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Green synthesis of magnesium oxide nanoparticles using *Trigonella foenum-graecum* leaf extract and its antibacterial activity

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Abstract
Synthesis of MgO Nanoparticles by Green synthesis using *Trigonella foenum-graecum* is a facile method which can be easily used for various biomedical application. Construction of nanoparticle by this method makes it compatible for Antibacterial studies. *Trigonella foenum-graecum* functions as a reducing and stabilizing agent and the precursor is Magnesium Nitrate. The synthesised MgO nanoparticles was characterized using UV-Vis spectroscopy. FTIR technique which was used to confirm the presence of functional groups, X-ray Diffraction (XRD) study was used to confirm the crystalline nature of the biosynthesised nanoparticle and confirm the size of the nanoparticle as 13nm.GC-MS technique was used to confirm the presence of stabilizing and reducing functional groups present in the leaf extract. SEM with EDAX was used to confirm the size, shape and composition of green synthesized MgO nanoparticle. The effect of green synthesised MgO nanoparticle against gram positive and gram negative bacteria was also studied. And it was observed that MgO nanoparticle shows significant antibacterial activity towards both the stains.

Keywords: *Trigonella foenum-graecum,* green synthesis, antibacterial activity, MgO nanoparticles

1. Introduction

Green synthesis of MgO is considered as a potential and eco-friendly way towards creation of Inorganic metal oxide nanoparticle. Inorganic materials such as metal and metal oxides have attracted a lot of attention over the past decades due to their ability to withstand harsh process conditions [1, 2]. MgO Metal oxides is of particular interest as it is not only stable under harsh process conditions but also generally regarded as safe materials to human beings and animals [3]. MgO is considered as a very good antibacterial agent compared to organic antibacterial agents [4-6]. There are numerous chemical methods available to synthesise MgO nanoparticles but as the usage of chemicals are highly toxic and hazardous it may lead to the environmental problems [7]. Today the methods available to synthesise metal oxide nanoparticles are solution combustion method, co-precipitation method, sol-gel method, hydrothermal method, Solvo thermal method, micro assisted sol-gel method and Green method [8]. Of all the methods mentioned above, synthesis of Metal Oxide nanoparticles by green synthesis is reported to be advantageous when compared to other methods in terms of less cost, reduced usage of toxic chemicals and the product and by products being Eco-friendly in nature [9].

Going Green in the construction of nanomaterials that is Biosynthesis of nanomaterials is considered an important area of research amongst researchers in today's world. The synthesis of metal and semiconductor nanoparticles by green route has potential applications towards the development of novel functional units. Various environmentally benign materials like plant extract, bacteria, fungi, enzymes etc are used as the starting material for the synthesis of metal oxide nanoparticle. Magnesium Oxide is an interesting basic oxide that has many applications in catalysis, adsorption and in synthesis of refractory ceramics [10-13]. It is a unique solid of high ionic character, simple stoichiometry and crystal structure and it can also be prepared widely in variable particle sizes and shapes. It has been reported that the shape and size of nanocrystalline magnesium oxide particles have high specific surface and reactivity because of the high concentration of edge/ corner sites and structural defects on their surface. Compared with TiO_2, silver, copper and other kinds of solid bactericides, Nano MgO has the advantage of being prepared from readily available and economical precursor and solvents and therefore is a considerable potent as a solid bactericidal material under simple condition [14-16].

Apart from all these, Magnesium Oxide Nanoparticles are very important because they have unique properties when compared to bulk materials. Its excellent properties like high chemical stability, high photocatalytic activity, high electrical permittivity, non-toxic nature makes MgO nanoparticles to be very unique.

It also finds extensive applications in catalysis, toxic waste remediation, paints, superconducting products, optical, electrical, electronic, antiseptic, antibacterial activities, semiconductors and catalytic devices [17]. In this paper for the green synthesis of MgO nanoparticle *Trigonella foenum-graecum* commonly called as fenugreek leaves is being used.

Trigonella foenum-graecum

Trigonella foenum-graecum commonly called as fenugreek is an annual plant in the family Fabaceae, with leaves consisting of three small obovate to oblong leaflets. This plant grows anywhere around the world and is an excellent medicinal plant which is used for producing blood lipids and helps in decreasing the sugar levels in diabetic patients. It also has antioxidant and antibacterial activity. The plant contains active phyto constituents such as alkaloids, amino acids, flavonoids, steroids, steroidal sapinogens, fibres, Saponins, lipids, polyphenols, carbohydrates etc [18]. Hence the presence of the above constituents can actively take part in the synthesis of nanoparticle. Thus in the present work, MgO nanoparticle is synthesised by green synthesis using *Trigonella-foenum graecum* and the synthesised nanoparticle will be applied to Gram positive and Gram negative bacteria to check the antibacterial activity.

2. Materials and Methods

All chemicals and reagents used in this study were of Analytical Grade. The chemical Magnesium nitrate was purchased from Merck, India. Double distilled water was used for the preparation of solutions.

2.1 Collection of plant material

Green leaves of *Trigonella foenum-graceum* was collected from the local market of Tambaram West, Kanchipuram District, Tamil Nadu, India.

Fig 1: Collected and washed leaves of *Trigonella foenum-graceum*

2.2 Preparation of plant extract

The roots of the plant fenugreek were cut. The leaves of the plant was washed thoroughly using tap water, followed by distilled water wash two to three times. The leaves were allowed to dry under shade for nearly three to four days. The obtained dry leaves was grined well and made into powder. The leaf powder was used for the preparation of the leaf extract. 5g of the powder was taken and 200 ml of the distilled water was added to it in a clean 500 ml beaker [18]. It was stirred continuously at 60˚C for an hour, cooled to room temperature and filtered using the Whatman filter paper. The colour of the extract was observed to be pale green.

2.3 Preparation of Magnesium Oxide Nanoparticles

The Magnesium Oxide nano particles prepared from Fenugreek leaf extract is as follows. 30 ml of the plant extract was taken in a 500 ml beaker and 150 ml of the freshly prepared 5mM Magnesium nitrate solution was added drop by drop using a burette and 1M NaOH was also added drop wise with continuous stirring for 2 hours at a temperature of 80˚C. With the addition of Magnesium nitrate solution a sharp change in colour from pale green to brown was observed confirming the formation of Mg $(OH)_2$ nanoparticle Fig 1.The solution was then centrifuged, the precipitate was washed with ethanol several times to remove the impurities and dried in the oven for 8 hours. It is finally calcined in the Muffle furnace at 600℃ for 4 hours and pale yellow coloured MgO nanoparticles was obtained.

(a) (b)

Fig 2a: Leaf Extract (b) MgO nanoparticle

2.4 Characterization

The MgO Nanoparticles prepared by the above method was characterized using Elico SL210 Double Beam UV-Vis spectrophotometer in the UV- Vis range of 200-800nm. FT-IR response was taken using Shimadzu IR Affinity1 spectrometer to confirm the presence of functional group in the plant extract and synthesised MgO nanoparticle. For the plant extract GC-MS response was taken using Joel GC mate II instrument. For GC-MS response the powdered sample of *Trigonella foenum- graecum* leaf extract was extracted with ethanol and the extract was used for analysis.X-ray Diffraction studies was carried out to know the whether the nanoparticle is crystalline or amorphous and alto also calculate the particle size.In the XRD technique the powdered sample of synthesized MgO nanoparticles was analysed using X-ray powder diffractometer in the low angle range (10 θ -70 θ). SEM-EDX characterization studies was carried out using FEI Quanta 200 F for studying the surface morphology of the nanoparticle. For the SEM- EDX analysis little amount of biosynthesized MgO NP was spread on the top of the sample holder followed by gold sputtering. The nano layer coated MgO nanoparticles was analysed for elemental studies.The Antibacterial Activity for the synthesized Nanoparticle was carried out using Resazurin Microtitre assay to determine the Minimum Inhibitory Concentration (MIC) values against various bacterial strains.

Determination of minimum inhibitory concentration (MIC) using resazurin microtitre assay

For screening natural products, that is crude extracts, chromatographic fractions or purified compounds for antibacterial activities, it is essential to employ an *in vitro* antibacterial assay that is simple, rapid, efficient, reliable, sensitive, safe and cost-effective. Moreover, most often the small quantities of natural products, especially purified compounds, that are available for antibacterial screening, can be a limiting factor in any viable screening programme. The conventional methods, e.g. disc diffusion method, may be time consuming and require significant quantities of the test materials, and there are also a few other problems associated with this method**.** Hence the resazurin microtitre assay utilising microtitre-plate, described by Drummond and Waigh in 2000, has been adopted in the determination of the Minimum Inhibitory Concentration (MIC) values of the synthesised Magnesum Oxide nanoparticles against various bacterial strains. The chemicals and the procedure adopted are described as follows.

Preparation of resazurin solution

The resazurin solution was prepared by dissolving 270 mg in 40 mL of sterile distilled water. A vortex mixer was used to ensure that it was a well-dissolved and homogenous solution.

Procedure

Test was carried out in a 96 well Plates under aseptic conditions. A sterile 96 well plate was labelled. A volume of 100 µL of sample was pipetted into the first three plates. To all other wells 50 µL of nutrient broth was added and serially diluted it. To each well 10 µL of resazurin indicator solution was added. 10 µL of bacterial suspension was added to each well. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. The plate was incubated at 37 °C for 18–24 h. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value.

Sample preparation

10 mg/1000µl distilled water, Std-Streptomycin

3. Results and Discussion

3.1 Synthesis of MgO nanoparticles

In the synthesis of MgO nanoparticle, it is seen form Fig1 that with addition of the leaf extract of *Trigonella foenum-graecum* which is added drop wise to colourless 5mM Magnesium Nitrate [$Mg(NO_3)_2$] solution followed by the addition of NaOH. the colour of the extract changes from pale green to brown confirming the formation of MgO nanoparticles [19].

3.2 UV-Vis Absorption Spectrophotometric Analysis

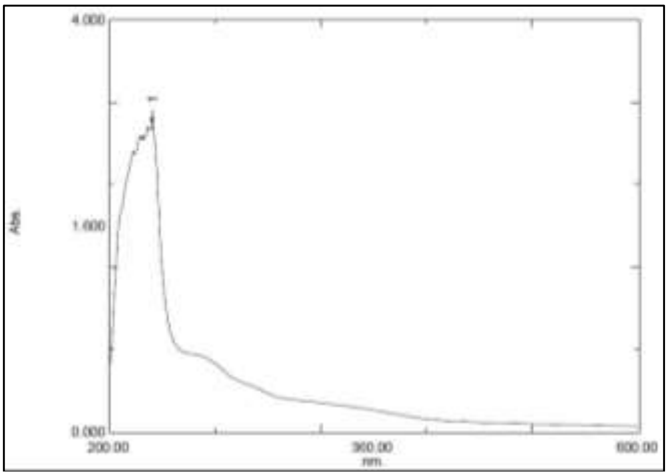


Fig 3: UV-Vis spectrum of MgO Nanoparticle

The above fig3 shows the absorption response of MgO nanoparticle (MgO NP). The specific absorption peak is observed at 267 nm which is in the range of 260-280nm specific for Magnesium Oxide nanoparticle. The optical band gap energy of the biosynthesised MgO NP is calculated from the formula $E=h\upsilon=hc/\lambda$ where h is the planck's constant, c is the velocity of light and λ is the wavelength observed from the UV- Vis response, is found to be 4.6eV which is similar to the earlier reported value based on the same time and temperature of calcination [20].

From Fig 2 and 3 it is clear that the phytochemicals like alkaloids, amino acids, flavonoids, steroids, terpenoids, vitamins, glycosides, ketones, alkenes, alkanes, aromatic and aliphatic components present in the leaf function as a reducing, capping and stabilizing agent towards the synthesis of biosynthesised MgO NP. The formation of Magnesium Oxide nanoparticle is confirmed by the visible colour change and the UV response observed in Fig 2.

3.3 Fourier Transform Infrared Spectroscopy

The FTIR response was carried through the wavenumber range from 500 -4000 cm^{-1} using KBr pellet method at room temperature. Figure 4(a) and 4(b) shows the FTIR responses for *Trigonella foenum-graecum* plant extract and MgO nanoparticles respectively.

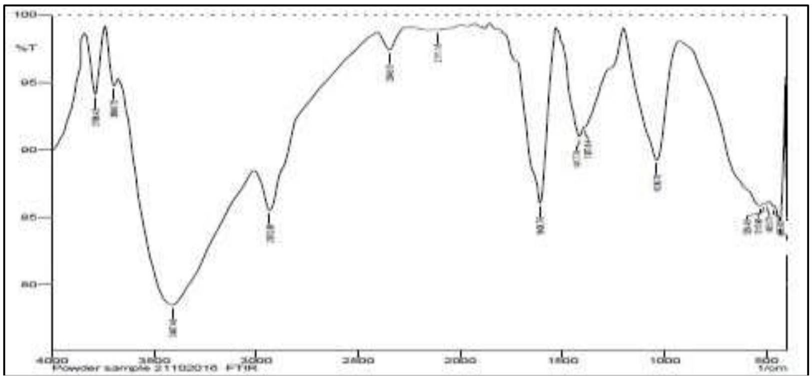


Fig 4(a): FTIR spectrum of *Trigonella foenum-graecum*

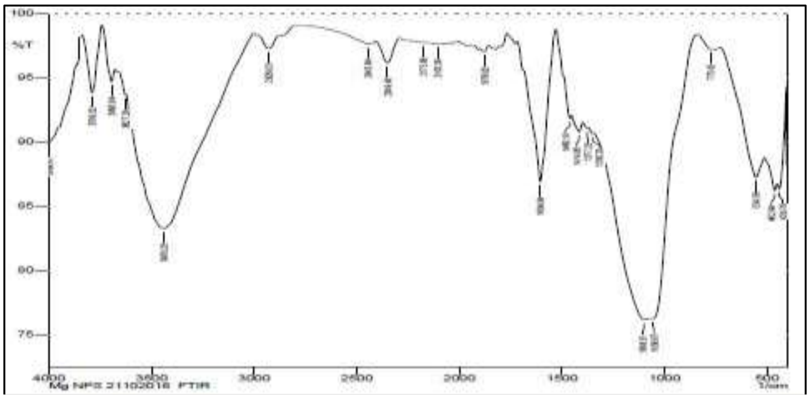


Fig 4(b): FTIR spectrum of Magnesium oxide nanoparticle.

The FTIR response for the plant extract *Trigonella foenum-graecum* confirms the presence of alkaloids, phenolic groups, polysaccharides, flavones, amino acids, terpenoids, flavonoids and steroids. The broad peaks in Fig 3a & 3b in the higher region 3600-3400 cm^{-1} is due to the presence of alcoholic or phenolic –OH groups. The strong –OH stretching vibrations represented at 3439 cm^{-1} are due to the water molecules. A reduction in peak intensity in fig3b compared to Fig 3a confirms that the organic molecules have been involved in the formation of MgO nanoparticles. Peaks in the range between 2100 -2500cm^{-1} indicate the stretching of alkynes. The peaks at 1876 cm^{-1}, 1604cm^{-1}, 1460-1350cm $^{-1}$ corresponds to C=O stretching(amide linkages), C=C stretching (alkenes) and –CH stretching (alkanes) respectively [21]. It is also observed from figure 3 (b) that with the formation of nanoparticle, intensity of the peak at 3400cm^{-1} and 1600cm^{-1} corresponding to N-H stretching and C-N stretching respectively is found to decrease. The peaks in the range between 450-560cm^{-1} is assigned to Mg-O stretching vibrations and the absence of peak at 694cm^{-1} confirms the absence of Mg $(OH)_2$.

3.4 Gas chromatography and mass spectrometry (GC-MS) analysis

The results pertaining to GC-MS analysis of the methanolic extract of Trigonella foenum-graecum leaves led to the confirmation on the presence of various components present in the plant extract analysed earlier by FTIR spectroscopic method. The compound prediction is based on National Institute Standard and Technological Database. The GC-MS results reveal the presence of different active components present in the *Trigonella foenum-graecum* leaves. The results of the present study is tabulated in Table 1. Figure 5 depicts the GC-MS spectrum of *Trigonella foenum-graecum* leaf extract.

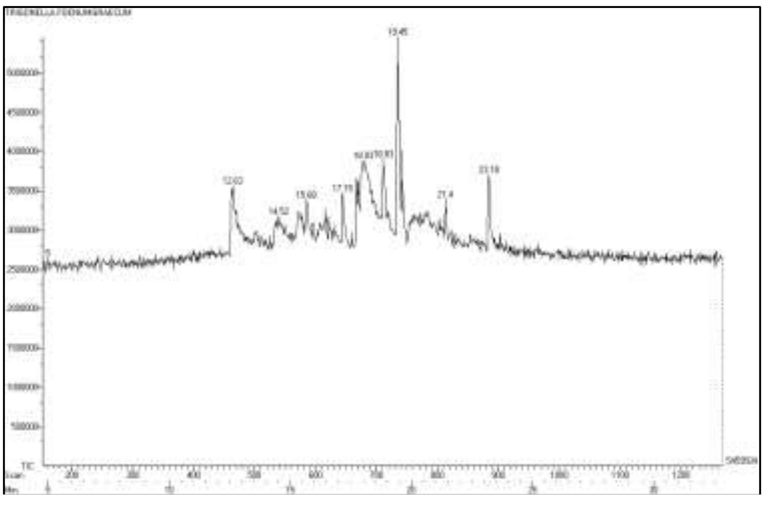


Fig 5: GC-MS Chromatogram of ethanolic leaves of *Trigonella foenum-graecum*

Table 1: Components detected in the plant of methanol extract of *Trigonella foenum-graecum* leaves, and its Biological activity

S.NO	*RT	Name of the compound	Molecular Formula	Molecular Weight(g/mol)	**Biological Activity
1	12.63	Phenol,2,4-bis (1,1-dimethylethyl)	$C_{14}H_{22}O$	206.32	-
2	14.52	4-(1,5-dihydroxy-2,6,6-trimethylcyclo Hex-2-enyl)but-3-en-2-one	$C_{13}H_{20}$	176.29	Endoanaesthetic, Endocrine protective, Energizer, Fertility Enhancing, enterotoxic, Memory enhancer, Stimulate PUFA Desaturase and Elongase Enzymes, Hexokinase-Stimulator.
3	15.68	3,6-nonadienedioic acid,5,5-dimethyl-, dimethylester	-	-	-
4	17.15	Pentadecanoicacid, 13-methyl-,methyl Ester	$C_{17}H_{34}O_2$	270.45	Catechol-O-methyl-Transferase-inhibitor, Methyl donor, methyl-Guanidine-inhibitor, Antioxidant, Acidifier, Urine acidifier, Increase the production of uric acid, Acidulant
5	18.03	Z,E-2methyl-3,13-octadecadien-1-ol	$C_{19}H_{36}$	264.48	Anticancer, Antidote, Antitumour, provides Oligosaccharides, Increases Zinc Bioavailability, Energizer, Methyl-Guanidine-inhibitor, Memory enhancer, stimulate Epinephrine production
6	18.83	8-octadecenoicacid, Methylester,(E)-	$C_{19}H_{36}O_2$	296	Antioxidant, Antimicrobial
7	19.45	(E)-9-octadecenoic Acidethylester	$C_{20}H_{38}O_2$	310.51	Acidifier, Acidulant, Anticancer, Urine acidifier, Antitumour, Antidote, Energizer, Memory enhancer, Decrease oxalate excretion, Ecbolic
8	21.4	6,11-eicosadienoic acid,methylester	$C_{21}H_{38}$	290.52	Anti inflammatory, Antioxidant, Antiarthritic, Anti-coronary
9	23.18	Isopropylstearate	$C_{21}H_{42}O_2$	326.56	-

*Retention time

**Biological Activity source: Dr. Duke's Phytochemical and Ethnobotanical Database

From the GC-MS data given in the table 1 and the FT-IR figure 4a and 4 b confirms the presence of functional groups depicted by the FT-IR response and hence clearly proves the presence of the phyto components such as alkaloids, steroids, saponins, terpenoids, flavonoids which have involved as capping agents and stabilization agents in the formation of MgO nanoparticle.

3.5 X-ray Diffraction Analysis

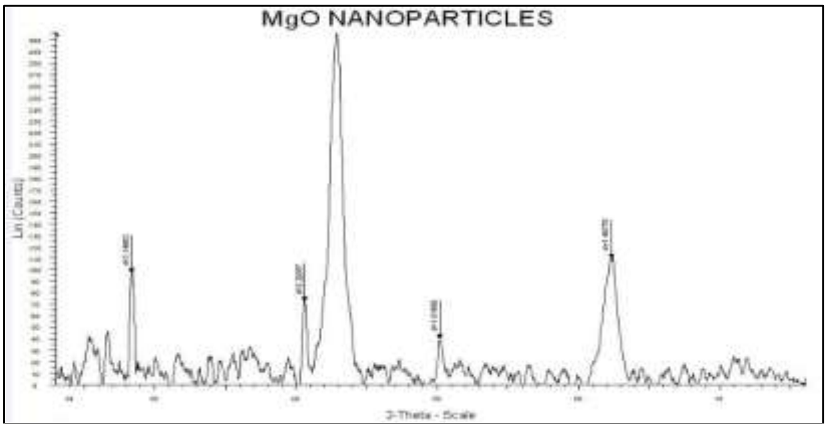


Fig 6: XRD pattern of MgO nanoparticles

The powdered MgO sample was analyzed by a Cu Kα – X Ray Diffractometer for confirming the presence of MgO nanoparticles. Fig 6 shows the peaks appeared at 2θ values ranging from 28.34˚, 40.58˚, 42.87˚, 50.20˚, 62.35˚ which corresponds to the presence of MgO nanoparticles and the location of the peaks in the graph are in good agreement with the literature report [22]. It is also clear from the XRD response that MgO NP is crystalline in nature.

The crystalline average size of the biosynthesized nanoparticle calculated by using Debye Scherrer's formula

$$D = k\lambda/\beta\cos\theta \, A^{\circ}$$

Where, D is the average crystalline size in A˚, k is the shape factor, λ is the wavelenghth of X-ray (0.1540 A˚) Cu-Kα radiation, B is the full width at half maximum (FWHM), and θ is the angle of diffraction.

The D values of the peaks which appeared at the 2 θ values are 20.13, 19.70, 8.41, 20.96, 7.67 nm respectively. From these values the average crystalline size of the MgO nanoparticle formed was calculated to be 13.89 nm.

3.6 Scanning electron microscopy (SEM) with energy dispersive X-ray diffraction (EDX)

SEM with EDX was used to study the surface morphology and the percentage composition of the nanoparticles. The SEM image in Figure 7(a) shows that the biosynthesised MgO NP's consisted of a mix of fine, spherical structures. Its dimensions were found to be in the range between 36.7 and 69.6nm and spherical in shape.The EDX response 7(b) depicts a high elemental composition of Mg and O in the nanoparticle. The presence of carbon is from the organic molecules present in the leaf extract which functions as both stabilizing and reducing agent. Other elements are in very negligible amounts.

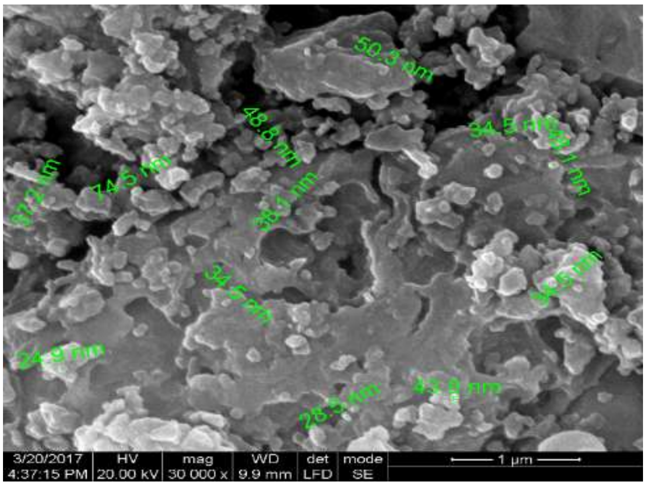
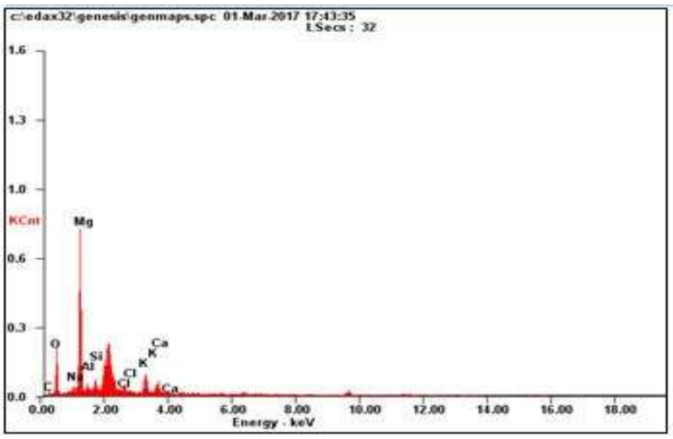


Fig 7(a): SEM image of MgO NP's



Element	Wt%	At%
CK	14.33	23.05
OK	31.28	37.77
NaK	01.89	01.58
MgK	34.97	27.79
AlK	02.07	01.48
SiK	03.44	02.37
ClK	01.42	00.78
KK	06.99	03.45
CaK	03.60	01.74
Matrix	Correction	ZAF

Fig 7(b): Elemental compostion of MgO NP's analyzed by EDAX

3.7 Antibacterial activity

Fig 8: Determination of MIC using Resazurin microtitre assay

Antibacterial studies was carried out to determine the Minimum inhibitory concentration (MIC) using Resazurin Microtitre Assay which is shown in Figure 8.

Table 2: Tabulation showing growth of inhibition on E.coli, Bacillus and S.aereus bacterial stains

S. No	Microorganisms/sample	Growth of inhibition									
		500µg	250 µg	125 µg	62.5 µg	31.2 µg	15.6 µg	7.8 µg	3.9 µg	Positive control 10µg	Culture
	MgO Nanoparticles										
1	*E.coli*	-	-	-	+	+	+	+	+	-	+

2	*Bacillus*	-	-	+	+	+	+	+	+	-	+
3	*S.aereus*	-	-	-	+	+	+	+	+	-	+

Table 3: Tabulation showing MIC values

Microorganisms/sample	MIC Value (µg)
MgO Nanoparticles	
E.coli	125
Bacillus	250
S.aereus	125

From the above tabulations 2 & 3, the change in the colour from purple to pink or colourless in Figure 8 were recorded as positive. The Minimum Inhibitory Concentration of the biosynthesised towards the various bacterial stains are found to be 125µg of MgO is the MIC for the bacteria *E.coli* and *S.aereus* which is in accordance with the earlier report [23-26] and the MIC value towards bacteria *Bacillus* is 250µg. It is observed that with just 125µg of biosynthesised MgO nanoparticle higher antibacterial effect is observed towards both gram negative and gram positive bacteria *E.coli* and *S.aereus*. A higher concentration of MgO NP is required for antibacterial activity in case of *Bacillus* which is according to the earlier report which stress that antibacterial activity by nanoparticle is dosage dependent [27]. It means *Bacillus* is more resistant to MgO nanoparticle compared to *E.colli.* Earlier reports [28-30] have indicated that antibacterial activity of MgO nanoparticle is due to lipid peroxidation and the formation of Reactive Oxygen Species (ROS) such as super oxide ion (O_2^-). It is due to the defects of Oxygen vacancy on the surface of nanoparticles [31]. As the MgO nanoparticles have large surface area, increase in surface area increase the concentration of ROS and hence effective destruction of bacterial cell wall. Thus the green synthesised MgO nanoparticle is found to exhibit antibacterial activity towards both gram positive and negative stains.

4. Conclusion

In the present study we report the green synthesis of Magnesium Oxide Nanoparticle using leaf extract of *Trigonella foenum-graecum.* The organic molecules present in the leaf extract functions as a reducing and stabilising agent. The synthesized MgO NP's were Characterizedusing various techniques which includes UV-Vis, FT-IR, GC-MS, X-ray Diffraction (XRD) and SEM with EDX. From the Uv-Vis response the optical band gap was calculated. It was found to be 4.6eV which is similar to the earlier reported values. The presence of functional groups present in the plant extract which function as both stabilizing and reducing agentnwas confirmed from the GC-MS and FTIR response. From the XRD analysis the average size of the biosynthesised MgO NP was found to be 13nm which was calculated by Debye-Scherer equation. SEM picture confirms the presence of spherical shaped MgO NP with nanometer sized dimension. The chemical composition of MgO nanoparticle was identified by EDX analysis. The Antibacterial activity of the biosynthesized MgO nanoparticle was studied by determining Minimum Inhibitory concentration (MIC) using Resazurin Microtitre Assay and the MIC values of MgO NP was found to be 125µg for both Gram positive and Gram negative bacteria. Thus the by green synthesis MgO NP can be synthesised and these MgO NP exhibits good antibacterial activity too.

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