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GREEN SYNTHESIS OF MAGNESIUM NANOPARTICLES FROM *ACTINOMYCETES* AND THEIR THERAPEUTIC APPLICATIONS

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ABSTRACT

Nanoparticles are becoming popular and taken the charge of novel research that will provide the significant role in resolving the problem easily. Different nanoparticles have been synthesized by number of various methods. Simultaneously microbial viz. actinomycetal nanoparticles were synthesized by using *Streptomyces* and magnesium nitrate hexa hydrate, $Mg(NO_3)_2 \cdot 6H_2O$ termed as Actinomycetal Mg NPS. These were microbially synthesized nanoparticles while antagonistic action of these nanoparticles was tested using pathogenic microorganisms present in water as one gram-negative and other gram positive microorganisms'. The results indicated that microbially synthesized actinomycetal Mg NPs were effective nanoparticles against water-borne pathogens. XRD patterns of prepared nanoparticles were characterized and XRD pattern revealed the formation of manganese nanoparticles. Mg nanoparticles were subjected to UV-Visible spectrum showed absorption peak which was lies in between 300 to 430 nm. TEM suggested that the particle size lies in the range of 100 to 125 nm.

Keywords: *Actinomycetes*, Mg NPs, Pathogen, Antagonism.

1. INTRODUCTION

The nanoparticles are innovation and very applicable in most of the fields. These are way synthesized chemically and recently it has been viewed as a way to synthesize by using microorganisms, including bacteria and fungus. Nowadays actinomycetes are in current use for the biosynthesis of nanoparticles and actinomycetal nanaoparticles are showing the verity of application in each field of technology where the term used as biological application. The actinomycetal nanoparticles are ecofriendly, do not cause any type of pollution or side effects and easy to handle. It is not hazardous for human being because the main source is biological rather than the other ways like chemical. Actinomycetes are the group of microorganisms. Nanoparticles synthesized by microorganisms in which actinomycetes were used for the synthesis of nanoparticles have a great importance and these nanoparticles are important to showing highly toxic against bacterial cells.

Compared to the chemically prepared nanoparticle, the microbially synthesized nanoparticles are effective and showing valuable results. Among them, nanoparticles prepared using actinomycetes are becoming more important as a concern with their applications. Different types of microbial cells and various metals are used for the preparation of microbially synthesized nanoparticles. Metal oxides are generally and mostly used for such type of synthesis and easy to synthesize by any route. The particles that fall in the range of 0.1 to 100 nm have shown the range of ideal properties such as identical strength as its resistant to crushing, having discrete energy levels, active surfaces which have important catalytic efficiency. As far concern with the size and shape of the nanoparticles it is simplified in the term of morphology which is important for their function in each field of their application.

The properties of nanoparticles are important for their functions, for example, smaller particles are more effective than larger nanoparticles. As they became smaller, it increases the capacity of influence to do their action. The microbially synthesized silver nanoparticles using actinomycetes were found to be highly toxic to bacteria and it was found that smaller silver nanoparticles synthesized by microbial route had a greater antibacterial activity when compared to their chemical priorities. In some cases some bacterial cells might be silver resistant, these bacterial cells can resist the action of silver, even these bacterial cells were accumulated the silver

nanoparticles in their periplasmic space [1-3]. This view focus on microorganisms that can synthesize nano-particles simply termed microbial nanoparticles. Mostly among microorganisms bacterial cells, fungi, and some yeast cells have synthesized nanoparticles. The ionic metals are effective against bacterial cells including pathogens. The oligodynamic effect of the metal ion is in use for inhibition of pathogenic microorganisms viz. copper, gold likewise some silver nanoparticles are in high demand which includes their wide applications. Microorganisms including saline soil actinomycetes are mostly involved in the degradation of carbofuran pesticide. The salt tolerant actinomycetes can be help for pesticide degradation. [4]

Along with magnesium are widely used in the preparation of nanoparticles. These MgO nanoparticles have been recorded for their antimicrobial activity. Magnesium ferrite ($MgFe_2O_4$) has shown the cubic, n-type semiconducting, good I-V characteristics. Property finds the various applications as heterogeneous catalysis, adsorption, sensors, magnetic technologies and used for the preparation of microbial nanoparticles using *Bacillus subtilis*. These biologically synthesized Mg nanoparticles are used for gas sensing properties, act as good biosensors, where they considerably sense CO_2 and different toxic gases with rapid response and helps in solving environmental pollutions and keep safe away from any toxicity [5].

The comparative study was made in which silver nanoparticles were synthesized through microbial route and chemical route, the results showed that chemical reduction of silver ions (Ag^+) using borohydride in aqueous solution yielded silver nanoparticles, and it was observed that the reduction process of silver is slow in the chemical method as compared to microbial synthesis method. The microbial synthesis of silver nanoparticles is rapid, which is one good advantage of microorganisms in the preparation of nanoparticles [6]. The nanoparticles are capable of showing a wide range of different properties; some of these properties are ideal and unique. The nanoparticles are identical in strength as they are more resistant to crushing. They have an active surface, which has important catalytic properties. The nano-particles have electronic properties. Microorganisms are also involved in the decomposition process, simply the process termed as bioweathering process as well as microorganisms are commonly involved in the degradation process; the term also refers to bio-degradation [7]. Resistance is shown by micro-organisms ranges from these microorganisms synthesize some of the redox enzymes that convert toxic metal ions to inert forms and structural proteins. These inert forms or structural proteins also show some other functions like they are involved in the transportation process where they transport the metal ions. For the transport of metal ions, these inert forms do create the proton motive force. For the transport of metal ions, these inert forms do create ATP hydrolysis along with proton motive force [8]. This type of mechanism and activity helps the synthesis of nanoparticles by microorganisms. Different microorganisms are involved in the biosynthesis of nanoparticles including fungi also. The fungi like *Fusarium oxysporum* and *Verticillium* were used for the biosynthesis of nanoparticles where these nanoparticles were used for the production of magnetite and silica nanoparticles [9]. It has been studied that when metallic nanoparticles are formed, they are well stabilized by the proteins where these proteins will try to bind with synthesized nanoparticles. In their binding, the free amino group of the protein is involved in binding and cysteine is one type of amino acid residue present in the protein responsible for the binding of protein with nanoparticles [10]. That indicates microorganisms and their metabolites are most actively involved in nanoparticle synthesis and have the affinity towards the nanoparticles is a key aspect of microorganisms.

2. MATERIAL AND METHODS

2.1. Source of actinomycetes

The soil sample is the major source of actinomycetes, the most and variety of actinomycetes present in soil samples. Hence the soil sample was selected for the isolation of actinomycetes, fresh soil samples were used for the isolation. Soil samples from the heavy metal polluted area and also soil samples from non-heavy metal polluted were selected for the isolation of actinomycetes. The glycerol asparagine agar media was used for the isolation of actinomycetes. Serially diluted soil samples were used for isolation of actinomycetes. Selected dilution was poured in petriplate along with the molted media. Plates were allowed to solidify and kept for incubation. Isolation of actinomycetes was carried out by pour plate technique. Media supplemented with griseofulvin 25µg/ml was supported for the isolation of actinomycetes that inhibits the growth of other contamination like fungi and most bacterial cell other than actinomycetes and favors the growth of actinomycetes. Plates were incubated at room temperature for 3-4 days, the incubation period is favored for the growth of actinomycetes. The colony of

actinomycetes was embedded in asparagine glycerol agar media. The powdery colony of actinomycetes appeared on media. The pigmentation, colony size, and powdery appearance to the colony is the primary indication of actinomycetes where the colony of actinomycetes was embedded in the media, such colony was used for further study. Once actinomycetes were isolated, the different strains of actinomycetes were preserved on glycerol asparagine agar slant as well as on nutrient agar slant. The growth of actinomycetes was well developed on nutrient agar medium also and then subjected to coverslip culture technique for identification of actinomycetes.

2.2. Identification of actinomycetes

The coverslip culture technique was used for the identification of actinomycetes, in which the growth of actinomycetes raises on the surface of coverslip and the coverslip was then observed in a microscope to get the structure and arrangement of the spore, which indicated as the genus of isolated actinomycetes. The method is useful for the identification of actinomycetes up to the genus level. The structure and arrangement of the spore gives an idea about the particular genus of actinomycetes. Further identification of actinomycetes was carried out by cell wall analysis and biochemical tests. The bio-chemical tests viz. sugars utilization, nitrate reduction, urea hydrolysis, amino acid deamination, amino acid decarboxylation, IMViC, H_2S, starch hydro-lysis, gelatin liquefaction, oxidase, etc. were carried out for the identification of genus up to species level.

2.3. Production of Biomass

The isolated strains of actinomycetes were subjected for biomass production because the biomass is required for the biological production of nanoparticles. The spores of isolated actinomycetes were inoculated on glycerol asparagine broth or nutrient broth. The nutrient broth was mostly used for the production of biomass in this study. The broth tubes were incubated at room temperature for 48 hours to 10 days. The broth was showing the actinomycetal biomass left on the edge of the test tube as well as on the surface of nutrient broth.

The biomass was then harvested by filtration system in which Whatman filter paper was used for the harvesting of actinomycetal biomass, the paper did trap the filamentous structure of actinomycetes. The trapped filamentous structure was actinomycetal biomass. The filtered biomass was then washed by using distilled water resulted to get pure biomass. Distilled water used for washing purposes was doubled diluted

The filtered biomass was then incubated along with doubled distilled water, the mixture was kept for 24 hours. With proper incubation, the cell biomass was then separated from the layer of distilled water. Washing of biomass and incubation of biomass in distilled water was carried out for getting the pure actinomycetal biomass which helps in further study to the synthesis of actinomycetal nanoparticles. Obtained biomass said to be an actinomycete mat, such prepared mat of actinomycetes biomass was used for the preparation of nanoparticles. The harvested biomass was then incubated along with doubled distilled water, the mixture was kept for 24 hours. Again, the mixture was filtered through the Whatman filter paper by filtration process to get the pure actinomycetal biomass. Such fresh biomass or mat was better to use for the preparation of nanoparticles. Such biomass or mat (actinomycetal) was weighted. Approximately 20 gm of fresh biomass was ground with the help of mechanical motor or mortar or pestle device to get uniformly blend. This uniformly blended biomass was then mixed with 200 ml of Millipore water in a 500 ml Erlenmeyer flask. All the material was allowed to be agitated while kept at 37°C or room temperature for 72 hours. For this, applied term was BIO.

The same process was repeated along with the previously obtained filtered water, where 20 ml of filtered water was taken along with 200 ml of Millipore water in a 500 ml Erlenmeyer flask. All material allows agitating while kept at 37°C or room temperature for 72 hours. For this, applied term was WAT. Used biomass for the preparation of nanoparticles termed as BIO and the filtered water sample used for the preparation of nanoparticles was termed as WAT. Twice samples were used in the study for the production of nanoparticles. Simultaneously microbial viz. actinomycetal nano-particles were synthesized by using *Streptomyces* and magnesium nitrate hex hydrate, $Mg(NO_3)_2{\cdot}6H_2O$ termed as actinomycetal Mg NPS.

These nanoparticles were synthesized by the microbial way. In which, microbial viz. previously synthesized BIO and WAT separately treated with magnesium nitrate hexahydrate $Mg(NO_3)_2{\cdot}6H_2O$ simply. A 50 ml of 1mili molar $Mg(NO_3)_2{\cdot}6H_2O$ mixed with 50 ml of BIO in Erlenmeyer flask and the mixture was agitated at 37°C in dark condition. 50 ml of 1mili molar $Mg(NO_3)_2{\cdot}6H_2O$ was mixed with 50 ml of WAT in Erlenmeyer flask and the mixture was agitated at 37°C in dark condition. Simultaneously one control was kept for BIO and WAT without $Mg(NO_3)_2{\cdot}6H_2O$.

The changes were recorded in the term of color change while and after incubation. The change in color indicated that various microorganisms are known to reduce the Mg^+ ions and to produce magnesium nanoparticles. The control was made by 50 ml of Distilled water mixed with 50 ml of WAT in an Erlenmeyer flask and the mixture was agitated at 37°C in dark condition; this was control made for WAT. Similar control was made by 50 ml of Distilled water mixed with 50 ml of BIO in Erlenmeyer flask and the mixture was agitated at 37°C in dark condition; this was control made for BIO. In the case of control, no changes were recorded in the term of change in color while and after incubation. While in the case of samples, the color change indicated that various microorganisms are known to reduce the Mg^+ ions and to produce magnesium nanoparticles, Change in color in case of samples matches with the control in which no change in color. Without any change in color in control matched with change the color in samples did predict that the nanoparticles were synthesized in samples tube by microorganisms.

In the case of sample, color changes in the sample test tube were observed at specific intervals like every 12 hours and up to 72 hours, eventually noted the change in color at 72 hours. Turn the color, this period was considered as supposed to be final reading.

2.4. Characterization of nanoparticles

The synthesized nanoparticles were characterized by XRD was performed with a Shimadzu XRD-6000 diffractometer with Cu-X radiation ($\lambda = 1.5405$ Å), UV-Visible Spectroscopy, A JEOL JEM-200CX transmission electron microscope was operating at 200 kV for the said analysis. This analysis was used for the characterization of microbially synthesized nanoparticles. This helps to know the different properties of nanoparticles that were synthesized by the microbial way.

2.4.1. TEM Study

The morphology and size of Mg nanoparticles were determined by TEM measurement. Mg nanoparticles were synthesized by the microbial way were analyzed by TEM. The detailed morphology and crystalline structure of two MgNPs samples, calcined at 600°C for 4 h were further studied using TEM.

2.4.2. UV- Vis spectroscopy

Magnesium nanoparticles synthesized by microbial way were analyzed through UV- Vis spectroscopy. The systronic double beam spectrophotometer was used for the analysis. Mg nanoparticles synthesized by microbial route, were analyzed in UV-Vis spectroscopy at the wavelength at 250 to 500 nm, which was a suitable wavelength for the analysis of nanoparticles synthesized by the microbial way. This characterization was applied for the nanoparticles which were synthesized by the microbial route. The study helps to know the differentiation between both types of nanoparticles. Change was appeared in both nanoparticles which were synthesized by the microbial route. In this study, the characteristics of nanoparticles that were synthesized by microbial route were taken into consideration.

2.4.3. Antagonism

It is one type of therapeutic analysis of prepared samples of nanoparticles. These nanoparticles were tested against human pathogens some of them were gram positive and gram negative were selected for the study. The antimicrobial activity was tested against gram positive bacteria like *Staphylococcus aureus* ATCC 25923 (multidrug resistance strain), *Bacillus subtilis* ATCC 6633, and gram negative bacteria like *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603. The antimicrobial activity was tested interms of zone of inhibition. The antimicrobial activity of synthesized nanoparticles was tested by the disc diffusion method, in which pure culture of gram positive and gram negative microorganisms was poured in molted sterilized nutrient agar media separately. All plates were allowed to solidify, future wells were prepared aseptically at a specific distance, in seeded nutrient agar plate with cork borer in nutrient agar media. Labelled the wells as BIO, WAT, and Mg chemical prepared nanoparticles used as a control. A 0.2 ml sample of synthesized nanoparticles was loaded as chemical, BIO, and WAT to respective well by micropipette. All plates were kept at low temperatures for diffusion purpose for 15 to 25 minutes. Plates were incubated at 37°C for 24 hours in the incubator, with proper incubation, the plates were observed for the zone of inhibition for each culture of gram positive and gram negative bacteria around the well. The results were recorded in cm or mm.

3. RESULT S AND DISCUSSION

Powdery colony on the plate was indicated as the presence of actinomycetes, and through coverslip culture technique and biochemical analysis, it was found that the isolated actinomycete was *Streptomyces. Streptomyces* was then subjected for production of nanoparticles termed as Mg NPs in the way of BIO and WAT. All the samples

viz. microbial (BIO and WAT) were well analyzed in this study. Distinct changes appeared in the case of both nanoparticles which were synthesized by the microbial route. In the study, the nanoparticles which were synthesized by microbial route were taken into consideration. The conversion of yellow to brown color appearance indicated the formation of nanoparticles by microorganisms like actinomycetal biomass. In this mechanism reduction of metal ions was carried out by microorganisms resulted in formation of magnesium nanoparticles. Mg^{+} ions react with actinomycetal biomass result in the reduction of metal ions, indicated by a change in color from light yellow to dark brown, this is the primary indication of nanoparticles. These nanoparticles were synthesized with BIO, WAT way. The dark brown color mostly appears to BIO than WAT way, indicating the presence of maximum magnesium nanoparticles henceforth the BIO was used for the future study; also considered as BIO actinomycetal Mg NPs. Now as a eco-friendly approach, most of microorganisms were used for the production of nanoparticles like microorganisms were used for production of silver nanoparticles Different microorganisms reduce the silver ions resulted to form silver nanoparticles [11-12]. Microbially synthesized nanoparticles with BIO were subjected to characterization by different patterns of analysis in which they were subjected to UV- Visible spectroscopy and found absorption peak at 410 nm, which was indicating the formation of magnesium nanoparticles as shown in fig.3.

3.1. XRD Study

The crystal structure and phase composition of MgO NPs were determined using the X-ray diffraction (XRD) technique (Fig.1). The XRD pattern suggests that the sample is probably nanocrystalline, which matches very well with that of the standard. X-ray diffraction (XRD) of actinomycetal Mg NPs was performed with a Shimadzu XRD-6000 diffractometer with Cu-X radiation (λ= 1.5405 Å). The data was collected at room temperature in the range of 2θ between 30 and 75°. This adsorbent has high crystallinity with d values. A similar result has been obtained in the literature [13-14]. Traditionally, the broadening of peaks in the powder's XRD patterns of polycrystalline solids is attributed to particle size effects. The mean crystallite size of a powder sample was estimated from the full width at half-maximum FWHM of the diffraction peak by the Debye-Scherrer equation:

$$d = 0.9\lambda / \beta\cos\theta$$

In this equation, d is the average particle size (nm), k is the X-ray wavelength (λ = 0.154 nm), k is the constant (k = 0.90), β is the FWHM of the most intense peak, and θ is the corresponding diffraction angle. The use of the Debye-Scherrer formula to estimate particle size is widely used, especially in the study of nanoparticulate materials [15-16]. We estimated the particle size of the synthesized Mg NPS from the line width as 90 nm using the Debye-Scherrer equation.

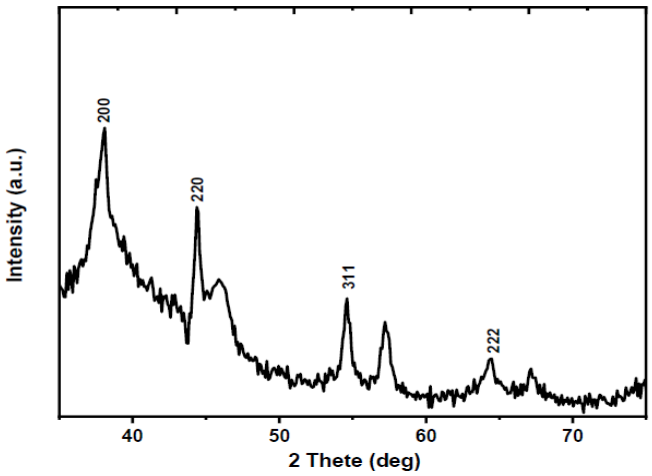


Fig.1: XRD analysis of BIO actinomycetal MgNPs

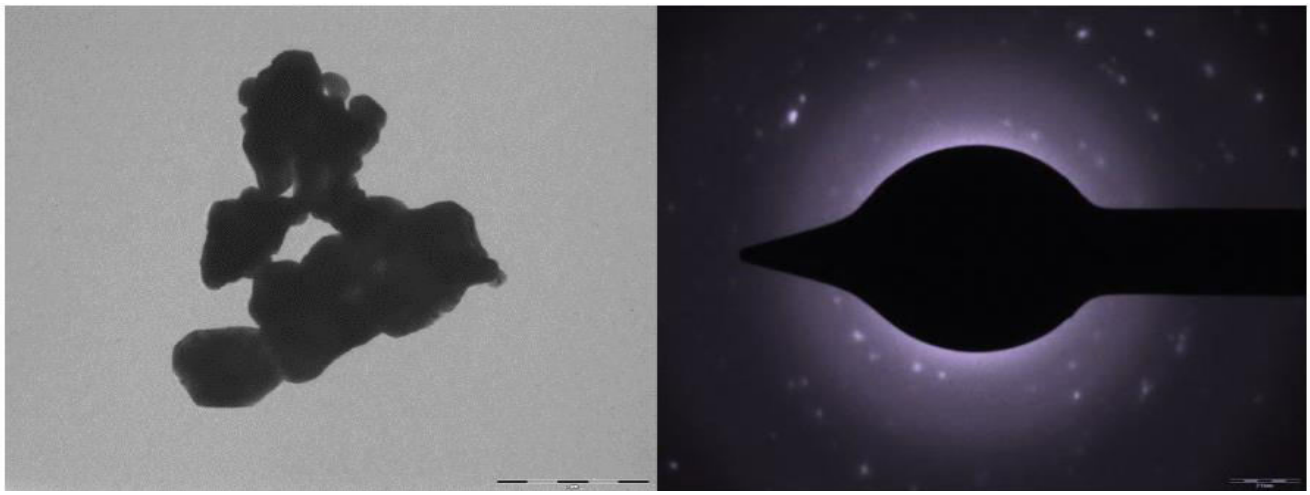
3.2. TEM Study

TEM bright-field images with corresponding selected area electron diffraction (SAED) patterns of the samples are shown in fig. 2. From the TEM bright-field images, it is seen that the samples consist of packed MgNPs crystallite particles of ~ 100-125 nm diameter and the particle size of MgNPs was seen to be uniform. From the TEM bright-field images, it was seen that the samples consist of packed microbial nanoparticles (BIO), and the size of synthesized nanoparticles by the microbial way (BIO) was near to 100 nm diameter.

3.3. UV- Visible spectroscopy

Microbially synthesized nanoparticles with BIO were subjected to characterization by different patterns of analysis in which they were subjected to UV- Visible spectroscopy and found to be absorption peak at 410 nm, which was indicating the formation of magnesium nanoparticles shown in fig. 3.

Rhodopseudomonas capsulata produced spherical nano-particles when they were treated with chloroauric acid ($HAuCl_4$) solution at the pH range of 4.0 to 7.0 for the incubation period of 48 hrs. The synthesized nano-particles are in the range of 10-20 nm [17].



a. Size of synthesized Mg nanoparticles by the microbial way (BIO) b. Size of synthesized Mg nanoparticles by the microbial way (WAT).

Fig. 2: TEM images with corresponding SAED patterns of the MgNPs samples calcined in air for 4 h at 600°C.

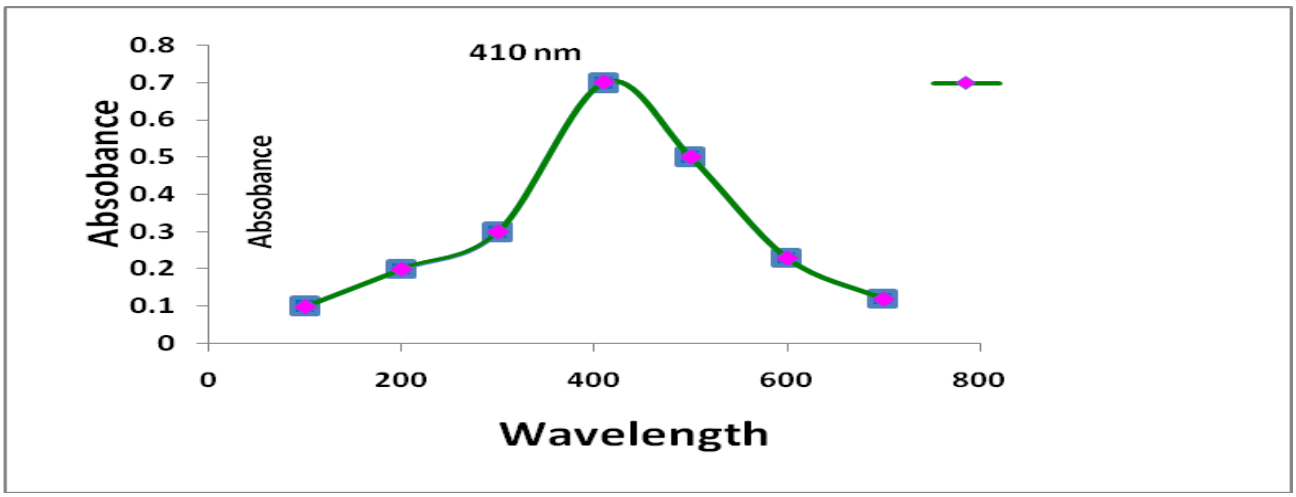


Fig. 3: UV-vis spectroscopy of microbially synthesized magnesium nanoparticles.

3.4. Antimicrobial activity of actinomycetal MgNPs

The antimicrobial activity of microbial magnesium nanoparticles was investigated for their therapeutic value in medicine. The antimicrobial activity was investigated against the different human pathogens. The antimicrobial activity was tested against gram positive bacteria like *Staphylococcus aureus, Bacillus subtilis,* and gram negative bacteria like *Klebsiella pneumonia, Escherichia coli*. All of them are human pathogens, some of them are water-borne pathogens. All the tests were carried out by the well diffusion method. The highest antimicrobial activity was observed against gram negative bacteria *Escherichia coli* followed by *Klebsiella pneumonia, Staphylococcus aureus,* and the least antimicrobial activity was noticed against *Bacillus subtilis.* It was shown that the antimicrobial activity of microbially synthesized nanoparticles was effective against all selected pathogens. These results were compared with results of chemically synthesized Mg nanoparticles used as a control was shown in table1and fig. 4.

The *Bacillus subtilis* is a human pathogen showing sensitivity only against microbially synthesized nanoparticles but not against control as chemically synthesized nanoparticles, the pathogen was resistant to chemically synthesized nanoparticles. The result comply with the results of previous studies in which the magnesium oxide nanoparticles shown antibacterial activity alone or with the combination of other

antimicrobial agents also [18-20]. It is also reported that the antimicrobial activity of magnesium oxide nanoparticles shown strongest activity against the gram positive microorganisms as compared to the gram negative microbial cells [21]. The same type of results was observed in the case of nanoparticles synthesized by silver nanoparticles, investigated against different human pathogens carried out by paper disc method in which the silver nanoparticles were tested against gram positive and gram negative microorganisms. The tested gram positive pathogen was belongs to *Staphylococcus aureus* and gram negative pathogens were belongs to *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia,* and *Proteus vulgaris*. The highest antimicrobial activity of silver nanoparticles was found against *Pseudomonas aeruginosa* than the *Staphylococcus aureus*, *Klebsiella pneumoniae* while least was noticed against *Proteus vulgaris* [6]. The main base of the research is a bioreduction process carried out by microorganism viz. actinomycetes and by the process of bioreduction actinomycetes were synthesized nanoparticles. This is an enzymatic process, magnesium ions were reduced by reductase enzyme which was extracellularly synthesized by actinomycetes. With the reduction process, magnesium ions were reduced to form magnesium metal which was in the nanometer range. NADH dependant reductase enzyme was involved and play the important role in this process to synthesize magnesium nanoparticles, which was detected from the protein assay of actinomycetes, NADH donates the electrons to the enzyme reductase from and through the oxidization process NADH gets oxidizes to NAD^+, while conversion of magnesium ions to form magnesium metal which was in nanometer form.

Table 1: Antagonistic property of synthesized nanoparticles against human pathogens

Sr. No.	Group of Human pathogen	Name of Human pathogen	Inhibition activity of Nanoparticles (mm)	
			Microbial Nano (BIO)	Chemical Nano
1	Gram negative	*Klebsiella pneumoniae*	17.5	15.6
2		*Escherichia coli*	18	17.2
3	Gram positive	*Staphylococcus aureus*	16.1	12.6
4		*Bacillus subtilis*	12.0	Resistant

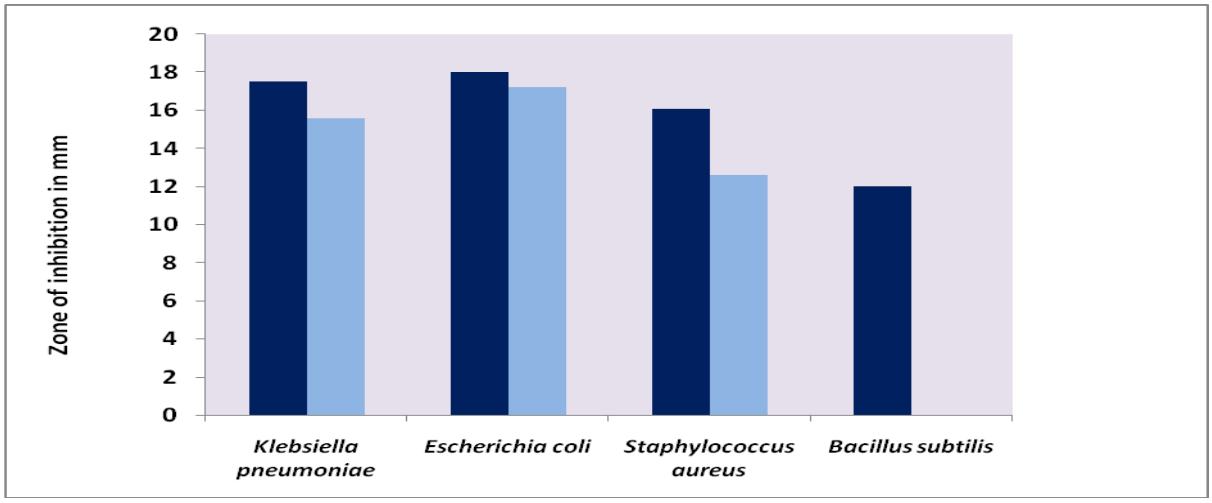


Fig. 4: Antagonistic activity of microbially synthesized nanoparticles (BIO) and chemically synthesized nanoparticles

The nanoparticles remained on the surface area of the actinomycetes but do not enter inside the solution where the actinomycetes were located. Therefore the actinomycetal biomass named as BIO is important in the study and selected for further research. In the mechanism, possibly the electrostatic interactions were carried out between the Mg^+ ions and negatively charged carboxylate group. This negatively charged carboxylate group was present in the enzymes which were present on the cell wall of the actinomycetes mechanism. It leads to the trapping of Mg^+ ions on the surface of actinomycetes. The term was applied for

overall mixture called BIO. This is a possible general process that may happen during the mechanisms of this research. This trapping mechanism has resembled with Ag^+ ions were trapped on the surface of the actinomycetes cell through the electrostatic interactions between Ag^+ and negatively charged carboxylate groups in the enzymes present in the cell wall of the mycelia [6]. Moreover, microorganisms involeved in the synthesis the nanoparticles. TEM analysis showed the presence of silver nanoparticles and their availability in both cytoplasmic membranes and the cytoplasm [22]. When metabolic nanoparticles were synthesized by the microbial cells, the proteins of the same microbial cells get surrounds and bind with the metallic nanoparticles. The cysteine is an amino acid of protein or amine group that might be involved in the binding process [23,24].

The chemically synthesized nanoparticles needed to stabilize because they were aggregates, need to prevent the aggregation of these fine nanoparticles and make them stabilized for a long time over. The stabilization is dependent on the microorganisms, these microbial cells can synthesize the protein which may involve in the stabilization process required for nanoparticles as they will not aggregate. The capping proteins are involved in the stabilization process, in which the fine particles don't have any contact with each other will not aggregate and because of that, these nanoparticles get to stabilize. One of the important enzyme is cytochrome C. Through this process the capping proteins are used for the stabilization of silver nanoparticles while these silver nanoparticles remained stable for 5 months more at 25°C [25]. There might be the same process happening in the case of magnesium nanoparticles synthesized by actinomycetes in this study as these nanoparticles were stable for a long period.

4. CONCLUSION

Magnesium nanoparticles were synthesized by micro-organisms like actinomycetes, by using *Streptomyces* and magnesium nitrate hexahydrate, $Mg(NO_3)_2{\cdot}6H_2O$ termed as actinomycetal Mg NPS. Antimicrobial activity of microbially synthesized magnesium nanoparticles was effective against all selected pathogens and the highest antimicrobial activity was observed against gram negative bacteria *Escherichia coli*, *Klebsiella pneumoniae* followed by *Staphylococcus aureus,* and the least antimicrobial activity was noticed against *Bacillus subtilis.* As the antimicrobial activity of microbially synthesized magnesium nanoparticles was more effective against *Escherichia coli, Klebsiella pneumonia,* therefore, synthesized nanoparticles may be used for the treatment of pneumonia disease, water, and water born diseases. After *Escherichia coli,* these microbially synthesized magnesium nanoparticles remained effective against *Klebsiella pneumonia, Staphylococcus aureus*, *Bacillus subtilis* hence these nanoparticles might be used against the diseases caused by these causative agents. Comparatively microbial synthesized nanoparticles were more effective rather than chemically synthesized nanoparticles. The microbially synthesized nanoparticles (BIO) were more sensitive against Gram negative human pathogens mostly present in water. From overall observations, it is predicted that the synthesized microbial nanoparticles are used for water treatment and in medicine as it can be used in the treatment of selected causative agents responsible for causing disease, that indicates microbiological nanoparticles was showing therapeutic applications in water and medicine. The presence of nanoparticles was confirmed by the TEM micrographs in which the size of nanoparticles was observed that size of synthesized nanoparticles by the microbial way (BIO) were smaller as compared with size of synthesized nanoparticles by the chemical way.

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Disclosure statement

There is no potential conflict of interest reported by any author.

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