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Synthesis, Characterization and Anti-Microbial Properties of Magnesium Oxide Nanoparticles by Using Corn Silk Extract

Hadia Tariq1st,NaeemUllah Khalil 2nd , Muhammad Arshad 3rd

[1]*Student, Women University Of Azad Jammu AndKahmirBagh*
[2]*Student, The University of Peshawar*
[3]*Student, Institute of chemical science Gomaluniversity*

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ABSTRACT: Metal oxides are significant technological materials to be used in photonic and electronic devices. They can also be used in various fields like catalysis, ceramics, petrochemical products, environmental remediation, drug delivery, medicine, cancer therapy and in antimicrobial activity as well. There are basically three methods to synthesis metal oxide nano-particles physical, chemical, and green methods. Fabrication of ecofriendly metal oxide nanoparticles using biogenic sources are currently used for their synthesis replacing the harmful and toxic chemicals. In present research work the main focus was on the facile synthesis of MgO-NPs by using Corn silk(Zea may) extract via Green method. Its non-toxic and eco-friendly method to synthesize Magnesium oxide nanoparticles (MgO-NPs). The precursor used in this method was Magnesium nitrate hexahydrate$Mg(NO_3)_2.6H_2O$ and corn silk extract. The precursors act as reducing and capping agent in the reaction. Thus, the obtained particles were then characterized by applying different analytical characterization techniques such as UV-Vis spectroscopy, SEM, XRD, FTIR and also study antimicrobial properties. X-Ray Diffractometer was used to confirm phase formation and to calculate average crystallite size and also to analyze the average particle size. SEM scanning electron microscope an advanced form of microscope was used for morphological study. UV-Visible spectroscopy was used to analyze absorption pattern and also Fourier Transform Infrared (FTIR) spectroscopy for analyzing functional groups involved in the reaction. Antimicrobial assay was performed to evaluate the effect of MgO-NPs against gram positive and gram negative bacteria. The zone of inhibition revealed that MgO-NPs inhibit the growth of both gram negative bacteria in a concentration dependent manner MgO-NPs form. In UV-Vis at 250 nm highest peak was observed which can be ascribed to Magnesium oxide nanoparticles and the cutoff peak for both the precursors used to synthesize MgO-NPs; corn silk extract and magnesium nitrate hexahydrate is around 221 nm. SEM showed that nanoparticles are uniformly distributed on the entire surface having size less than 20nm. SEM scanned images showed that nanoparticles are present in the form of assemblies that are round in shape. X-ray diffractogram showed multiple distinctive peaks indexed to various crystal planes of cubic phase MgO. No secondary peak was detected in the diffractogram indicating that the obtained final product is pure. FTIR spectra of MgO-NPs synthesized by using corn silk extract is recorded in the range of 500 to 4000 cm^{-1}. IR spectrum also showed that corn silk specimen is rich in carboxylic acid, polyphenol, amino acid, polysaccharide and proteins.

KEYWORDS: Nanoparticles, MgO-NPs, Corn silk extract, Antibacterial assay.

I. INTRODUCTION

Synthetic approach of metal and metal oxide nanoparticles that is based on plant materials is extremely efficient, low cost and ecofriendly. These metal oxides nanoparticles due to their vast number of properties are used in number of fields like chemical, ceramics, pharmaceutical, electronics and biological fields. Nanoparticles having particle size less than 100nm have been used in manufacturing drugs, polymer science and mechanical engineering. The inorganic nanoparticles that are based on plant extract are ecofriendly and also safe for all living beings because of their composition that includes biochemical compounds and phytochemicals. From past couple of years' nanoparticles manufactured from synthetic methods have attain tremendous attention of the people and the researchers. All the researchers are now more concerned to synthesize nanoparticles that are hazardous free chemicals. Biologically prepared nanoparticles are safer as compared to the ones that are synthesized chemically and have the ability to show numerous pharmaceutical and biological properties. The most common metal oxide nanoparticles synthesized from green synthesis are magnesium oxide (MgO), Copper oxide (CuO), Calcium oxide (CaO), Zinc oxide (ZnO), and Iron oxide (Fe2O3). Nanoparticles of magnesium oxide have tremendous importance in the field of pharmaceuticals due to their good antimicrobial activities against microorganisms. These MgONPs are non-toxic in nature having high purity and high melting point and are odorless as well. In our research we have studied the synthesis and characterization of MgO using corn silk extract. (Singh, Joshi et al. 2019).

1.1 NANOTECHNOLOGY

Nano science is the most emerging science branch that is dealing with the structural study of unbelievably minor things, particles and materials having size as well as dimensions less than 100nm on a Nano scale and their applications range between 1-100 nm. Basic idea and key concept of Nano science and technology was founded by a well-known scientist Richard Feynman and according to him "There is plenty of room at the bottom".

Figure: 1. 1 Nanotechnology originator Richard Feynman

The phrase "Nano" describes the concept of Nano technology referring to the prefix -9 and it is $10^{-9}th$r billionth part of meter. We encounter naturally occurring nano materials mostly in nature fall in Nano order and can also be attained by top-down and bottom-up approaches. Nano science controls structure of a material at atomic scale. The properties of material and their functionality can be affected by dimensions. Materials become more reactive at nano scale when surface to volume ratio increases. So, it is expected to say that at nano level materials will improved material properties. Nano technology depends on basic science, analytics and methodologies of various disciplines like physics, molecular biology, chemistry and material science. It includes different scientific fields and also has enormous usages in the field of nano biotechnology, nano medicine, energy, combat, art, electronics and also in many other fields including lithography and nano robots. (Roco, Mirkin et al. 2011)

1.2 NANO MATERIALS

Nano technology is restructuring the materials at atomic and molecular level ranging from 1-100nm. So the chemical substances that are formed and used at nanoscale are known as nanomaterials. These nanomaterials play an important role in regeneration of tissues and are considered to be the most advertised area of nano medicine development. Nano silver is used in antimicrobial coatings and ceramic nanoparticles are used to strengthen and enhance the dental composites (Jandt and Sigusch 2009). Recently, an increase in the practice of nanomaterials is witnessed and advancement in the nanotechnology in almost every field which then lead to the uncertain environmental impacts (Klaine, S. J, Alvarez ~~et al~~et al. 2008). Some uncertainties regarding adverse effect to the environment and health are reported by the Royal engineering society in 2004. But nanotechnology being an emerging field of science and technology having components at nano scale can have the ability to easily remove such adverse environmental effects.

1.3 PROPERTIES OF NANO MATERIALS

Electrical, mechanical, antimicrobial and Nano materials magnetic properties are considerably distinguishing at Nano scale as compared to the bulk materials and their properties. They own intrinsic property of thermodynamics. They are quite hard in nature and also have melting point in the decreasing order.

In recent years' research and publications on nanoparticles mechanical properties has increased. They exhibit number of mechanical properties comparative to their bulk materials and micro particles providing quite effective routes for modifying the surfaces of many devices in strengthening mechanically or else by improving nanofabrication quality. The mechanical nanoparticles effects have the ability to affect lubricants tribiological properties and reinforcing composite coatings with the help of nanoparticles. A good control over nanoparticles mechanical properties and their interaction with the polished surface is significant for refining the surfaces' quality. For successful applications of nano-particles a good understanding of basic mechanical properties like hardness, interfacial adhesion, friction, movement and size dependent effects are required (Guo, D. et al 2013).

In water disinfection, medicine, textile industry and food packaging antibacterial agents are of significant importance. For disinfection organic compounds were used but they have some disadvantages which also includes human body toxicity as a result interest in metal oxides nano particles (inorganic disinfectants) is increasing (Hajipour, M. J et al 2012).

1.4 TYPES OF NANO PARTICLES

Like any other compound nanoparticles are also categorized into a number of types depending on their sizes, structures, physical as well as chemical properties. Some of the known categories of nanoparticles include ceramic, carbon based, semiconductor, polymeric and lipid based nanoparticles. The general idea of these nanoparticles is discussed below.

Ceramic nano particles

Ceramic nanoparticles basically comprises of oxides, carbides, phosphates, metal carbonates and metalloids like calcium, titanium, silicon etc. They have high heat resistance and chemical inertness. Ceramic nanoparticles are widely used in biomedical field and are considered as outstanding drugs, genes, proteins carriers. These nanoparticles work as excellent drug delivery systems various diseases which include glaucoma, cancer, bacterial infections and many more (Thomas, Harshita, Mishra, &Talegoankar et al 2015).

Carbon-based nano particles

Carbon based nanoparticles comprises of two main materials are present one of them is carbon nanotubes and other one is fullerenes. These nanoparticles exhibit quite high antimicrobial activity. From the recent researches it has been shown that fullerenes, single wall carbon nanotubes and GO nanoparticles show potent antimicrobial properties. These allotropic carbons were discovered in last 2 decades and since then they have been used in numerous fields of sciences. The important parameters of carbon nanomaterials are size and surface area which affect the antibacterial activity for instance by increasing surface area and decreasing the size of nanoparticles leads to improvement in their interaction with bacteria (Dizaj, S. M et al 2015).

Yang et al 2010 investigated the SWCNTs length effect on nanoparticles antimicrobial activity. And revealed that stronger antimicrobial activity was directly related to longer SWCNTs because of their improved bacterial cells aggregation. These carbon-based materials are mainly used for structural reinforcement.

Semi-conductor nanoparticles

Semiconductor nanoparticles show optical, electrical, thermal and catalytic properties and because of these properties they attracted interest and concern of the researchers and are used in photo catalysis, electronic devices, electro chemical sensors and photo optics (Wang, F., & Hu, S. et al 2009).

Polymeric Nanoparticles

Polymeric nanoparticles are actually organic nanoparticles. Some of the advantages of polymeric include protection of drug molecules, diagnostics, drug delivery and the ability to combine therapy and specified target. Because of flexibility their structure, can be modified with complex composition and properties. Therapeutics based on polymeric nanoparticles show amazing promises in treatment of wide range of diseases (Elsabahy, M., & Wooley, K. L. et al 2012).

Lipid Nanoparticles

Lipid nanoparticles basically have spherical shape with diameter ranging from 10 to 100nm. In developing nano technology they have a number of potential applications which include drug delivery in biomedical field, clinical research and medicine and in a number of other sciences as well (Lingavat, V. J. et al 2017).

1.5 SYNTHESIS OF NANOPARTICLES

There are two approaches to synthesize nano particles namely 'bottom up' and 'top down' approach.

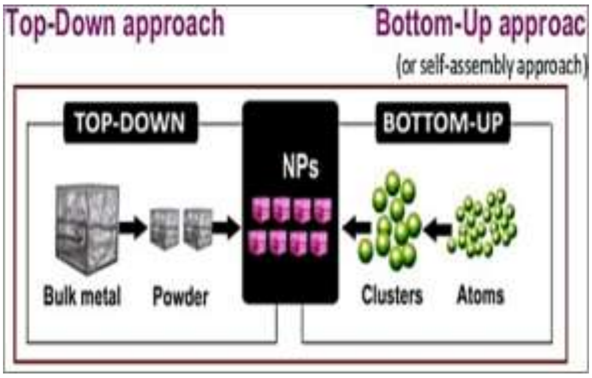

Figure: 1. 2 Nano material synthesis

"Top down" approach is the one in which material available in bulk form is sliced/cutted down into minor pieces and is then further processed into powder form which then undergoes nucleation process to form Nano particles by mechanical grinding process and destruction however "bottom up" approach - a method that nature follows. In this methodology material is build up by adding small building blocks forming self-assembly clusters.

A number of methods are used to synthesize and process Nano materials. Name of these methods are mentioned below:

PHYSICAL METHOD	CHEMICAL METHOD	BIOLOGICAL METHOD
MECHANICAL METHODS	CO-PRECIPITATION METHOD	SYNTHESIS USING PLANT EXTRACTS
VAPOUR DEPOSITION	SOL-GEL METHOD	SYNTHESIS USING ENZYMES
SPUTTER DEPOSITION	MICROEMULSIONS	SYNTHESIS USING AGRICULTURAL WASTE
ELECTRIC ARC DEPOSITION	HYDROTHERMAL SYNTHESIS	
ION BEAM TECHNIQUE	SONOCHEMICAL SYNTHESIS	
MOLECULAR BEAM EPITAXY	MICROWAVE SYNTHESIS	

Figure: 1. 3 Methods for synthesizing Nanomaterials

There are various methods that are used to synthesize nanoparticles but the chemical methods that are available to synthesize MgO NPs protocol may contain the chemicals that are toxic and may lead to environmental problems. The other methods that can also be used to synthesize metal oxide nano particles include co-precipitation, combustion, sol-gel, hydrothermal, micro assisted sol-gel and green method. Green synthesis is not like other conventional methods; it is a green chemistry principle that makes the researchers to create synthetic strategies using biological methods where the major role would be played by enzymes, microorganisms and also plant extracts in nanoparticles formation. Because of environmental concerns plants extract is given more priority over chemical methods (Yuvakkumar, R., Suresh et al 2014). These biological methods that are used for NPs are cheap, ecofriendly and also has less chance of lethal chemicals exposure and their products (Okitsu, Mizukoshi, Yamamoto, Maeda, & Nagata, et al 2007). Biological methods using plants for NPs synthesis are more biologically compatible because of presence of functional biomolecules which will vigorously lessen metal ions. MgO-NPs were synthesized by biological method from Costuspictus and then their antimicrobial property was tested against two grams of positive bacteria (S. aureus, B. subtilis), two grams of negative bacteria (E. coli, S. paratyphi) and also (A. niger, C. albicans) two fungal species under laboratory conditions (Suresh, J.,Pradheesh, G., Alexramani, V et al. 2018). The present work is mainly focused on the synthesis, characterization and antimicrobial properties of MgO-NPs using Corn Silk Extract.

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1.6 INTRODUCTION TO CORN SILK

Kingdom:	Plantea (Plants)
Scientific name: Zea mays	
Phylum:	Magnoliophyta
Class:	Magnoliopsida
Order:	Poales
Family:	Poaceae
Genus:	Zea L. (corn)
Species:	Zea mays L. (corn)

Figure: 1.4 (a) Corn Silk (b) Corn (Zeay May)

Zea mays is a scientific name of corn which is an annual grass whose origin is Central America and have its place in a grass family of Poaceae. In the world among rice and wheat it is considered to be the third top cereal. At the top ear of the corn long and shiny fibers called corn silk are present. They are whitish to green in color and are silky single threads like that grow from seeds eye and fall outside the husk. Because of their characteristic of hanging down outside the husk they are capable of catching pollen. They are quite useful for medicinal purposes like treating diabetes, heart failures, lethargy, kidney stone, high blood pressure and high levels of cholesterol. It comprises of vitamins, fibers, carbohydrates, mineral and proteins and can be used in fresh form as well as in dried form. Corn silk contain high amount of potassium in it and slight sweet flavor making summer tea refreshing and nutritious. China and France are the countries that use corn silk for treating ailments as it works to strengthen and restores mucous membranes of urinary tract helping in preventing incontinence (Wang, K. J et al 2019).

1.7 PROPERTIES OF CORN SILK

Corn is generally known as maize having a scientific name Zea mays L. is a yearly crop that belongs to the grass family i.e. Poaceae. Maize crop can be recognized by different names like zea, corn, silk corn etc. It is considered to be the mother grain of Americans and also considered as the earliest cultivar of new world. It is a plant that is widely distributed plant in the entire world. Multitudes of maize subspecies are identified as well as classified into the categories depending on the amount of starch present in each. It has short life cycle and needs warm weather and appropriate management for growth and cultivation. It is a valuable livestock feed and is used as human food and raw material for several industries. Medicinally corn silk has been used to pacify anorexia, general debilities, emaciation and hemorrhoids. It is a potent antioxidant that guards a body from harming by free radicals responsible for cellular damage or cancer. It has the potential to alleviate pain and possess analgesic activity as well. It improves symptoms of rheumatism as B-complex and is able to improve joint motility. Main nutrient of corn silk is potassium that is powerful diuretic (Kumar & Narayan Jhariya, 2013).

APPLICATIONS OF MgO NANOPARTICLES

Nanoparticles being used as drug carriers basically have technological benefits which include high stability, high carrying capacity, various routes of administration, feasible combination of hydrophilic and hydrophobic substances. They are used in the manufacturing of various things like transparent sunscreens, ceramic coatings, stain repellent fabrics, scratch proof glasses, superconductor products, antimicrobial materials, photonic and many more. MgO-NPs are basic oxide and have number of applications in catalysis, adsorption and also in synthesis of refractory ceramics. It is widely used because of its chemical, optical, magnetic, electric, catalytic and antimicrobial properties. MgO-NPs act as antimicrobial agents and are used for therapeutic purposes like blood collecting vessels, coated capsules and band aids.

MgO is the most known and efficient metal oxide comparable to the others because of its optical, electrical, chemical, thermal and mechanical properties. At nano scale it shows high reactivity (Fakhri&Adami, 2014). Because of its high melting point along with low heat capacity it is considered to be the best material for insulation applications.

MgO is the most significant material for numerous applications for instance paint products, additives refractory and environmental remediation by scavenge flouride from drinking water (Xiao, Singh, Chaffee, & Webley, 2011).

1.9 SIGNIFICANCE OF THE STUDY

This study based and focuses on exploring potential use of corn silk for MgO-NPs synthesis which is a unique method. After synthesis we performed characterization and evaluated antimicrobial properties of MgO-NPs. This study focuses on the formation of MgO-NPs via biological method. Magnesium oxide nanoparticle synthesized from this method is free from hazardous effects as compared to the nanoparticles synthesized from physical and chemical methods. Biological method is assumed to be cost effective, ecofriendly and safe from harmful effects that take place during the production of NPs.

AIMS AND OBJECTIVES

- To find out the potential of silk corn extract for the synthesis of MgO nanoparticles.
- To characterize MgO-NPs by different characterization techniques.
- To calculate antimicrobial potential of MgO-NPs against different bacterial species (Gram positive, Gram negative).

II. CHAPTER 2

LITERATURE REVIEW

The magnesium salt counterions effect on the phase precipitation reaction was taken to produce NPs. The results showed that different parameters like morphology, crystallinity, size and mono disparity effected by magnesium counterions. The particle size was increased from 50 to 2000 nm. The Mg (OH)2 NPs were applied successfully on conservation of paper. The efficacy of NPs as de-acidifying agent preserved the chemical features of paper as they are less aggressive and are easily converted to carbonate. Results showed that by taking the proper composition and counterions quantity, the desired NPs will get (Giorgi, R., et al., 2005).

Microwave-assisted method has extensively used due to its advantages like higher reaction rate, rapid volumetric heating, increasing yield of products and reducing reaction time as compared with heating methods Lately, the synthesized numerous nanomaterials with different morphologies using microwave heating. The magnetite and hematite NPs was prepared by the method of simple microwave heating. The morphology was affected by this method (Wang, W. W., et al., 2007).

Purified flavonoids (FCS) from corn silk and its activity was obtained by microporous resin technology. Different properties of eight kinds of macroporous were tested by static adsorption. The study proves that FCS can raise the exercise tolerance and anti-fatigue activity of mice by preventing the making of blood lactic acid, checking the creation of BUN and increasing hepatic glycogen concentration (Hu, Q. L., et al., 2010).

The antibacterial activities of MgO nanoparticles (nisin and ZnO-NP against Escherichia coli and Salmonella) were examined. The results showed that it has strong bactericidal activity against the pathogens, attaining more than 7 log reductions in bacterial counts. Though, few reports on the antimicrobial activity of MgO-NP relating the use of MgO-NPs alone or in combination with other antimicrobial agents to destroy or prevent the growth of foodborne pathogens. Antimicrobial activity of MgO-NP increased as the concentrations of MgO increased (Jin, T., & He, Y. et al., 2011).

Corn silk is the stigma of Zea mays Linne. Presently, small amount is used as medicine. Flavonoids from stigma were testified to, regulate lipid, metabolism in mice, scavenge hydroxyl and have anti-fatigue and anti-aging effect. The abstraction of corn silk total flavonoids (CSTF) was considered for the growth of corn silk as natural food antioxidants and medicine. It has significant social and economic value (Yan, Z., et al., 2011).

Pleurisy, the pleural layers that at the edge of the lungs. In spite of much research into inflammatory diseases, no drugs with favorable safety was available. Corn silk has been used for the treatment of kidney stones nephritis, prostitutes, gout, cystitis, and edema. Though, no reports were on the anti-inflammatory effects of corn silk were found. It has been shown to test the anti-inflammatory efficacy of corn silk extract (CSEX) in a rat model (Wang, G. Q., et al., 2012).

Magnesium oxide NPs has a strong antimicrobial potential. With the beginning in antibiotics progress, the medical applications of Mg as antimicrobial were failed. By working on their size at nano-level antimicrobial effects of Mg can be increased (Rai, M. K., et al., 2012).

MgO nanoparticles has many applications. e.g., MgO nanoscale particles, with ultrafine and high specific surfaces area has shown as destructive adsorbent for toxic chemical. Nanoscale MgO displays unique optical magnetic, electronic, mechanical, thermal, and chemical properties, due to its distinctive structures. Consequently, nanoscale MgO extensively used in catalysis, refractory materials, toxic waste remediation based on its properties (Camtakan, Z., et al., 2012).

Nanotechnology implies in growth of science and technology, and materials with unique physical, biological and chemical properties. Interest in nano sized silver NPs increased by their antibacterial applications. Silver nanoparticles (AgNP) having inhibitory effect to many microorganisms and bacterial strains. These nanoparticles can proposal unexpected interactions with biomolecules both inside the body cells and on the surface. which may use for treatment of many hazardous diseases (Bhat, R., et al., 2013).

It has been shown that corn is useful not only as food or medicine but has number of exclusive importance for animals and also for industries. Together from that it also helps keeping away health connected disorders in human and resistance against diseases and offers continuing assistances (Kumar, D., &Jhariya, A. N. et al., 2013).

Growing environmental concerns over chemical synthesis routes resulted develop bio-mimetic approaches. One of them, is the synthesis using plant extracts. The MgO nanoparticles were synthesized by Nepheliumlappaceum L. peels, as a ligation agent. The particle size was obtained at 60-70 nm. The as-synthesized MgO powder, particle size was 100 nm (Suresh, J., et al., 2014).

By using the wet chemical method, MgO-NPs were synthesized. Results showed that the structure of MgO was FCC. The final product was well dispersed with the particle size of 16 nm. The formation of spherical shaped of MgO was revealed. The morphological and optical study revealed that MgO with size distribution of 7-38 nm and indicates quantum confinement effect. These antibacterial properties will have utilized in the purification of water (Bindhu, M. R., et al., 2016).

The magnesium oxide NPs were prepared by environmental friendly, cost-effectiveness, and the green technique through white mushroom aqueous extract. The result showed the gardened size was obtained at (20, 18.5, 18, 16.5, and 15 nm) of biosynthesized magnesium oxide. The smaller size was 15 nm which enhanced the seed growth and germination as compared to other sizes. Further, MgO enters into peanut seed and affects its growth rate and germination. This germination found to be high in seed as compared to other sizes. The study was useful in growing peanuts at large scale (Jhansi, K., et al., 2017).

The recent year shows NPs an alternative to antibacterial reagents because they show antibacterial activity in many biomedical applications like gene delivery, drug, tissue engineering and in imaging. It has been shown the synthesis technique advancement for copper, gold, titanium, silver, magnesium oxide and zinc oxide nanomaterials was reviewed and also focus on the toxicity of multi-dimensions of nanomaterials. The remunerations of choosing each material or metal-based composite for definite applications although the effects of toxic nanomaterials on the environment were discussed. These materials could be used as a vehicle for transporting a variety of therapeutic agents, plus drugs, antibodies and pharmaceuticals (Vimbela, G. V., et al., 2017).

The (MgO) magnesium oxide nanoflakes using Bauhinia purpurea leaf extract were biogenic synthesized by alkaline precipitation method. The average particle size was obtained around 10-11 nm. The morphology was investigated by Electron microscopy. The results revealed high efficiency of MgO as a potential antibacterial agent against S. aureus at dose size of 250 µg/ml. Antibiotics are extensively used to control microbial infections. Nonstop experience to antibiotics resulted in antibiotic resistant that leads to the existence of deadly infectious diseases spreading worldwide (Das, B., Moumita, S., et al., 2018).

Green synthesis is a technology and an attention taking area for the creation of metal oxide NPs in chemical and bio-medical applications. The green outlook includes reductants, solvents found from the resource because they are ecofriendly. The magnesium oxide NPs were synthesized by green strategy. Presence of metal oxide and biomolecules were definite. Pure cubic crystalline structure and hexagonal shaped MgO was obtained. The average size was obtained upto 5 nm. The particles of magnesium oxide exhibit inhibition rate upto 200 µg with efficient anticancer activity (Suresh, J., et al., 2018).

It has been shown, the MgO NPs with the use of plant extract were synthesized by chemical method. Nanoparticles, which are inorganic materials and ranged from 1 and 100 nm in size. The pure monoclinic crystalline structure with average size of 10 nm were obtained. NPs due to their small size, differentiate them from their bulk parts The single characteristic of oxygen and magnesium were

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confirmed. The presence of active compounds was accountable for the maintenance of MgO. It has been used for many fields and are functional metal oxide like refractory materials, superconductor, catalysis, and paints (Dobrucka, R., et al., 2018).

The MgO NPs have attained attention in the field of chemistry, material sciences and physics due to their significant properties. It has been shown that MgO were synthesized by facile approach using Aloe vera (A. vera) and Pisidiumguvajava (P. guvajava). Another study was on antimicrobial activity of MgO. The absorbance showed peaks on 221 nm. The results confirm that the used precursors act as capping agents and reducing. The final product was FFC phase and pure. It indicates that MgO has cubic shape and made of MgO. The MgO used in opto-elecetric devices, in biodiesel synthesis and as an antibacterial agent (Umaralikhan, L., &Jaffar, M. J. M. et al., 2018).

It has been shown, the MgO nanoflowers were prepared/synthesized by biological method without capping agent. The particle size obtained was less than 20 nm. MgO have established a countless ability to alter cell membranes of E. coli that cause cell death due to leakage of intracellular content. The antibacterial activity against Xoo strain GZ 0005 attributed to biofilm formation, motility, and its inhibition on growth of bacteria (Abdallah, Y., et al., 2019).

The MgO NPs were synthesized using a reducing agent (marine brown algaeSargassumwighitii). The product was characterized by microscopic and spectroscopic analyses. The structure was FCC and crystalline in nature. The EDX showed presence of oxygen and magnesium. The existence of functional group for sulfatedpolysaccharides was confirmed. The stability of MgO showed at 19.8 eV with size of 68.06 showed antibacterial and antifungal activities. MgO showed cytotoxity. (Peripheral blood mononuclear cells) PBMCs showed non-toxic in nature. The effectiveness of antibacterial might be due to smaller size and its show high bacterial activity with smaller size as reported. The MgO NPs exhibit application in multifaceted biological (Pugazhendhi, A., et al., 2019).

III. CHAPTER 3

MATERIALS AND METHODS

3.1 METHODS

3.1.1 Preparation of corn silk extract

Figure 3. 2 corn silk extract

In present study the main focus was on the synthesis of MgO-NPs from corn silk extract. Green method was used to synthesize MgO-NPs using Magnesium nitrate hexahydrate ((MgNO_3)_2.6H_2 O) and Corn silk extract precursors as reducing agents in order to synthesize corresponding NPs. Small amount of corn silk was used for the extract and rest of it was thrown away as a waste material. For corn silk extract preparation fresh corn silk was collected from a village named Birpani District BaghAj& K. The collected sample was then washed up from a tap water followed by distilled water and then dried at room temperature. Dried corn silk was then grinded using pestle and mortal. In order to prepare extract solution 5g of dried corn silk powder was dissolved and boiled in a 500ml beaker containing 200ml distilled water for 1 hour at 100℃. Acquired extract solution was filtered out with the help of Whatmann's No.1 filter paper. The freshly prepared filtrate/corn silk extract was then stored at 4℃ and further used for the MgO-NPs synthesis. In present study the main focus was on the synthesis of MgO-NPs from corn silk extract. Green method was used to synthesize MgO-NPs using Magnesium nitrate hexahydrate

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$MgNO_3)_2.6H_2O)$orn silk extract precursors as reducing agents in order to synthesize corresponding NPs. Small amount of corn silk was used for the extract and rest of it was thrown away as a waste material. For corn silk extract preparation fresh corn silk was collected from a village named Birpani District BaghAj& K. The collected sample was then washed up from a tap water followed by distilled water and then dried at room temperature. Dried corn silk was then grinded using pestle and mortal. In order to prepare extract solution 5g of dried corn silk powder was dissolved and boiled in a 500ml beaker containing 200ml distilled water for 1 hour at 100 ℃. Acquired extract solution was filtered out with the help of Whatmann'sNo.1 filter paper. The freshly prepared filtrate/corn silk extract was then stored at 4℃ and further used for the MgO-NPs synthesis.

Figure 3.3 corn silk extract

3.1.2 Synthesis of MgO Nanoparticles

Nanoparticles were synthesized by using the method mentioned below:

First of all, 5 gram of dried corn silk extract was added to a beaker containing 200 ml distilled water and then heated at 100℃ for 1 hour. 5 grams of (MgNO3)2 .6H2O was added in to a beaker to prepare 0.1 M solution. After that corn silk extract was added drop wise into the 0.1 M solution of (MgNO3)2 .6H2O heated at 80^0C with continuous magnetic stirring for 2 hours. After half an hour 10ml solution of 1M NaOH was added drop wise in order to adjust the pH 10-12. By using corn silk extract magnesium ions were reduced to MgO-NPs or Magnesia. Color of the solution changed from yellow to yellowish brown indicated the formation of MgO-NPs. The solution was then centrifuged at 10,000 rpm for 10 min and the precipitates were then washed for couple of times with ethanol to remove impurities. The precipitated out complex solution was the dried in an oven at 40℃ for 8 hours and then grinded in mortar and pestle to form powder (Awwad et al., 2014).

Mg (NO3)2 .6H2O + 2 NaOH → Mg (OH) 2 + 2NaNO3 ⟶ Mg (OH) Δ MgO +H2O

Figure 3. 4 (a) dropwise addition of chemicals in corn silk extract (b) change in color after adding chemicals

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Figure 3.5 Complex sample centrifugation

Figure 3. 3 (a) Incubating sample until completely dried (b) dried sample

3.2 CHARACTERIZATION TECHNIQUES

Nano particles were characterized by using different characterization techniques for instance scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), UV/ VIS spectroscopy and X-ray diffraction (XRD).

3.2.1 Scanning electron microscope (SEM)

Scanning electron microscope is a form of electron microscope. It is the advanced form of microscope that is used for scanning and producing images. It produces image of sample by scanning the surface with focused beam of electrons. In the sample the electrons interact with atoms and produce number of information containing signals about topography of a surface and sample composition. Beam of electron is scanned in raster scan pattern. The beam position is then combined with the detected signal intensity in order to form an image. In common SEM mode, atom emits secondary, electrons; these electrons are then excited by beam of electron and are detected by Everhart-Thornley detector. Signal intensity depends upon the number of detected secondary electrons amongst other things on sample topography. It has the ability to achieve a resolution that is better than one nanometer. For MgO-NPs sample was firstly sonicated for about 5 mins in order to form a suspension of MgO-NPs in DMSO and after that a suspension drop was placed on double carbon coating conductive tape and then was placed under a lamp to dry. SEM was performed on a specimen in order to study the morphology of a sample. To visualize the size and shape of MgO-NPs.

Applications that are linked with SEM are as follow:

- One of the most important usage of SEM is it produces high resolution imaging.
- It is used to study microstructures.
- It is also used for examining surface contaminations (Ebnesajjad, S. et al 2011).
- SEM analysis was performed in COMSATS University, Lahore Campus.

3.2.2 UV-Vis Spectra Analysis

UV-Vis spectroscopy is a characterization technique that is used in determining analyte concentration either once or oftenly over desired time period. This technique is used to measure the light absorption across ultraviolet and visible light region wavelength via liquid specimen. The synthesis of MgO nano particles in a reaction solution was monitored at room temperature with the help of UV-Visible spectrometer which was the first ever confirmation test. The solution color was changed and turned from pale yellow to brown color signifying the formation of MgO-NPs. MgO-NPs spectral analysis was documented as function of time along with a distilled water as reference. MgO-

NPs UV spectrum was measured with the wavelengths ranging from 300-700 nm. MgO-NPs absorption spectra formed in reaction media was 20 when recorded at 24 hours. This characterization technique was performed in COMSATS University, Lahore Campus.

Figure 3. 4 Scanning electron microscope

The UV-Vis spectrum was used for detecting structure and sample functional group by using formula:
Aαcl or A=εcl
Where
A= Absorbance
ε = Molar absorptivity
c = Concentration
l = Path length

3.2.3 X-Ray Diffraction (XRD)
X-ray diffraction is used to confirm phase formation and MgO-NPs quality. With the help of XRD analysis the crystal structure, composition and size of centrifuged sample of purified MgO nanoparticles were determined. In COMSATS University Lahore campus this technique was performed. To find out the particles size from width of XRD peaks Debye Scherer formula is used that is:

$$D = \gamma 0.9/\beta \cos\theta$$

Where
D = Domain size of crystalline
K = Scherer constant (0.9-1)
λ = X-ray wavelength
β = full width at half maximum
Cos θ = Bragg diffraction angle

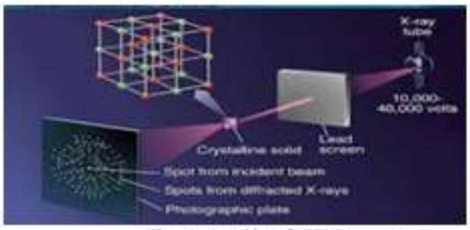


Figure 3. 5 working of UV-Vis spectroscopy

Objectives of X-ray diffraction are stated below:

- It is used to determine crystalline structure.
- It is used to determine lattice parameter information.
- It is also used to evaluate crystal defects and crystalline size. (Chauhan, A. et al 2014)

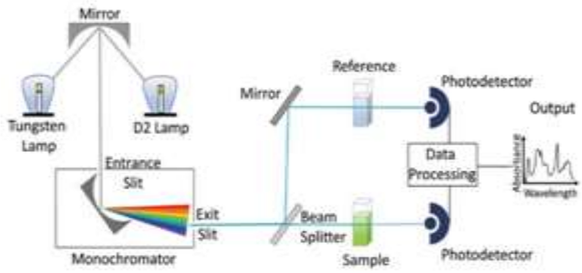


Figure 3. 6 Instrumentation of XRD

Fourier-transform Infrared Spectroscopy

FTIR is an acronym used for Fourier Transform Infrared Spectroscopy which is a characterization technique used to get infrared spectrum of emission and absorption of solid, liquid or gas sample. FTIR collects high spectral data over wide spectral range at once. It has a vital advantage over dispersive spectrometer which can measure intensity over narrow range of wavelength. FTIR is used to find out the functional group of corn silk and MgO-NPs by using KBr pellet method.

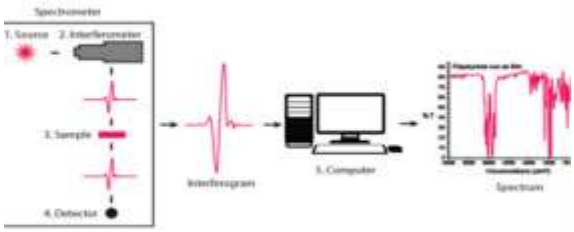


Figure 3. 7 instrumentation of FTIR

To determine absorption spectra at different wavelengths Beer-Lambert law is used which is written as:

��� = ���������

Where,

��� = molar absorptivity (m2mol-1 or M-1cm-1) or how strongly light is absorbed by the chemical species at given wavelength.

b = path length (distance travelled by light through chemical species)

c = chemical species molar concentration

A = absorbance (amount of light absorbed by the sample) (C., Hienerwadel et al 2009)

This part of characterization was done in COMSATS University, Lahore Campus.

3.2 ANTIBACTERIAL STUDIES

The agar well diffusion method was used to testify antibacterial activity of green synthesized MgO-NPs. Organism was inoculated on nutrient agar plates and was spread uniformly. Sterile polystyrene tip (4mm) was used to make wells. Different concentrations of MgO-NPs (5,10,15,20,25 mg/-ml) were prepared separately and used in study. By measuring diameter of zone of inhibition around wells the antimicrobial activity was determined. For antimicrobial assay both positive and negative bacterial strains were used. Overnight grown culture of Gram-negative bacteria: Escherichia coli and Gram-positive bacteria: Staphylococcus Aureus were used to assess MgO-NPs antibacterial activity. Antibiotic Clindamycin phosphate was used as a positive control to determine antimicrobial activity of MgO-NPs. All glassware, reagents and media used were autoclaved at 121˚C for 20 minutes. The turbidity of bacterial culture was adjusted to 0.5% McFarland standard, equivalent to density of 1.5x108 CFU/mL.

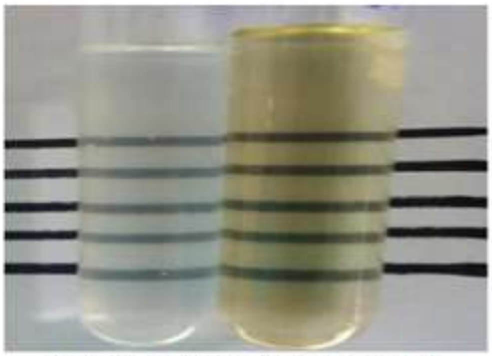


Figure 3. 8 image of McFarland test

3.2.1. Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) is a lowest chemical concentration, that has the ability to prevent bacterium visible growth. For each microbial strain MIC was measured by following the well diffusion method in replicates (N = 3). This is a standard method for antimicrobial study and was employed in a tube serial dilutions of MgO-NPs (100mg/mL) in bacterial growth media.

Figure 3. 9 image of McFarland test

At 37°C pathogenicmicrobes were incubated for about 24 hours and the lowest inhibitory concentration was scored. All experiments were applied in replicates (N = 3) and the mean or average value along with standard deviation was calculated by the descriptive analysis on SPSS statistics version 17.0.

IV. CHAPTER 4

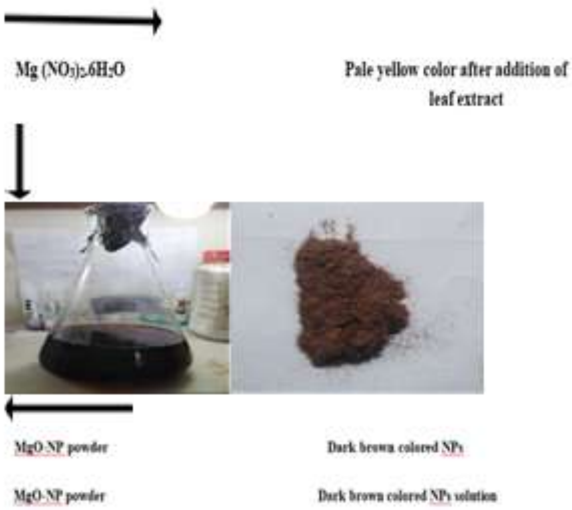
RESULTS AND DISCUSSION

4.1 RESULTS

In nanotechnology synthesis, characterization and uses of biologically prepared nanostructures have remarkable applications. This research study consists of MgO-NPs synthesized from corn silk extract. The experiment was carried out in order to analyze the corn silk extract effect on MgO-NPs synthesis and assessment of antimicrobial activity on magnesium oxide nanoparticles.

4.1.1 Synthesis of MgO-NPs

During the synthesis process of MgO-NPs magnesium nitrate hexahydrate [Mg (NO3)2. 6H2O] was reacted with corn silk extract and sodium hydroxide (NaOH). Solution was continuously stirred on a magnetic stirrer 80˚C for about 2 hours. The ions of magnesium nitrate were reduced to MgO. The extract solution changes its color from pale yellow to brown indicating and confirming the MgO-NPs formation. For each different plant the time duration of color change is different depending on the reducing agents present in that particular plant. In this case of Corn Silk, it took 2 hours to change its color. The phenomenon of color change occurred because of excitation of (SPR phenomenon) Surface Plasmon Resonance. Change in color of a solution indicated reduction of Mg (NO3)2 .6H2O and formation of MgO. The complex solution was then centrifuged at 10,000 rpm for 10 minutes. Complex was then dried at 40˚C for 8 hours to remove the impurities and get MgO-NPs.



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4.1.2 Characterization Results

4.1.2.1 UV-Vis absorption spectrophotometric analysis

In order to determine and confirm that MgO nanoparticles were synthesized successfully, UV-Vis is a technique that was used for determining structural characterization of a synthesized MgO-NPs

Absorbance at the wavelengths that are ranging from 200-800 nm were determined. This research is based on UV-Vis spectroscopy results in which it was found that there is only one peak of UV-Vis spectrum in range of 200 nm to 400 nm. At 250 nm the highest peak was observed which can be ascribed to MgO nanoparticles. UV-Vis spectrum of synthesized colloidal MgO-NPs using corn silk extract and the cutoff peak is around 221 nm for both precursors. Following relation can be used to find out the band gap.

Eg= 1240/wavelength

By using the above mentioned formula, MgO-NPs bandgap energy was found out to be 5.6 eV. Particle size value and the MgO bandgap clearly shows that these nanoparticles can be used as antimicrobial, optical device fabricator and photo catalytic activator.

4.1.2.2. Scanning electron microscope

SEM analysis is basically used to study the morphology of a sample, size and shape of MgO-NPs was find out by SEM analysis. This analysis showed that MgO-NPs particles are well dispersed as shown in figure. The scanned images of a sample show that morphological surfaces are in form of nanoparticles assemblies having round shapes. MgO-NPs are uniformly distributed over entire surface. SEM images show that the size of nanoparticles is less than 20nm.

4.2.1.3. X-Ray diffraction study (XRD)

XRD characterization technique is used to check phase formation and quality of MgO-NPs. The X-ray diffractogram contains multiple peaks which are clearly distinctive from one another. All diffraction peaks were indexed readily to various crystal planes of Cubic phase MgO. There are no secondary peaks that can be detected, indicating that prepared product through this route is highly pure. Moreover, MgO NPs average crystalline size that is calculated from XRD was 50 nm according to the Scherer's equation

4.2.1.4. FT-IR analysis

FT-IR spectroscopy is a technique that is used for absorption measurement of Infrared radiations by plotting the sample against wavelength. Interpretation of IR spectrum involves correlation of absorption bands with chemical compounds in a sample. That way, biomolecules present in the extract of plant that are responsible for the reduction and stabilization processes of nanoparticles green synthesis can be identified. FTIR spectra of MgO nanoparticles synthesized by corn silk are recorded in range of 500 to 4000 cm-1 as shown in Fig.

IR spectrum of corn silk shows band at 3290 cm-1 which is because of stretching vibrations of O-H group in water, phenol, alcohol and N-H stretching in amines. At 2917cm-1 and 2849 cm-1 C–H stretch in alkanes and O–H stretch in carboxylic acid appear respectively. C=C stretch in aromatic ring and C=O stretch in polyphenols is attributed as strong band at 1649 cm-1. Band at 1367 cm-1 is given by C-N Stretch of Amide-I in protein. C–O–C stretching in polysaccharides causes a band at 1732 cm-1. C–O stretching in amino acid gives a band at 1045 cm-1. Weak band at 835 cm-1 resulted because of plane bending. As a result, it can be observed from IR spectrum that corn silk specimen is rich in carboxylic acid, polyphenols, amino acid, polysaccharide and proteins. The peak present at 600-835 cm-1 strongly confirm stretching vibrations of Mg-O.

4.1.3. Antimicrobial study

Agar well diffusion method was used to measure bacterial resistance of green synthesized MgO. Microorganisms used in experiment were gram positive bacteria- Staphylococcus aureus, Streptococcus pyogenes and gram negative bacteria Escherichia coli, Klebsiellapneumonia, Serratia Marcescens. The efficiency of the antibacterial agent to inhibit the growth of bacteria was determined by the diameter of zone of inhibition.

Agar well diffusion method was used to measure bacterial resistance of green synthesized MgO. Microorganisms used in experiment were gram positive bacteria- Staphylococcus aureus, Streptococcus pyogenes and gram negative bacteria Escherichia coli, Klebsiella pneumonia, Serratia marcescens. The efficiency of the antibacterial agent to inhibit the growth of bacteria was determined by the diameter of zone of inhibition.

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Organisms	No of samples	Antibiotic (CliPO4)	ZOI (mm) of NPs against isolations at different concentration			
			0.5 mg/ml	1mg/ml	5mg/ml	10mg/ml
S. aureus	5	21.33±1.24	7.33±1.24	14.33±1.24	26.33±1.24	28.33±1.24
E. coli	5	18.33±1.24	9.33±1.24	21.33±1.24	23.33±1.24	28.33±1.24
S. Mre	5	27.33±1.24	8.33±1.24	16.33±1.24	25.33±1.24	26.33±1.24
S. pyo	5	18.33±1.24	8.33±1.24	16.33±1.24	26.33±1.24	27.33±1.24
Klebsiella	5	27.33±1.24	7.33±1.24	15.33±1.24	24.33±1.24	25.33±1.24

Table 4.1: ZOI of different concentration of MgO-NPs against Staphylococcus aureus, Escherichia coli, Klebsiellapneumonia, Serratia marcescens and Streptococcus pyogenes.

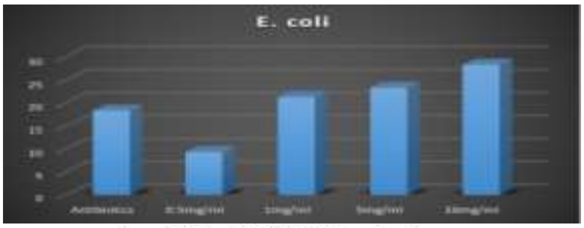

Figure 4.1 Mean ZOI of MgO-NPs against S. aureus

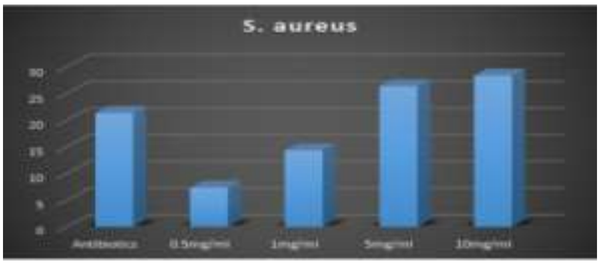

Figure 4.2 Mean ZOI of MgO-NPs against E. coli

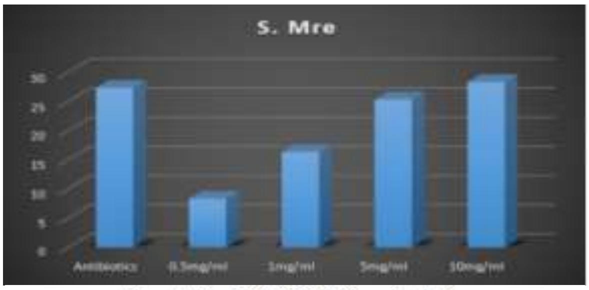

Figure 4.3 Mean ZOI of MgO-NPs against S. Mre

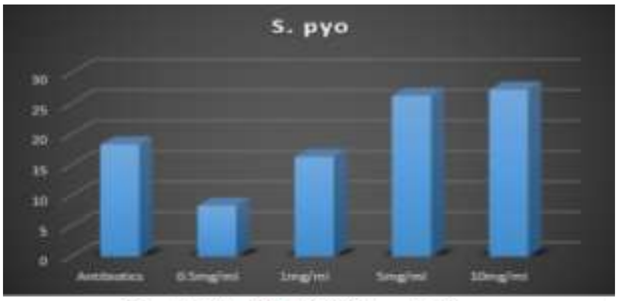

Figure 4.4 Mean ZOI of MgO-NPs against S. pyo

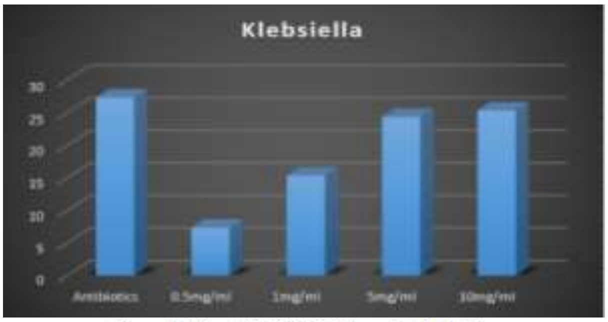

Figure 4.5 Mean ZOI of MgO-NPs against Klebsiella

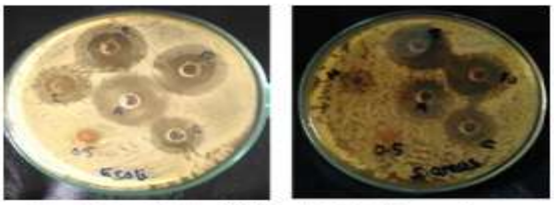
Figure 4.6 Antibacterial activity of MgO-NPs against E. coli and S. aureus pathogen

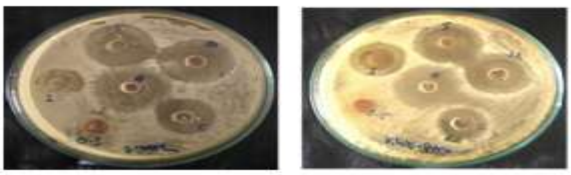
Figure 4.7 Antibacterial activity of MgO-NPs against S. Mre and Klebsiella

Antibacterial activity of a green synthesized MgO nano particles was determined by agar well diffusion method against gram positive bacteria- S. aureus, Streptococcus pyogenes and gram negative bacteria E. coli, Klebsiella and S. Mre. They are bacterial pathogens. Clindamycin phosphate was used as a standard antibiotic in agar well diffusion method. The ZOI of antibiotics was observed at (21.33±1.24) in case ofS. aureus, (18.33±1.24) in E. coli case, S. Mre at (27.33±1.24), in Streptococcus pyogenes (18.33±1.24) and in case of Klebsiella (27.33±1.24) ZOI was observed.

Figure 4.8 Antibacterial activity of MgO-NPs against S. pyo

Mean inhibition zone indicates maximum antibacterial activity of a prepared test sample of MgO-NPs and it ranged from 15-25 mm. The MgO-NPs synthesized from corn silk displayed maximum ZOI against S. aureus and E. coli (28.33±1.24 mm) and minimum ZOI was observed (7.33±1.24) at Klebsiella. In case of S. aureus ZOI was observed at (28.33±1.24) and minimum at (7.33 ±1.24). In case of E. coli maximum ZOI was observed (28.33±1.24) and minimum ZOI was observed as (9.33 ±1.24). In Streptococcus pyogenes maximum ZOI was observed (27.33±1.24) and minimum ZOI was observed as (8.33 ±1.24). In S. Mre case maximum ZOI was observed (26.33±1.24) and minimum ZOI was observed as (8.33 ±1.24). Klebsiella has case maximum ZOI was observed (25.33±1.24) and minimum ZOI as (7.33±1.24). As per mentioned in Table 4.1.

4.2 DISCUSSION

In present years more focus is given by researchers to the synthesis of MgO nanoparticles because of their novel applications in advanced technology. Metal oxides are very significant in technological devices. MgO has good bactericidal show because of formation of super oxide and various properties like catalytic property can be enhanced if used in nano sized particles compared to micro sized particles. These MgO-NPs can be synthesized using extract obtained from unicellular organisms for instance bacteria and fungi and also from the extract of different plants (J. Suresh et al., 2014).

This research reports the facile synthesis of MgO-NPs using extract of Pisidiumguvajava (P. guvajava) and Aloe vera (A. vera). For synthesis antibacterial activity was studied using gram negative and E. coli and gram positive S. aureus. Precursors used act as reducing agent and controlled MgO-NPs well enough. XRD in this study confirms the formation of MgO-NPs using precursors MgO and plant extract. UV-Vis showed cutoff peak at 221mm for both precursors. FTIR showed strong O-H bond stretching at 3433cm-1 because of water and alcoholic content and peaks at 2372, 2084, and 2079cm-1 are cause of alkenes and carboxylic acids (Umaralikhan& Jamal Mohamed Jaffar, 2018).

In present research work FTIR of MgO-NPs showed wide range stretch of O-H group at 3290 cm-1, C=C group at 1649 cm-1, C-H at 2917 cm-1, and C-O-C, C-N groups at 1732 and 1367 cm-1 respectively which matches nearly to that of extract. Oxidized polyphenols were examined on the synthesized MgO-NPs. May be it is supposed that polyphenols in corn silk extract function as capping and reducing agent. (Discov&Ksv, 2017)

The formation of ecofriendly metal oxide nanoparticles using biogenic source is used currently for the synthesis purpose replacing toxic chemical methods. In this study green synthesis of MgO-NPs take place using aqueous extract of S. wighitii and brown marine as reducing and capping agent, UV-Vis of MgO-NPs showed strong absorption peak at 322nm and evaluation using PBMCs illustrated MgO-NPs as non-toxic in nature (Pugazhendhi et al., 2019).

This research work exhibited antibacterial activity of MgO-NPs against S. aureus whereas against E. coli it showed less antimicrobial activity related results were also found in this work showing that MgO particles had effective antibacterial activity towards E. coli and S. aureus by piercing into bacterial cell wall and cell membrane (Tang &Lv, 2014).

Excellent antibacterial efficiency was exhibited by MgO-NPs (Palanisamy&Pazhanivel, 2017). Research study in this indicates that against both E.coli and P. aeruginosa pathogens MgO-NPs exhibited efficient antibacterial activity (Nguyen et al., 2018).

Clear size effect was revealed in results showing that the amount of bacteria killed was strongly dependent on the size of particle (Gandhi et al., 2012). E. coli's cell membrane consists of several layers of proteins, lipids and lipopolysaccharides thus inhibiting against magnesium oxide (MgO) while S. aureus's cell membrane consists of only one thick peptidoglycan layer which contains mixture of sugars and amino acids. Hence the interaction between S. aureus and MgO are stronger than with E. coli (Ramanujam&Sundrarajan, 2014). MgO-NPs have the ability to alter and damage cell membranes of E. coli which then results in the leakage of intracellular content of the MgO-NPs and die eventually (Jin& He, 2011).

In this research work MgO-NPs antimicrobial properties were evaluated by their MIC against gram positive and gram negative bacteria. Amongst different MgO-NPs concentration S.aureus showed 7.33mm ZOI at 0.5mg/ml while E.coli, S. Mre, S. pyo and Klebsiella showed 9.33mm, 8.33mm, 8.33mm, 7.33mm ZOI respectively at 0.5mg/ml (Zhu et al., 2016).

In present study S. aureus, E. coli, S. Mremarcenes, Streptococcus pyogenes and K. pneumonialebsiella showed maximum ZOI at 28.33mm, 28.33mm, 26.33mm, 27.33mm, and 25.33mm respectively. It can be seen that the zone of inhibition was found to be more in gram positive bacteria as compared to gram negative bacteria's. Zone of inhibition effects of MgO nanoparticles take place by inhibiting the growth of microorganism using electrochemical mode of action to penetrate and disturb the cell walls.

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