste to Iraqi soft cheese, increasing customer approval. According to researchers (Farhadi et al., 2015), it may help probiotics and helpful microbes survive in improper storage circumstances and enhance the product's tissue and sensory characteristics. It has been shown that its active elements defend against damage caused by dangerous substances, reduce blood cholesterol, provide a fundamental condition for specific physiological activities, and serve as a vasodilator (Donna et al., 2014). They observed that *Allium porrum* has antitoxic, antioxidative, immunostimulatory, and anti-inflammatory properties, making it a medicinal plant at a time when people are suffering from the negative side effects of synthetic pharmaceuticals. observed that aqueous leek extract increases insulin in white female rats while having a negative impact on cholesterol, urea, creatinine, and weight of the rats (Dwivedi et al., 2017).

2. AIM AND SCOPE OF THE STUDY

The aims of the study are given as,

2.1 Synthesize the magnesium oxide (Mgo) NPs by using leek plant leaf extract (*Allium porrum*) as a reducing or stabilizing agent.

2.2 Studying its antimicrobial efficiency on clinical gram-negative bacteria (*Klebseiella pneumoniae*)

2.3 Discuss the mechanism of MgO's role as an antimicrobial agent.

3. MATERIALS AND METHODS

The materials listed below were used to perform the experiments included in the study, with a description of their origin and manufacture companies, as in the list below.

3.1 Equipment and Apparatus

Table 3.1. List of equipment and devices.

No	Apparatus	Manufacturer (Origin)
1	Autoclave	Takizawa (Japan)
2	Centrifuge	Gallenkamp (England)
3	Electrical oven	Memmert (Germany)
4	Electrical Blender	Dragon Lab (China)
5	Incubator	Memmert (Germany)
6	Micropipettes	Dragon Lab (China)
7	Shaking incubator	Gallenkamp
8	Field emission scanning electron microscopy (FESEM)	Kern (Germany)
9	Atomic force spectroscopy	USA
10	Sensitive scale	USA
11	UV-Visible spectrophotometer	China

Table 3.2. Equipment used in the study, manufacturer, and origin.

No	Equipment	Manufacturer (Origin)
1	Cell culture plates	Santa Cruz Biotechnology (USA)
2	Cotton swabs	AFCO (Jordan)
3	Disposable sterile syringes (10 ml)	Medeco (UAE)
4	Loop	HiMedia (India)
5	Microscope slides with coverslip	Sail brands (China)
6	Petri dishes	AFCO (Jordan)
7	Plain tubes and Screw tubes	AFCO (Jordan)
8	Bunsen burner	GallenKamp (England)
9	Volumetric flasks	LAB (Germany)

Table 3.3. Readymade media.

No	Culture media	Manufacturer (Origin)
1	Brain-Heart Infusion Agar (India)	Biomark (India)
2	Nutrient agar	Mast (England)

Table 3.4. List of chemicals.

No	Chemicals	Manufacturer (Origin)
1	Deionized water	Baghdad (Iraq)
2	MgO	AFCO (China)
3	Normal saline (NaCl)	Baghdad (Iraq)
4	DPPH	China

Table 3.5. The antibiotic discs

No	Antibiotics	Code	Concentration	Manufacturer (Origin)
1	Meropenem	MEM	10 mcg	Bioanalyse (Turkey)
2	Ciprofloxacin	CIP	30 mcg	Bioanalyse (Turkey)
3	Amikacin	AK	30 mcg	Bioanalyse (Turkey)
4	Ceftazidime	CAZ	10 mcg	Bioanalyse (Turkey)
5	Carbapenem	CRO	30 mcg	Bioanalyse (Turkey)
6	Chlorophenamine	CPM	10 mcg	Bioanalyse (Turkey)

3.2 Sterilization Methods

All culture mediums and solutes used in the research were sterilized by autoclave for 15 minutes at 121 °C and 15 lb/in² pressure. The glassware, forceps, and caps used in the investigation were sterilized using dry heat (oven) at a temperature of (160-180 °C) for 2 hours. The inoculating loop, the tip of the forceps, and the point of the forceps were sterilized by holding them over a red-hot gas burner flame. The working surfaces were sterilized with ethyl alcohol at a 70% concentration (Guangyou et al., 2018).

3.3 Culture Characteristics

The samples were cultured and diagnosed using microscopy, biochemical assays, and antibiotic sensitivity testing. The isolates were initially diagnosed depending on the phenotypic characteristics of suitable culture media, such as the shape of the colonies, their texture, color, and edges, as well as their ability to analyze red blood cells on blood agar media and their ability to ferment lactose sugar on MacConkey Agar media (Turkmen et al., 2005).

3.4 Source of Bacterial Strain

We collected 100 isolates of *K. pneumoniae* from urine samples of adult patients at Medical City Hospital in Baghdad, Iraq, between May and September 2022. The samples were collected under sterile procedures, inoculated on Nutrient agar, MacConkey Agar, and blood agar, and then incubated for 24 hours at 37 °C. The samples were isolated and diagnosed at the University of Baghdad's nanolabs and College of Science and Biotechnology. The samples were cultured and diagnosed using microscopy, biochemical assays, and antibiotic sensitivity testing, according to those steps, as *Klebseiella pneumoniae*.

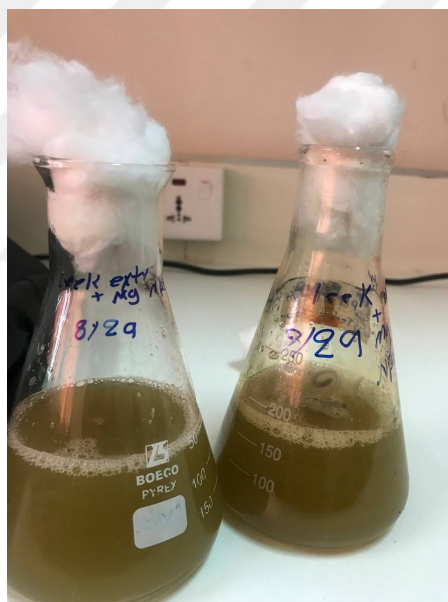
3.5 Preparation of Ultrasonicated Aqueous Extracts of Leek

250 g of fresh leaves were purchased from the markets and repeatedly rinsed with distilled water. Mix with 500 ml of DDW in a ratio of 1:2. They were grinded with an electric grinder at 2X speed for 10 minutes and passed through a filter paper to exclude plant fibers. Plant extract was put in a plastic tube and centrifuge it for 10 minutes at room temperature to get pure leek extract, as seen in picture 3.1.

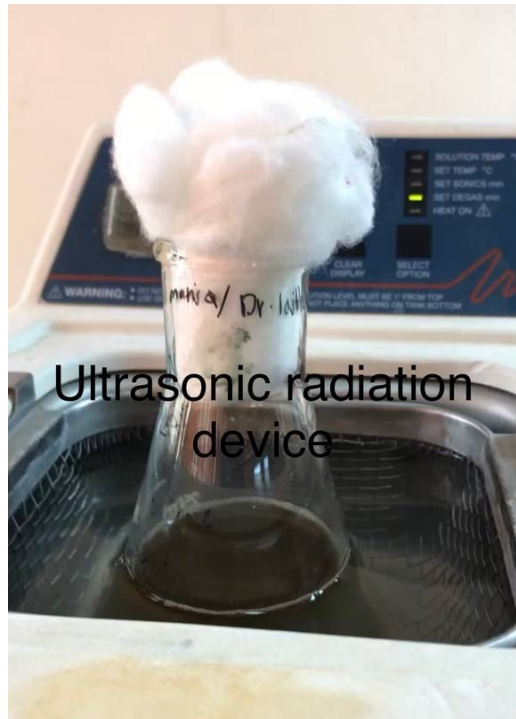


Picture 3.1. The centrifugation process of plant extract.

After centrifugation 500 mL of solution was put in a sterile glass flask (see picture 3.2) and 50 g of MgNPs powder was disseminated for 10 minutes in an ultrasonication bath at 5.35 V, as seen in Picture 3.2 and 3.3.



Picture 3.2. Mixing plant extract with MgCL salt.



Ultrasonic radiation device

Picture 3.3. Ultrasonication of the solution of plant extract and magnesium salt.

The solution was put in an incubator and shaken at 300 rpm for 24 hours, as seen in picture 3.4.



Picture 3.4. Overnight incubation in a shaker

The MgO nanoparticle-containing solution was separated, centrifuged for 30 minutes Picture 3.5 at 6000 rpm to concentrate and twice washed with DDW.



Picture 3.6. After a double wash with DDW

The substance was then dried at 40°C for 24h to produce a white powder, which has been stored in a dark container for future characterization and uses.



Picture 3.7. Preparation of Ultra-Sonicated Aqueous Extracts of Leek.

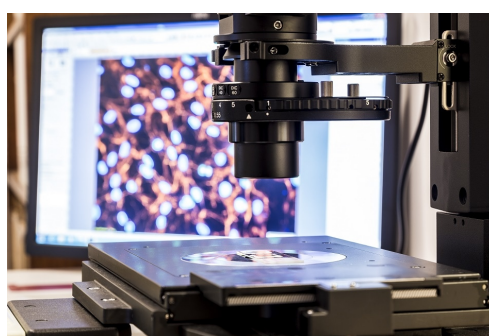
500 mL of reducing leek extract with 50 g of MgO, then sonication for 10 minutes at 5.39 V in the flask in the shaker in the darkroom overnight, followed by a ten-minute centrifugation at 5000 rpm to recover the precipitated material and a DDW wash to remove it. Additionally, the solution was centrifuged at 6000 rpm, deionized distilled water (DDW) was added to the precipitate, and then it was put in the oven at 40 °C for 24 hours. MgO NPs were successfully prepared in Picture 4.7 via the green synthesis method using *Allium porrum* extract, and This opens up new possibilities for exploring the biosynthesis of MgO NPs.

3.6 Characterization of MgO NPs

Characterization is a crucial point in the green manufacture of nanoparticles. A key step is to identify each Magnesium NP's shape, surface chemistry, surface area, and nature of the difference (Ma et al., 2016). Multiple methods were used to characterize MgO NPs.

3.6.1 Atomic Force Microscopy (AFM)

An atomic force spectroscopy system model (AA-3000, USA) was utilized to analyze various characteristics of MgO NPs, including size, surface properties, roughness, granularity, and volume distribution. A thin coating of MgO NPs was produced on the slide by dispersing 50 liters of the particles there and allowing them to dry for five minutes. The slides were scanned using the AFM apparatus. AFM height pictures were used to extract typical data, such as particle size and root mean square roughness. At the University of Baghdad in Iraq (Picture 3.7), the clear factor was tested at the Chemistry Department of the Science College.



Picture 3.8. Atomic force microscopy (AFM).

3.6.2 Field Emission Scanning Electron Microscopy (FESEM)

The size and morphology of MgO NPs were examined using scanning electron microscopy (FESEM-ZEISS SIGMA VP). SEM makes use of a high-energy electron beam that is transmitted to photograph and analyze the microstructure of materials with atomic-scale resolution from a very thin sample, as seen in Figure 3.1. To show the setup and photographs of the SEM, electrons were focused on electromagnetic lenses, and a picture was captured on a digital CCD camera. The samples were produced by spreading them on a glass slide in accordance with the usual procedures of the device.

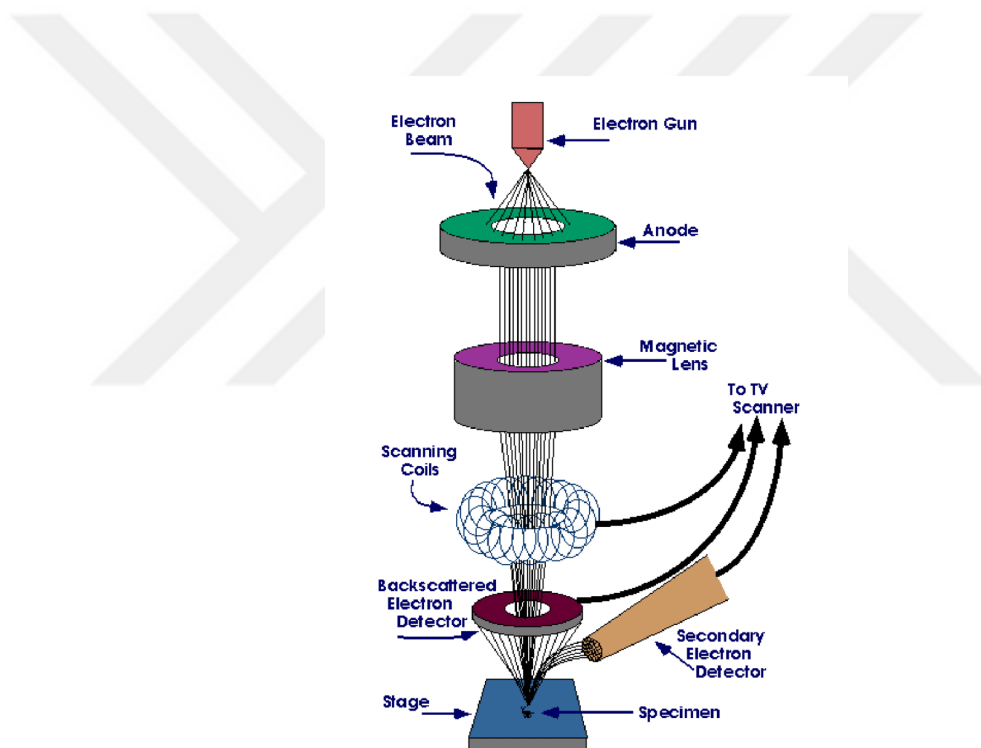


Figure 3.1. SEM Device diagram (Danilatos, 1980) .

3.7 Media Preparation

3.7.1 Brain Heart Infusion Broth

It is used as a medium for the growth of microorganisms. It is a nutrient-rich medium that may be used to cultivate a wide range of discerning organisms and provide a surface on which bacteria may grow. It is a rich source of nutrients

that may be utilized to grow a variety of discriminating organisms. To make BHI, prepared hearts and brains from pigs or cows are mixed with a variety of different nutrients. It was made by combining 37 g of agar with 1 L of distilled water, bringing the pH level down to 7.4, then sterilizing for 15 minutes at 121°C (Duckenfield, 2013).

3.7.2 Nutrient Agar

It is a multipurpose solid medium that promotes the development of a variety of organisms. Nutrient agar (28 g) is dissolved in 1 L of distilled water. As shown in Table 3.6, it has been used to support bacterial growth and enumeration, enabling the generation of pure colonies and the creation of microhabitats to temporarily protect isolates from the agar medium. (Tanaka et al., 2014) (Roesch et al., 2007)

Table 3.6. Nutrient Agar contact

Ingredients	Benefits
Beef extract	Nutrient substance
Peptones	Main source of organic nitrogen
Agar	Gelling agent.

3.7.3 Mueller Hinton Agar

It is a liquid medium that was initially developed for the isolation of pathogenic organisms but is commonly used to test antibiotic susceptibility (Murray & Zeiting, 1983) (composition in Table 3.7).

Table 3.7. Mueller Hinton Agar Composition

Ingredients	Benefits
Beef infusion solids	Supplying carbon, nitrogen, vitamins, amino acids, sulfur, and other vital nutrients
Starch	Adds a colloid that serves as a sorbent

	for any produced toxic metabolites.
Casein hydrolysate	Supplying carbon, nitrogen, vitamins, amino acids, sulfur, and other vital nutrients
Agar	The solidifying agent.
Final pH 7.3 +/- 0.2 at 25°C	

To prepare the medium, 38 g of the substance were suspended in 1 liter of distilled water, heated to boiling to ensure complete dissolution and sterilized through autoclaving at 121°C for 15 minutes.

3.8 Solutions Opreation

3.8.1 Turbidity Standard (McFarland No. 0.5)

It was prepared by combining 99.5% (w/v) sulfuric acid and 0.5% (w/v) barium chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$) in a graduated cylinder. A sterile test tube was then filled with 10 mL of the liquid and maintained at room temperature and in the dark. A spectrophotometer was used to measure the absorbance at a wavelength of 600 nm. An absorbance of 0.08 to 0.1 is acceptable for the standard. Before 1.5×10^8 CFU/mL, the criterion for bacterial cell counts, the ingredients were well mixed (Lee et al., 2015).

3.8.2 Normal Saline

Sodium chloride (NaCl) (8.5 g) were dissolved in 1 L of deionized distilled water to create normal saline solution (0.85% NaCl), and kept away from direct light (Blumberg et al., 2018).

3.9 Culture Preparation

Klebsiella pneumoniae were transferred to brain heart infusion (BHI) agar and re-cultured there. The agar plates were then incubated for 24 hours at 37 °C while being held upside-down. After 24 hours, bacteria were isolated as single colonies from each agar plate and subcultured into their respective broth media.

3.10 Antibiotic Susceptibility Testing for *Klebsiella pneumoniae*

The modified Kirby-Bauer method to help clinicians choose the best course of treatment for their patients, the Kirby-Bauer disk diffusion susceptibility test, examines the sensitivity or resistance of pathogenic bacteria to various antibiotic drugs. The pathogenic organism is grown on Mueller-Hinton agar using disks of filter paper that have been antibiotic-treated. The ability of a drug to suppress an organism can be inferred indirectly from the presence or lack of developmensurrounding the disks (Bovo et al., 2023).

3.10.1 Inoculums Preparation

(Standard for turbidity) To create inoculums, 3-5 colonies were transferred into a tube with 5 mL of normal saline solutions (NaCl 0.85%), stirred thoroughly, and adjusted to the McFarland tube's turbidity standard of 0.5 to produce a culture with 1.5×10^8 CFU/mL suspensions that were employed within 30 minutes of production (Vijayalakshmi et al., 2021).

3.10.2 Inoculation of the Test Plates

Inoculation of the test plates:

A) A sterile swab was used to inject the inoculums into the plates. After each application, the swab was rotated through the plate at an angle of 60 degrees and wiped three times over the surface of the medium to remove any excess by squeezing and otherwise rolling the swab strongly on the tube's highest side, above the liquid level. Finally, the corners of the agar plate were pushed with the swab (Abo Saif & Sakr, 2020).

B) Prior to analysis, the inoculums were allowed to stand for a few minutes with the lid closed.

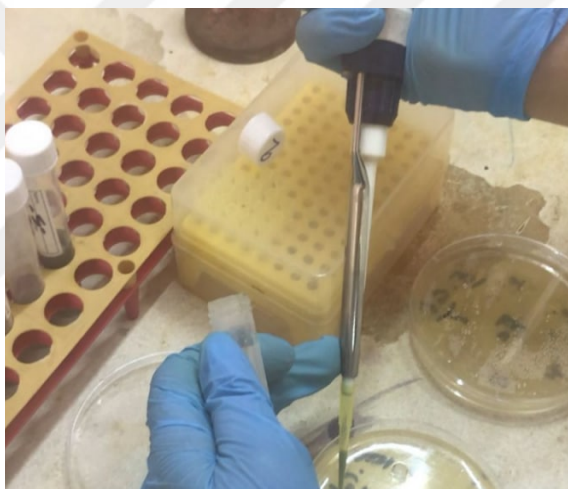
C) A sterile pair of forceps were used to insert the antibiotic disc on the inoculation plate (each plate included 5–6 discs).

D) The plates were incubated for 18 to 24 hours at 37 °C while inverted.

3.11 Antimicrobial Activity of Leek Extract MgO

3.11.1 Agar Well Diffusion Method

It was used to assess the in vitro antibacterial activity potential of phytochemically reduced MgO NPs against 10 typical MDR *Klebsiella pneumoniae* strains. On nutrient agar, *Klebsiella pneumoniae* Petri dishes were swabbed with MDR *Klebsiella pneumoniae* (0.5 McFarland standard). With the aid of a sterile cork borer, 6 mm-diameter wells were created. Individual aqueous extracts of green-synthesised MgO NPs LLE in various concentrations (100, 50, 25 g/ml) were utilized (Picture 3.9). The positive control was ciprofloxacin, while the negative control was DDW.



Picture 3.10. Method of Agar well diffusion

3.11.2 Determination of the Minimal Inhibitory Concentration (MIC) of nanoparticles of Magnesium Oxide (MgO)

The MIC of MgO NPs was established using a modified version of a CLSI-recommended technique as below:

A microtiter polystyrene plate with 96 flat-bottomed wells was used to test the antibacterial activity of MgO NPs against the pathogenic *K. pneumoniae* (gram-negative) bacterium. Before beginning the experiment, the newly formed bacterial subcultures were created by inoculating the bacteria in a test tube containing 10 ml of nutrient broth and allowing them to grow there for 18 hours at

37 °C. Different MgO NP concentrations were utilized (250, 500, 1000, and 2000 g/mL), the overnight growth of test species was carried out and dispersed throughout the agar medium, and wells were created. Then, various concentrations of biological MgO NPs (0.2, 0.4, and 0.8 g/mL) were added (Kowalska-Krochmal & Dudek-Wicher, 2021).

3.12 The MgONPs Anti-Oxidant Abilities

In accordance with (Kainat et al., 2021), the antioxidant activity of NPs was assessed by utilizing stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals. MgO NPs were utilized to study the scavenging activity at concentrations of 6.5, 15.5, 30, 55, and 100 g/mL. 50 µL of the sample and 450 µL of the DPPH solution were mixed, and then 100% ethanol was added to get the combined volume to one milliliter. Control was provided by ascorbic acid. The samples and the control were both stored at room temperature in the dark for 30 minutes. The absorbance was measured using UV light at 517 nm. Scavenging activity was determined by using the formula:

Scavenging % =	$\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}$	* 100 %
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3.13 Statistical Analysis

For the statistical analysis of the data GraphPad Prism was used and 2 way ANOVA was applied and the statistical results are presented in Figure 4.6 (.12 (ns), .033 (*), .002 (**), .001 (***)).

4. RESULTS AND DISCUSSION

4.1 Distribution of Bacteria in UTIs

Following the isolation and treatment of 100 urine samples for individuals with urinary tract infections (UTI), According to the results of the distribution of 100 bacterial isolates, in Figure 4.1. 35% of the patients' cases of urinary tract infections in Iraq were caused by *Klebsiella spp.*

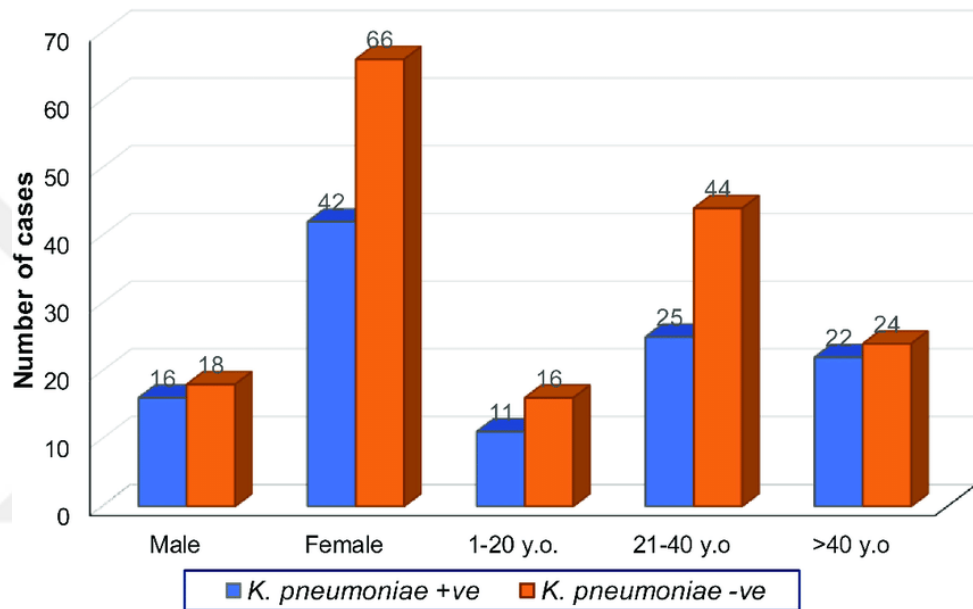


Figure 4.2. Gender and age distribution of *K. pneumoniae*-positive and negative UTI patients.

4.2 Culture Characterization

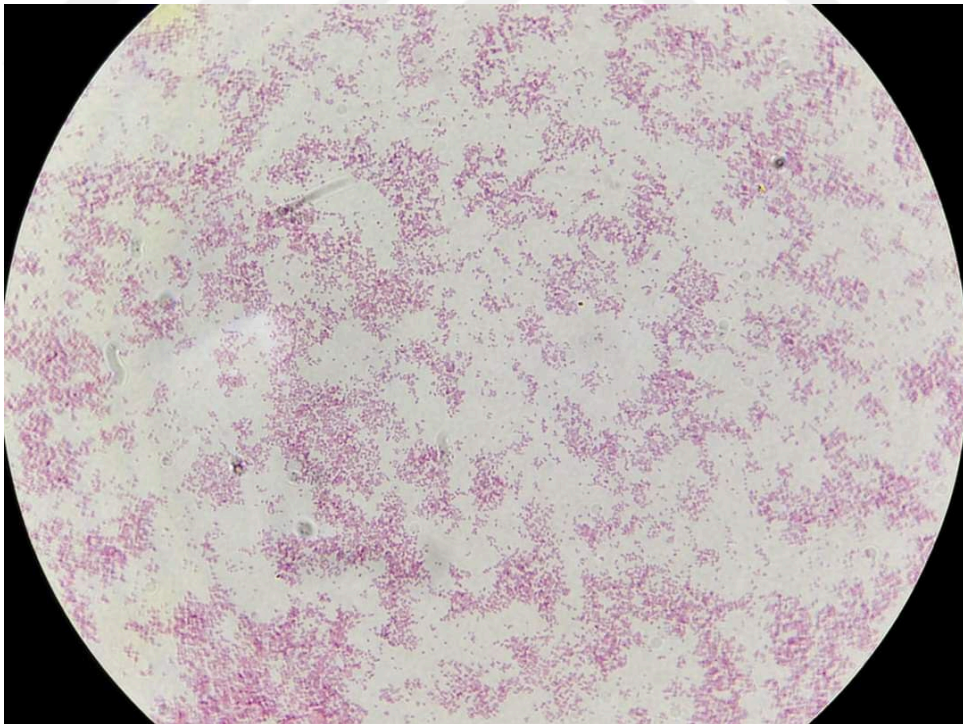
Klebsiella pneumoniae colonies on nutritional agar were developed efficiently on standard medium to an ideal temperature of 37°C in 18 to 24 hours, resulting in colonies that are extensive, mucoid, and white, as seen in the picture 4.1. Colonies' mucoid appearance is caused by the presence of capsular material that the organism produces, according to (S. Zhang et al., 2018) they conclude that this mucoid phenotype is definitely an important virulence factor of *K. pneumoniae*. It is due to the plasmid-encoded production of a substance which is different from colanic acid and the capsular polysaccharide of *K. pneumoniae*.



Picture 4.1. *K. Pneumoniae* colonies on nutritional agar.

4.3 Microscopic Examination

The initial diagnosis is made through microscopic examination and by observing the characteristic features of the bacterial colonies. After staining of *K. pneumoniae* bacteria with Gram stain. *K. pneumoniae* bacteria appeared under the microscope, in red-colored (Gram negative), rod-shaped, arranged in pairs and short chains. The results are also in line with another previous study done by (Ryberg et al., 2011) as shown in picture 4.2.



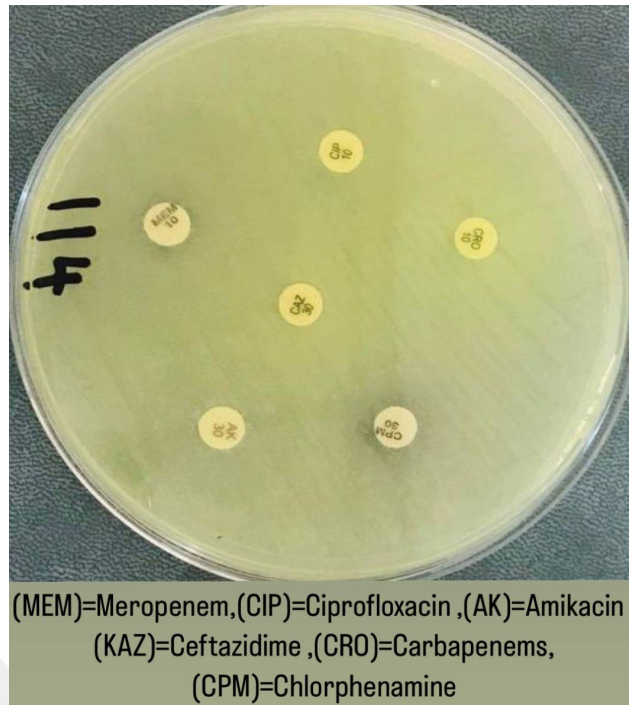
Picture 4.2. *K. Pneumoniae* bacteria appeared under the microscope.

4.4 Antibiotic Susceptibility Test

Samples from 100 *Klebsiella spp.* isolates were examined by using microscopy and biochemical assays. As seen in Table 4.1, a sensitivity test to six separate board spectrum antibiotic types was examined by using a millimeter-measuring ruler. *Klebsiella spp.* bacteria exhibited the highest levels of resistance to Ciprofloxacin (with an average inhibition diameter of 6 mm), Amikacin, Ceftazidime (7 mm), and Carbapenem (6 mm), but were still sensitive to other medicines. (Chlorophenamine 19 mm and Meropenem 11 mm) Figure 4.3, according to the study be (Kurzynski et al., 1976) found a mutation in these molecules will interfere with or negate the drug's destructive action since it is so particular, leading to the development of antibiotic resistance mechanisms, picture 4.3 ,in deal with (Peterson & Kaur, 2018), multiple families of therapeutic drugs were developed to combat a host of infectious agents. The effective use of these drugs.

Table 4.1 Sensitivity (I: Inter mediate, R: Resistant; S: Sensitive) of pathogenic bacteria to antibiotics with inhibition diameter

Antibiotics	<i>K. pneumoniae</i> Sensitivity	Inhibition Diameter (mm)
Meropenem (MEM)	(I)	11
Ciprofloxacin (CIP)	(R)	6
Amikacin (AK)	(R)	7
Ceftazidime (CAZ)	(R)	7
Carbapenem (CRO)	(R)	6
Chlorophenamine (CPM)	(S)	19



Picture 4.3 The antibiotic susceptibility test.

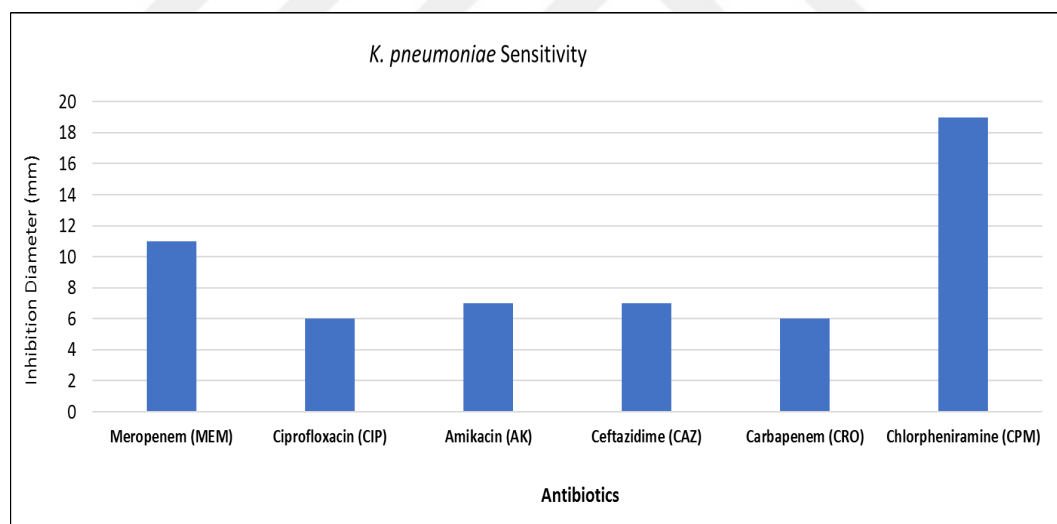


Figure 4.4. Sensitivity test to different antibiotic types

4.5 Characterization of MgO NPs

The dispersion of MgO NPs is homogenous and almost uniform, according to the MgO NPs' characterization according to (Eaton et al., 2008), determine

their bulk characteristics, many individual particles must be described. The nanoparticles were essentially cylindrical in both AFM and FESEM microscopies.

4.5.1 Atomic Force Microscopy

Atomic force The plot topographies that show the surface elevation and surface structure may be recognized by microscopic investigation. This method applies to digital photographs that enable the study of images from many angles, including 3D simulation and quantitative measurements of surface properties such as root mean square roughness (Rq) and average roughness (Ra) (Low et al., 2021).

Figure 4.4 illustrates the three-dimensional picture and the distribution of MgO NPs' granularity buildup. MgO NPs have an average grain size of 44.34 nm, The nanoparticles were roughly cylindrical, as can be observed as seen in Figure 4.5. It is significant to remember that, depending on where the measurements were made on the samples, the mean values were determined and displayed a statistical variance, On the other hand there is a study (Zomorodian et al., 2016), they compared the various mechanisms of action of aqueous garlic extract with the -lactam antibiotic ampicillin using AFM. The findings highlight how important AFM is for supplying qualitative and quantitative data on the antibacterial properties of garlic (*Allium sativum* L.) against *E. coli*.

As shown in Table 4.2, five slides were examined to show the Avg. Diameter of MgO NPs , first sample was 43.00nm with volume 10.52 and cumulation 5.52, second sample was 40.00nm with volume 12.34 and cumulation 27.85, third sample was 44.00nm with volume 12.34 and cumulation 40.1 ,Fourth sample was 45.00nm with volume 11.04 and cumulation 43.2 and the last sample was 48.00nm with volume 8.76 and cumulation 40.0 , The Avg diameter of the MgONPs was 44.34nm ,Figure 4.6 shown The distribution of grain size.

Table 4.2. MgO NPs average grain size

Avg. Diameter:44.34 nm <=10% Diameter:0 nm		
<=50% Diameter:40.00 nm <=90% Diameter:45.00 nm		
Diameter(nm)<	Volume (%)	Cumulation(%)
43.00	10.52	5.52
40.00	12.34	27.85
44.00	12.34	40.1
45.00	11.04	43.2
48.00	8.76	40.0

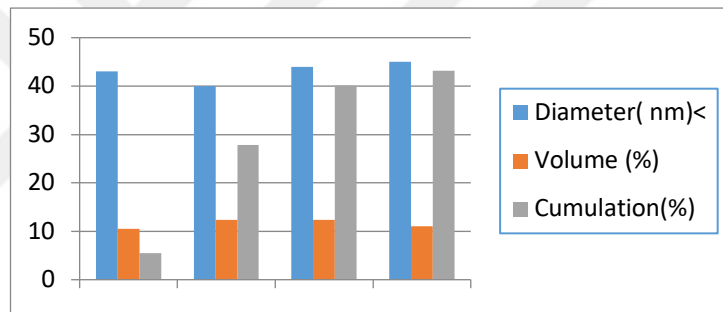


Figure 4.7. The distribution of grain size.

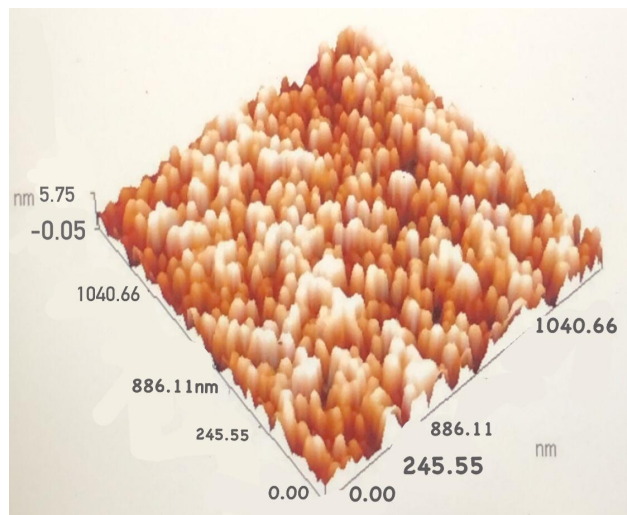


Figure 4.8. 3D AFM images of MgO NPs, It can be seen that the nanoparticles were approximately cylindrical.

4.5.2 Analysis Using Field Emission Scanning Electron Microscopy

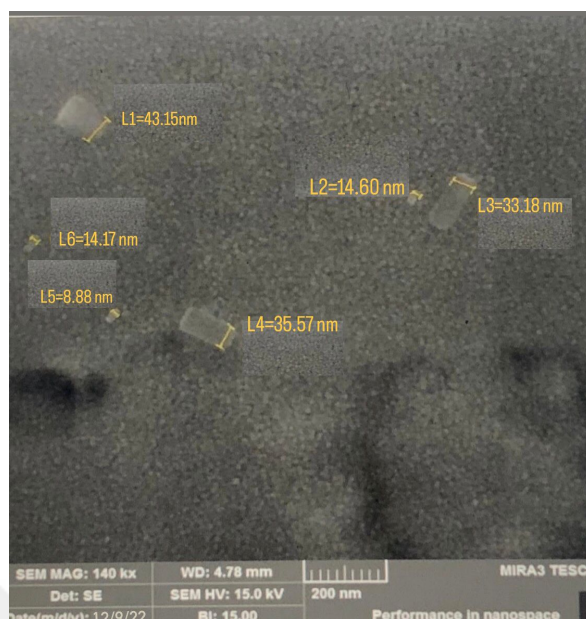


Figure 4.9. MgO nanoparticles' surface shape as shown in the FESEM microscope that work with performance under 200nm and more 4.78 , picture shown diameter in nanometer.

Analyzing Figure 4.5 reveals that the synthetic MgO NPs had an average diameter of 35.57 nm yield from six diameters.

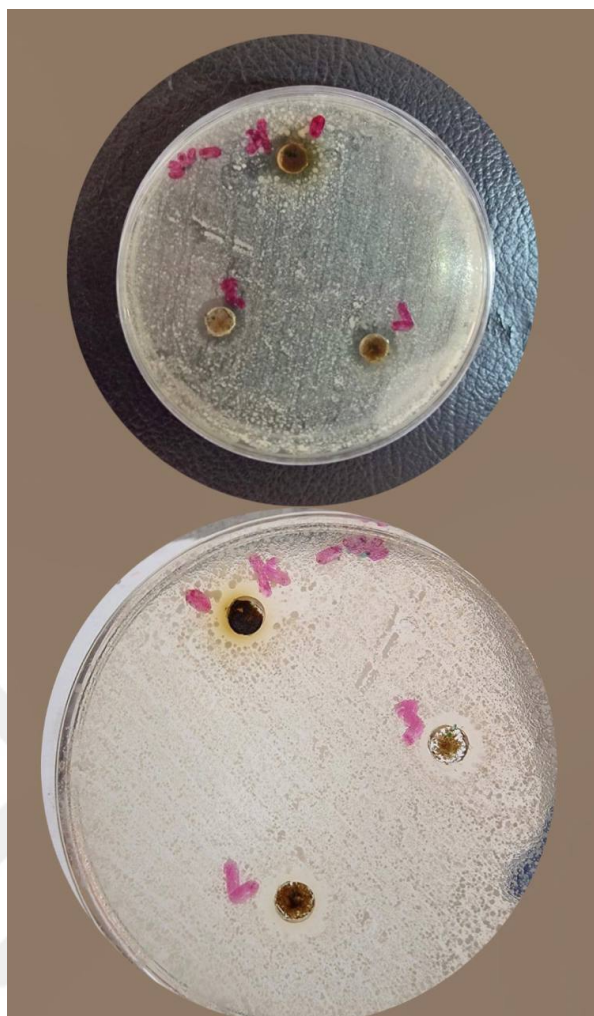
The shape cylindrical flakes with a homogeneous and almost uniform distribution for MgO NPs they were aggregated to create flakes that were mostly thick and uneven and the result corresponds with that of the AFM analysis and were roughly cylindrical.

4.6 Evaluation of the Antibacterial Activity of MgO Against Tested Bacterial Strains

The agar-well diffusion technique is commonly employed to evaluate the antimicrobial activity of plant or microbial extracts (Rotti et al., 2023b). The antimicrobial/bactericidal action of green synthesised MgO from leek leaf extract against human pathogenic bacteria (*K. pneumoniae*) was assessed utilizing an agar well diffusion test using an inhibition zone measurement (mm), as stated in table 4.3, by marking the "0" in the antibiotic disk's center with a millimeter-measuring ruler. The edge of the region where there has been no growth is measured from

the disk's center. The distance should be measured in millimeters, as shown in pictures 4.4 and 4.5. The results depicted a strong result at phytochemically reduced NPs with MgO leek extract against MDR *Klebsiella pneumoniae*.

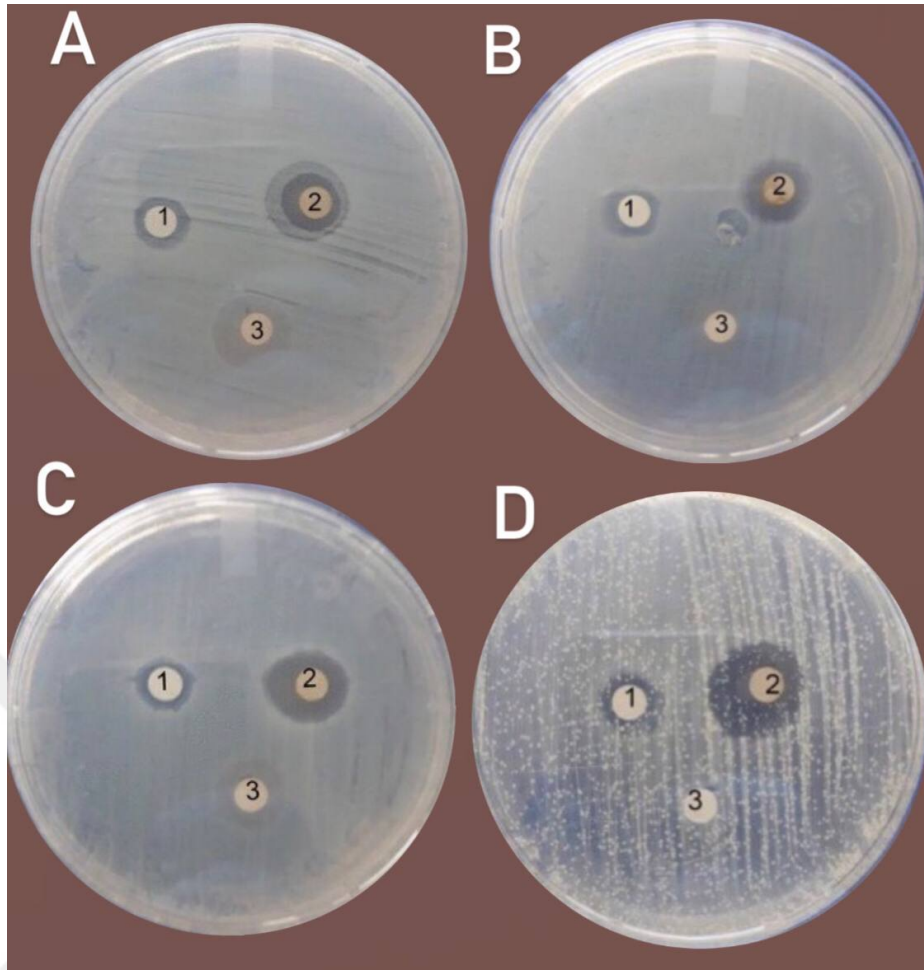
The highest effect of the MgO NPs against *K. pneumoniae* was obtained from 100 µg/ml concentration (inhibition zone: 17.00 ± 1.1 mm) and it was followed by 50 µg/ml (inhibition zone: 15.23 ± 0.57 mm). The lowest effect of the MgO NPs was obtained from the 25 µg/ml (inhibition zone: 13.56 ± 0.57 millimeter). As the concentrations of magnesium nanoparticles decreased from 100 µg/ml to 25 µg/ml, the effect on inhibition zone for the *K. pneumoniae* bacteria also decreased from 17.00 ± 1.1 mm to 13.56 ± 0.57 mm. All results were compared to negative control DIW (0 mm) and positive control ciprofloxacin (12.55 mm). The effectiveness of Magnesium nanoparticles against *K. pneumoniae* bacteria was evident, and these results are consistent with those of a recent study (Emamifar et al., 2011), that found MgONPs to be antibacterial against Gram-negative bacteria. Overall, green-synthesised MgO with leek extract showed significant enhanced bactericidal action against MDR *Klebsiella pneumoniae*. Also (Huang et al., 2005), reported that bacterial death has been linked to the release of ions from MgO nanoparticle surfaces that bind to cell membranes, Numerous research have shown that the generation of ROS such superoxide anion (O_2^-) is what gives MgO nanoparticles their antibacterial properties (Alharbi et al., 2022).



Picture 4.4. Antibacterial activity of MgO against tested bacterial strains

Table 4.3. Antimicrobial effect of green synthesised MgO from leek leaf extract against human pathogenic

Green synthesised MgO concentration	Inhibition zone in diameter (mm)
100 µg/ml	17.0 ± 1.11
50 µg/ml	15.23 ± 0.57
25 µg/ml	13.56 ± 0.57



Picture 4.5. A, B, C, D. Antibacterial activity of MgO against tested bacterial strains

Table 4.4. Antibacterial activity of Magnesium oxide against tested bacterial strains

MgONPs concentration	inhibition zone in diameter
100 µg/ml	18.0± 1.21
50 µg/ml	14.15± 1.11
25 µg/ml	11.0± 1.05

MgONPs concentration	Inhibition zone in diameter
100 µg/ml	20.0± 1.06
50 µg/ml	18.0± 1.08
25 µg/ml	6.0± 1.17

MgONPs concentration	inhibition zone in diameter
100 µg/ml	19.0± 1.08
50 µg/ml	18.0± 1.13
25 µg/ml	5.0± 1.03

MgONPs concentration	Inhibition zone in diameter
100 µg/ml	22.0± 1.18
50 µg/ml	11.0± 1.12
25 µg/ml	4.0± 1.1

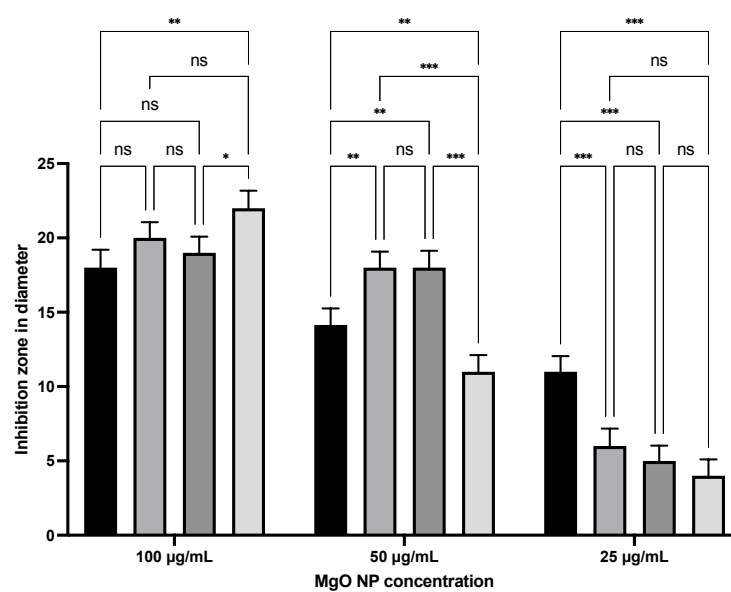


Figure 4.10. Whole results comparison of the inhibition zones (.12 (ns), .033 (*), .002 (**), .001 (***)).

4.7 MIC test for Measuring Antibacterial Activity

The lowest concentration at which bacterial colony development is most effectively inhibited is known as the Minimum Inhibitory Concentration (MIC). In order to determine the impact of Magnesium nanoparticles on the development of *Klebsiella* spp. bacteria, this investigation was carried out by culture in liquid culture media. MgO NPs were discovered to have antibacterial activity. The MIC value of MgO NPs against *Klebsiella pneumoniae* after ultrasonic treatment was 500 g/ml. The MgO NPs in general had already been shown to display antibacterial activity (van Hal et al., 2012), despite the fact that a high concentration was used (2000 g/ml), which had the same MIC value against *Klebsiella* spp. at 500 g/ml. Many experts agree that this oxidized metal's green generation of nanoparticles is superior to chemical synthesis (van Hal et al., 2012). In this study's green MgO NP synthesis, we used leek extract, which showed antibacterial qualities, notably against Gram-negative bacteria (*Kebsiella* species) (Parvekar et al., 2020).

The inhibitory effect of MgO NPs green synthesized with *Allium porrum* showed promising antibiotic activity against the tested *Klebsiella pneumoniae* isolates, and this was done through the use of the turbidity test. As a consequence of additional dilution of the sub-MICs, the findings demonstrated that the Minimum Inhibitory Concentration at various ultrasonic treatment doses of 0.2, 0.4, and 0.8 g/mL had the maximum inhibition rate in hole No.2. (See Picture 4.6,4.7). Significantly less inhibition was seen, along with a rise in the dilution of sub-MICs (Table 4.5).

Table 4.5. MIC activity of green synthesised MgO from leek leaf extract against human pathogenic

MIC of MgO NPs	Inhibition zone
0.2 µg/ml	1 mm
0.4 µg/ml	18 mm
0.8 µg/ml	36 mm
8 µg/ml	12mm
16 µg/ml	19.5mm
32 µg/ml	34.5mm
0.5 µg/ml	2 mm
1.0 µg/ml	16mm
2.0 µg/ml	32mm

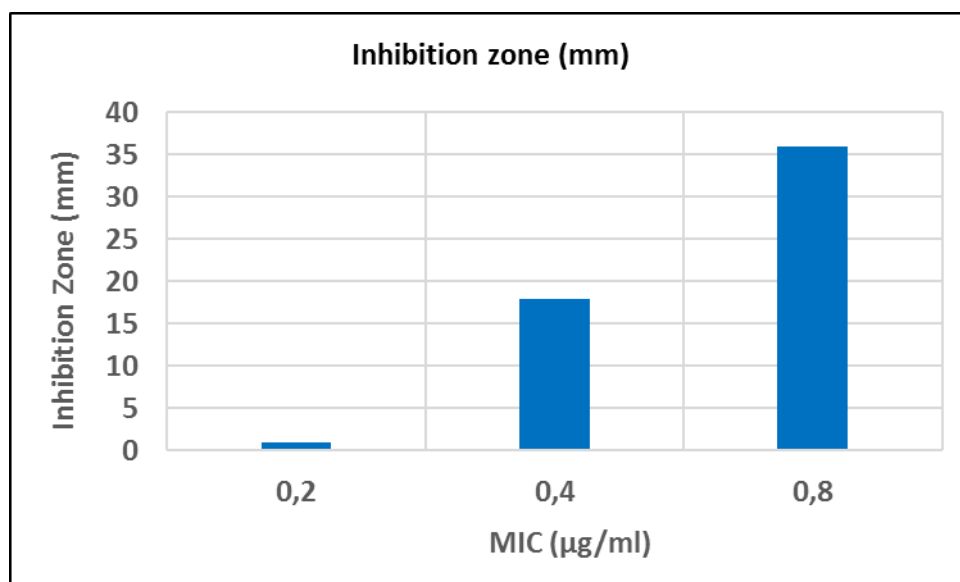
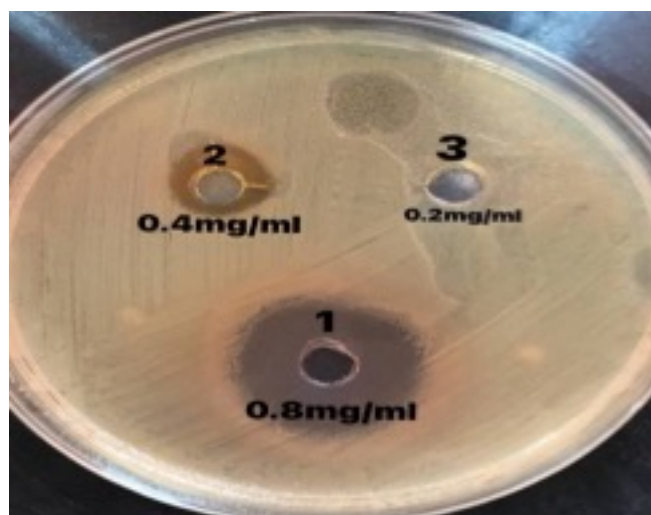
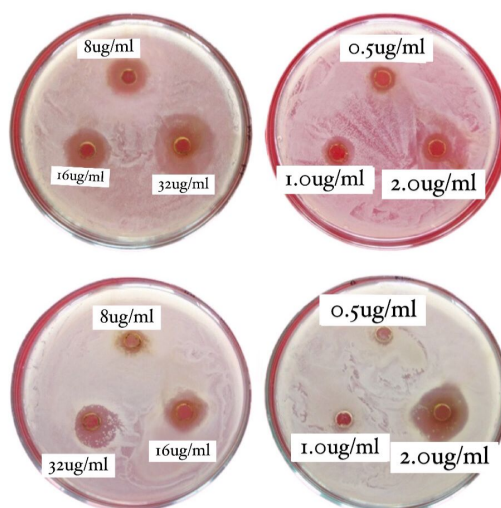


Figure 4.11. Analysis of the MIC concentration of synthesized MgO nanoparticles for 0.2, 0.4, and 0.8 µg/ml .



Picture 4.7. The inhibitory effect of MgO nanoparticles green synthesized with plant extract



Picture 4.8. Different concentration MIC of MgO nanoparticles green synthesized with plant extract

4.8 The Antioxidant Properties of MgO NPs

The DPPH technique is a reliable, quick, and simple way to measure the activity of free radical scavengers. As shown in (Fig. 4.8), the green synthesis magnesium oxide nanoparticles have antioxidant properties. The greatest antioxidant activity was 88.7% at a concentration of 100 g/ml, while the lowest antioxidant activity was 18.0% at a concentration of 6.50 g/ml, as shown in Tables

4.6. This characteristic improved as the concentrations of MgO nanoparticles increased.

Numerous cells release reactive oxygen species (ROS), such as hydrogen peroxide, irritant carbonyl (C=O), and single oxygen (O₂). These ROS can harm DNA, proteins, lipids, and other cell components, leading to cell death. Reactive oxygen species (ROS) have been combated effectively with MgO nanoparticles. The capacity of antioxidants to scavenge free radicals is well established and safeguard the body against several diseases (cancer and cardiovascular disease). The use of MgO nanoparticles to counteract ROS produced unique results. At a concentration of 100 g/ml, the green-synthesised MgO NPs by LLE demonstrated maximal antioxidant activity of 88.8%. This concurs with findings from previous research (He et al., 2016).

Table 4.6. Antioxidant activity.

Concentration (µg/ml)	Antioxidant Activity (%)
6.50	18.0 %
15.5	30.6 %
30.0	50.3 %
55.0	68.4 %
100.0	88.7 %

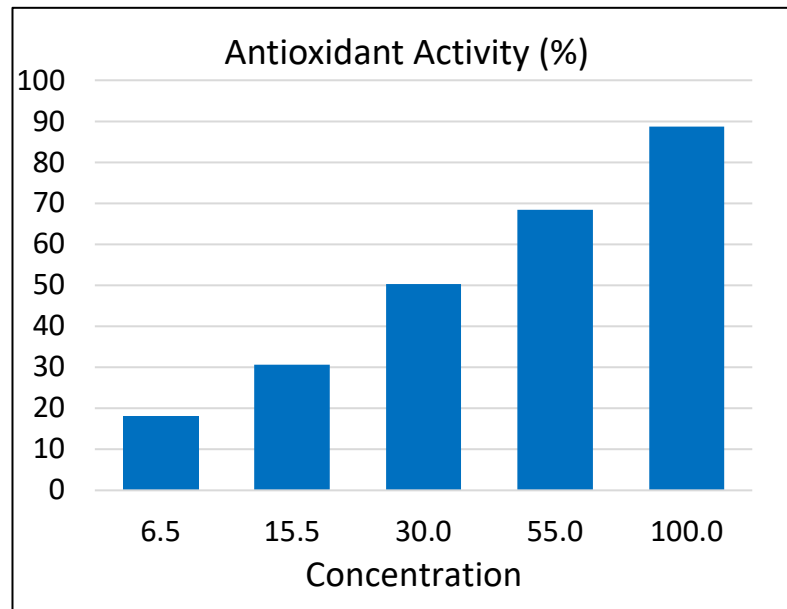


Figure 4.12. Antioxidant activity of NPs using DPPH assay

In this investigation, we demonstrated the potent antibacterial action of MgO NPs against gram-negative bacteria by using LLE in high concentrations of this plant extract with MgO nanoparticles. According to (Goyal et al., 2016), various phytochemicals in Leek aqueous extracts (Flavonoids, Alkaloids, Tannins, Phenolics, Fatty acids, and Saponins) acting as capping agents are responsible for the average crystallite size of metal oxide NPs and were required to inactivate increased numbers of bacterial cells (Shrivastava et al., 2007).

A second reason for the high antibacterial activity might be the presence of active oxygen, such as superoxide, on the surface of the MgO NPs, which causes cell death and cell membrane damage as mentioned in DPPH assay that shown high antioxidant activity of MgONPs (Sutradhar et al., 2011).

Also, the high MgO concentration leads to the death of cells, as well as the results of the MIC test that show the first and second dilutions of stock cause the bacteria's death.

5. CONCLUSIONS AND RECOMMENDATIONS

The conventional approaches to NP development are costly and result in extremely hazardous byproducts. As a result, the different substances utilized in the physical and chemical techniques may cause environmental toxicity. Recent advancements have made ecologically friendly metal oxide MgONP synthesis a particularly desirable field for scientific investigation. Green chemistry and the use of green ways to manufacture nanoparticles are expanding daily in order to produce an ecologically friendly procedure. MgO nanoparticles were created using plant extracts (leek leaf extract). When MgO NPs are sonicated with plant extract in ultra sonic device to create nanoparticles, these plants are known for their efficiency as capping, reducing, and stabilizing agents ,Some gram-negative bacterial strains were resistant to the antibacterial effects of NPs. Therefore, green or phytochemically reduced MgO from leek leaf extracts may be employed without affecting the environment in modern medicine to replace antibiotic resistance and in the textile sector to operate as a catalytic agent. MgO nanoparticles can harm bacteria's cell walls through a variety of processes. The effect must be investigated and characterized because it is unknown. Further research is necessary for the characterization of this impact. Future research will benefit from this study's insights and understanding of a variety of difficulties.

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