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# SYNTHESIS, MICROSTRUCTURAL AND ANTIBACTERIAL ACTIVITIES OF NANOSTRUCTURE $MgFe_2O_4$ AGAINST DIFFERENT BACTERIAL STRAINS

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***Abstract:*** *Bacterial pollution is a great risk for human health. Nanotechnology offers a way to develop new inorganic antibacterial agents. Magnesium ferrite ($MgFe_2O_4$) was prepared by the sol-gel process. The effect of thermal densification treatment at different temperatures in the range of 350-650$^0$C on antibacterial properties was examined. The structural characterization of the Magnesium ferrite ($MgFe_2O_4$) was performed by using X-ray diffraction (XRD), Fourier transform infra-red spectroscopy (FTIR) and Transmission electron microscope (TEM). The antibacterial activity of the different calcined nanoparticles was determined against Escherichia Coli, Streptococcus aureus, Bacillus substillus and Pseudomonas aeruginosa by Well Diffusion Method. The result show that a high level of antibacterial activity was observed for the calcinied at 350$^0$C or lower temperatures allowing accommodation but during calcination at higher temperatures (550 or 650 $^0$C) resulted in apparent degradation in the antibacterial activity. But it will be important for investigation bacterial-sensitivity in wells with very low calcination efficiencies. Each bacteria discriminated between a range of sensitivities in the well examined, and with some minor differences, the ordering of sensitivities using the five bacteria was different. Possible explanations for the differences between results obtained with the five bacteria are discussed.*

***Keywords:*** *$MgFe_2O_4$ Nanoparticles, XRD, FTIR, TEM and Pathogenic bacteria.*

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## I. Introduction

A resurgence of interests on spinel ferrites magnetic nanoparticles is observed in recent years due to their unique optical, electrical, magnetic properties and promising applications in biomedicine [1]. Magnesium ferrite ($MgFe_2o_4$) is an important functional magnetic material which crystallizes in the spinel structure type. Recent progress in nanostructure of inorganic nanoparticles for biomedical application has received more and more attention due to their novel material and pronounced application [2-4]. They are significantly different from those of their bulk counter parts [5-7].

Among the different ferrites, Magnesium ferrite ($MgFe_2O_4$) has special magnetic and physical properties which lead to its wide application in medicine. Bacterial pollution is a great risk for human health. Nanotechnology offers a way to develop new inorganic antibacterial agents. Nano-inorganic metal oxide has a potential to reduce bacterial contamination. The search for new good antibacterial material and study of the new properties of conventional material has become an active research field. Iron oxide has various applications regarding biomedicine due to its different properties.

Ferrite nanoparticles are suitable material for biomedical application because of their wide range of size, diversity and chemical stability as compared to metal nanoparticles. The small scale size of the well-known spinel ferrites offer potential a applications for intensive research to utilize their properties for biomedical applications [8]. Nanoparticles that have been found in many commercial applications in fields such as magnetically guided drug delivery, magnetic hyperthermia and magnetic resonance imaging [9-10]. The vast scope of nanomedicine, we will focus on their apeutic application in particular, drug delivery application of nanoparticles. Many advantages of nanoparticle-based drug delivery have been recognized [11]. Previous reports show that considerable antibacterial activity of magnesium based nanoparticles is attributed to the generation of reactive oxygen species [12].

Magnesium ferrite ($MgFe_2O_4$) is one of the important functional magnetic materials with a cubic normal spinel structure type. It is used as a catalyst and humidity sensor. Among various materials used for sensing application, ferrite is used as a good class of sensing materials, but they suffer a drawback of being at higher temperature [13]. M. Sunderarajan show that Antibacterial activity of nanoparticles decreases with increases of calcinating temperature. [14]. Previous studies show that nanosized particles with their size comparable to that of biological structure are very smart material for the manipulation, sensing and detection of biological systems. Relationship between particle size and activity was also reported and this has led to our interest in Mg based materials for applications in health and water [15].

Activity of nanoparticles is directly dependent on the bacterial strain, i.e., gram positive and gram negative as they have differences in their cell wall. Yamamoto et al [16] Enumerated the influence of particle size on the antibacterial activity of nanoparticles and showed that the activity increased with decreasing particle size. However, not many results have been reported on bacterial activity at different temperature of magnesium ferrite nanoparticle.

In this paper, the nanocrystalline Magnesium ferrite was synthesized by sol-gel technique. The effect of calcinations conditions on the crystallite size and on bacterial strains. of nanosize $MgFe_2O_4$ were investigated. All the results obtained provide a basis for further application of $MgFe_2O_4$ in relevant fields. The present investigation deals with the structural and biological characterization of Magnesium ferrite. The sol-gel technique allows good control over stoichiometry and produce nanoparticles with small size and narrow size distribution. The main objective of this study was to evaluate the antibacterial effects of nanoparticles at different calcinations temperature against various strains such as E. Coli, S. Aureus, Bacillus Substillus and P. Aeriginosa. Magnesium ferrite ($MgFe_2O_4$) is chosen because it has various application regarding biomedicine due to its different properties such as Paramagnetic, sensing, transformer etc. In most research reports, synthesis technique aim to limit the size of the nanoparticles to below 30nm and to have uniform size distribution.

In present work Magnesium ferrite is characterize by X-ray diffraction (XRD), Transmission electron microscope(TEM), and Fourier transform infrared (FTIR) and from XRD and TEM Magnesium ferrite has been size 20nm. These nanoparticles have extensive application in drug delivery system. It was a high yielding, low cost and facile synthesis method. In this study the applicability of various ferrites nanoparticles as a drug delivery systems. Therefore, in this paper, the main synthesis methods, antibacterial activity and antibacterial mechanisms of Magnesium ferrite nanoparticles are reviewed.

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## II. Materials And Methods

**MATERIAL:**

Magnesium nitrate tetra hydrate ($Mg(NO_3)_2.4H_2O$), Iron nitrate non hydrate ($Fe(NO_3)_2.9H_2O$) and citric acid was obtained of analytical grade. All experiment was done by using ethyl alcohol. $MgFe_2O_4$ were synthesized by sol-gel method.

**Synthesis of $MgFe_2O_4$nanoparticle:**

Cobalt doped $MgFe_2O_4$ nanoparticles were synthesis by sol-gel method. All chemicals add in beaker and continuous stirring on magnetic stirrer for 2 hours then form gel and calcinite at different temperature 350, 550 and 650 to form nanoparticles.

**Assay to Evaluate Antibacterial Activity/ Screening for antibacterial activity**

**Bacterial Culture**

The following bacterial pathogens are gram positive and gram negative namely Escherichia Coli (NCIM-2256), Streptococcus aureus (NCIM-2901), Bacillus substillus (NCIM-2063) and Pseudomonas aeriginosa (NCIM-5031). All the cultures were grown on nutrient agar plates and maintained in the nutrient agar slants at $40^0$C. Overnight culture in the nutrient broth was used for the present experimental study.

**Well diffusion method for Antibacterial activity**

The antibacterial activity of the synthesized $MgFe_2O_4$ nanoparticles was assessed against above mentioned test strains by agar well diffusion technique. The overnight bacterial cultures grown in nutrient broth was spread evenly over Mueller Hinton agar (MHA) plates. Dip the swab into the broth culture of the organism. Gently squeeze the swab against the tube inside to remove excess fluid. Use the swab to streak a nutrient agar plate for a lawn of growth. This is best accomplished by streaking the plate in one direction, then streaking at right angles to the first streaking, and finally streaking diagonally by using sterile cotton swab. The inoculated plates were incubated at appropriate temperature for 24 hours only for determining any contamination. Next day Wells of 6 mm diameter were cut on the MHA plates using sterilize cork borer and 20 μl of nanoparticles suspension was dispensed in each well. The plates were left overnight at $37^o$C in incubator. The antibacterial activity was evaluated by measuring the zone of inhibition against the test organisms. Zone of inhibition is the area in which the bacterial growth is stopped due to bacteriostatic effect of the compound and it measures the inhibitory effect of compound towards a particular microorganism. Here the set is doublet for determine exact result. Finally results were recorded by measuring the diameter zone of inhibition (mm) of the control strain and test nanoparticles with ruler calliper [17] .

## III. Result And Disscution

**Structural Studies:** The XRD patterns of the $MgFe_2O_4$ ferrites are shown in Fig. 1. All the reflection peaks were identified and indexed in good agreement with the referred data base of (JCPDS No. 89-4924). Figure 1 show a typical X-ray diffraction pattern of $MgFe_2O_4$, well defined diffraction peaks, confirmed the formation of pure single phase with cubic spinel phase. X-ray diffraction patterns of the MgFe2O4 samples were calcinated at $350^0$C, $550^0$C and $650^0$C are presented in Fig. 2(a), 2(b) &(c). The miller indices show planes of cubic spinel structure at (220), (311), (400), (511), (440) of prominent peaks are appear at $2\Theta = 31^0, 35^0, 43^0, 53^0, 57^0, 62^0$ indicates the formation of pure single phase $MgFe_2O_4$ with cubic spinel phase. The well-defined (311) peak appears to be more intense. Fig. 2 also shows that the peaks become sharper and narrower with increasing calcinating temperature, indicating the enhancement of crystallanity. The average particle size (D) is Calculated from peak broadening using Debye–Scherrer formula [18]

$$D = \frac{0.89\lambda}{\beta cos\Theta}$$

Where D is the particle size, β is the half maximum width, λ is the X-ray wavelength and Θ is Bragg angel. Hence average particle size from the XRD is vary from 10 to20 nm was observed for $MgFe_2O_4$ nanoparticles. The line broadening of the X-ray diffraction pattern gives a clear evidence for the nanometer range of the synthesized powder.

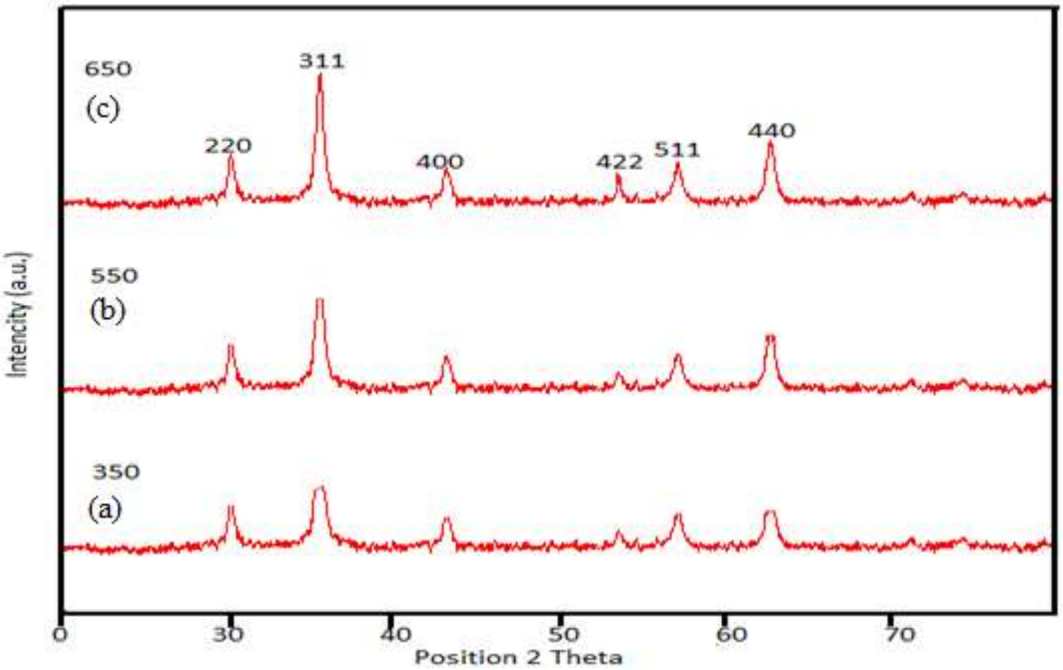



**Fig. 2(a, b & c) XRD analysis of Magnesium ferrite sample calcinate at (a) $350^0$C (b) $550^0$C and (c) $650^0$C.**

**FTIR Spectra:** The The formation of spinel MgFe2O4 structure in the calcined at different temperature such as $350^0$C, $550^0$C and $650^0$C was further supported by FT-IR spectra (Fig.3). The intensive band at~˜1626 $cm^{-1}$ is due to O–H stretching vibration interacting through H bonds. The band at ~ 2925 $cm^{-1}$ is C–H asymmetric stretching vibration mode. 1438.38 $cm^{-1}$ and the band at ~1019.42 $cm^{-1}$ was corresponded to nitrate ion traces. The broad band between about 3300 and 2600 $cm^{-1}$ in the spectrum of the (Mg-Fe) gel. The vibrations disappeared when calcinations temperature was increased. In the range of 1000– 450$cm^{-1}$ a typical metal–oxygen absorption band for the spinel structure of the ferrite at ~565 $cm^{-1}$ 1 was observed in the FT-IR spectra of all of the calcined MgFe2O4 samples. This band strongly suggests the intrinsic stretching vibrations of the metal (F↔ O) at the tetrahedral site.

From fig. 3 show that at increasing temperature the peak becomes more intensive.

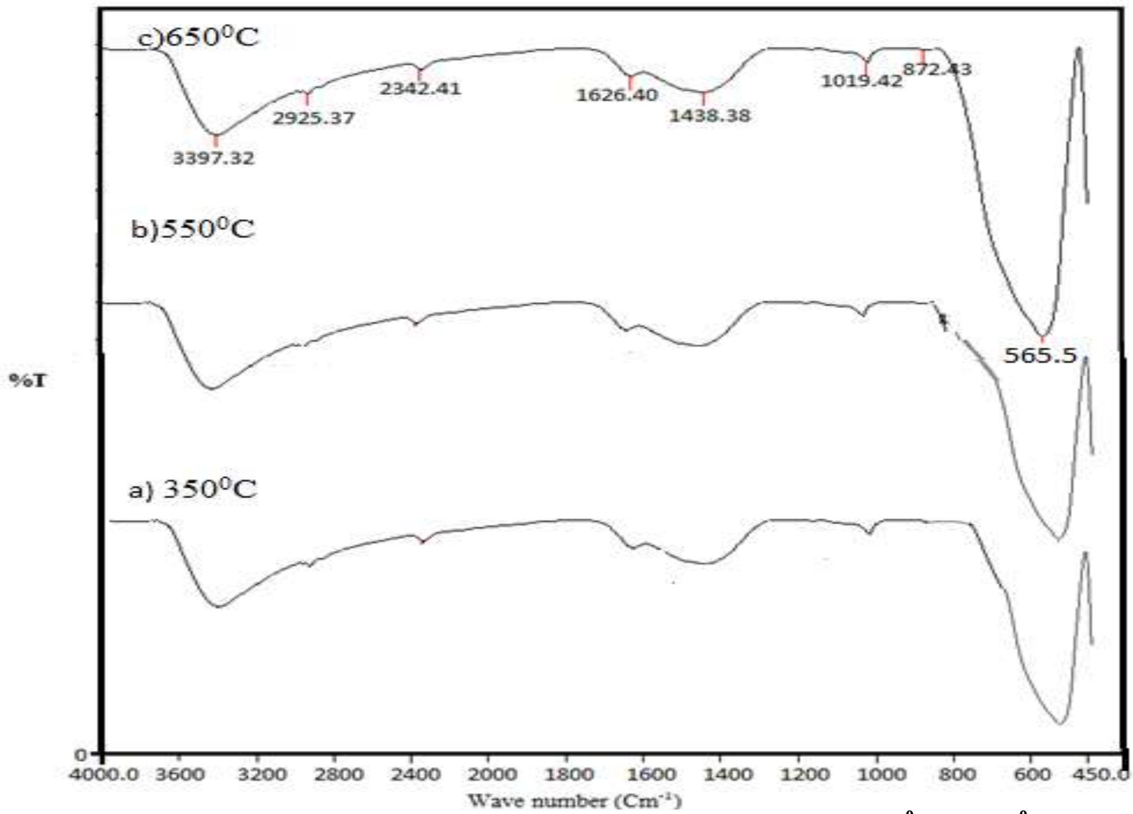
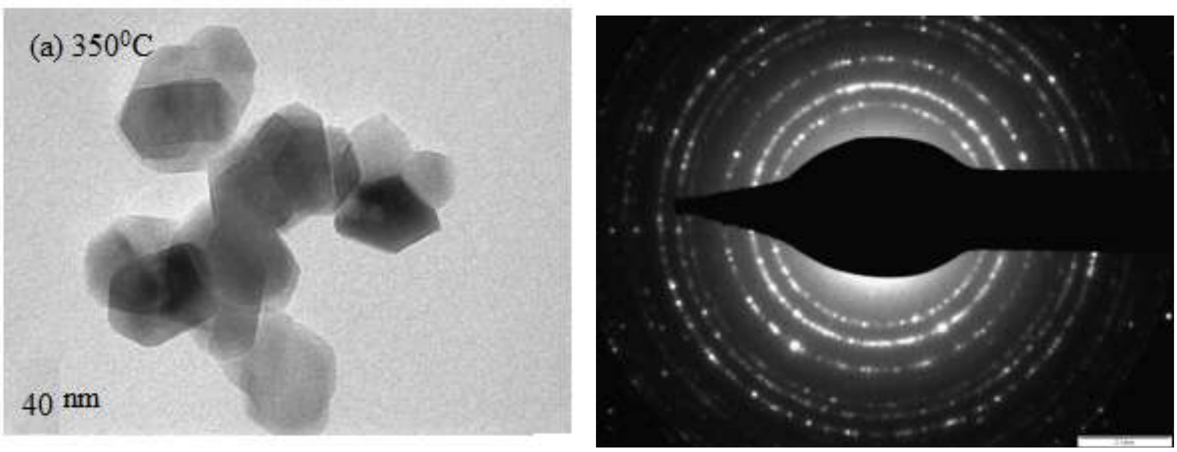



**Figure 2(a, b & c) FT-IR spectra of Magnesium ferrite sample calcinate at (a) $350^0$C (b) $550^0$C and (c) $650^0$C.**

**Morphological Analysis:** The images of nanocrystalline MgFe2O4 are shown in fig. 3 (a,b,c). The detailed morphology and crystalline structure of the MgFe2O4 calcined at $350^0$C, $550^0$C and $650^0$C for 3hrs was further investigated by TEM, and the TEM bright-field images with corresponding selected-area electron diffraction (SAED) patterns of these three samples are shown in Fig.3. It is clearly seen from the TEM bright field images that three samples consisted of packed MgFe2O4 particles with particle sizes of 20 nm in diameter for the samples of $350^0$C, $550^0$C and $650^0$C calcined, respectively. It is seen that the particle sizes of MgFe2O4 contained in the calcined MgFe2O4 are quite uniform and crystalline nature of MgFe2O4 nanoparticles are increased at higher calcinied temperature. The particles of MgFe2O4 are rhombic in nature. Since this concurs with the results of XRD presented in Fig. 2.



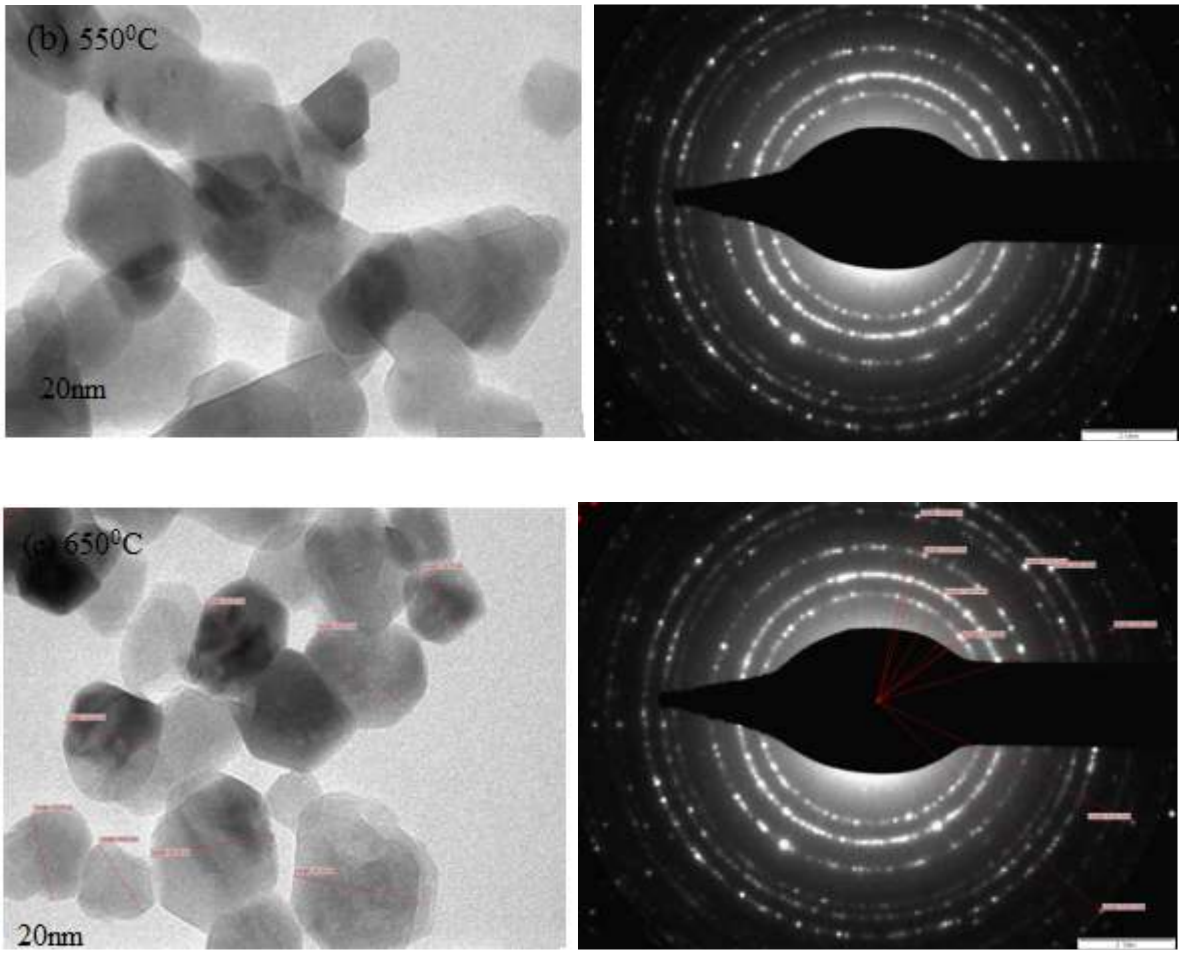


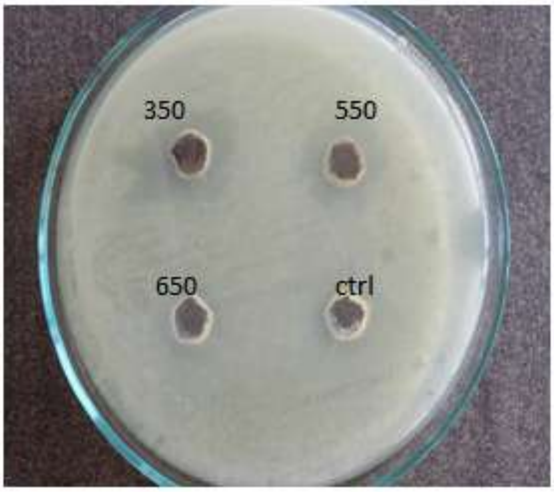

**Fig.3 TEM images with corresponding SAED patterns of the $MgFe_2O_4$ samples calcined for 3hrs at a temperature 350$^0$C, 550$^0$C and 650$^0$C.**

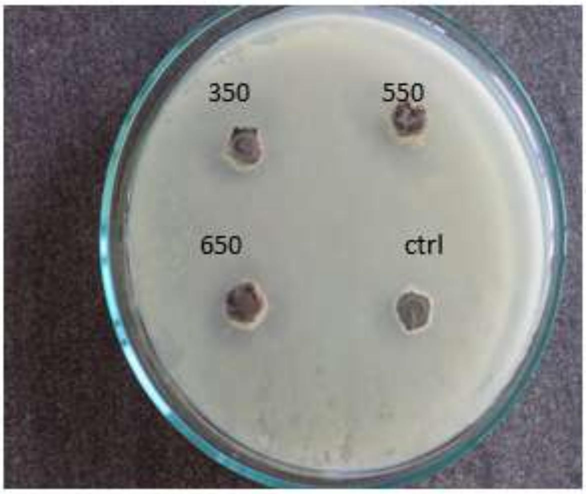
## ANTIMICROBIAL STUDY

Antibacterial activity of pure $MgFe_2O_4$ nanoparticles calcined at different temperature 350$^0$C, 550$^0$C and 650$^0$C was determined by Well diffusion method. The inhibitory activity of pure $MgFe_2O_4$ was evaluated against various pathogenic bacteria are presented in Table 1 and Fig. 4 (a , b, c, d, e) Different classes of bacteria exhibit different susceptibilities to nanoparticles. In this the distilled water is used as control. Antibacterial activity towards bacteria E. Coli (NCIM 2256), S. Aureus (NCIM 2901), B. Substillus (NCIM 2063) and P. aureginosa (NCIM 5031) of magnesium ferrite at different calcinations temperature are shown in Table 1 and Fig. 4. From table 1 and fig. 4 shows the difference in the zone of inhibition for the five bacteria's at different calcinations temperature. By observing the result we conclude that pure $MgFe_2O_4$ nanoparticles calcined at 350$^0$C shown zone of inhibition 15mm,15mm,16mm, 15mm have excellent bacterial activity against four bacteria than the calcinations of 550$^0$C and 650$^0$C . Sine antibacterial activity of prepared magnesium ferrite is decreases with increases of calcinations temperature. Zone of inhibition effects of magnesium ferrite nanoparticles occur by inhibiting the growth of microorganisms using an electrochemical mode of action to penetrate and disrupt their cell wall. Yamamoto et al enumerated the influence of particle size on the antibacterial activity of nanoparticles and showed that the activity increased with decreasing particle size. Previous studies have shown that antimicrobial formulation in the form of nanoparticles can be used as effective bactericidal agents [19]. The result also observe that zone of inhibition is maximum in gram positive bacteria as compared to gram negative bacterium. These types of nanoparticles are used as drugs in pharmaceuticals.

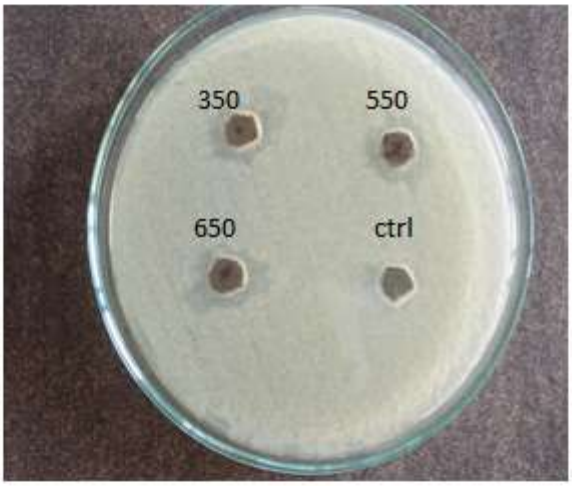
| | | Zone of Inhibition in mm | | | |
|---|---|---|---|---|---|
| **Samples** | **Calcination tempreture** | **E. Coli** | **S. aureus** | **Bacillius substillus** | **Pseudonomus areginosa** |
| $MgFe_2O_4$ | $350^0C$ | 15mm | 15 mm | 16 mm | 15 mm |
| | $550^0C$ | 13 mm | 14 mm | 15 mm | 13 mm |
| | $650^0C$ | 11 mm | 13 mm | 14 mm | 14 mm |
| | Control | 10 mm | 9 mm | 8 mm | 10 mm |

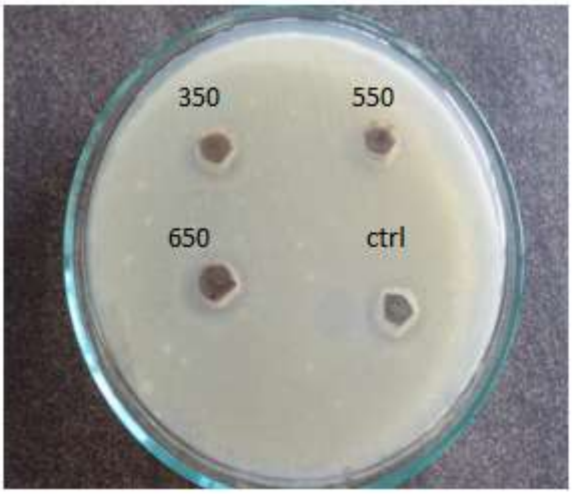
**Table-1 Antibacterial assessment by Well diffusion method**




**(a) E.coli** **(b) S. aureus**




**(c) Bacillius substillus** **(d) P. aerigenosa**

**Fig. 4 Antibacterial effect of $MgFe_2O_4$ nanoparticles at three calcinating range against E.coli (a), S. aureus (b), Bacillius substillus (c), P. aerigenosa (d) and Bacillius Streptothermophillus (e).**

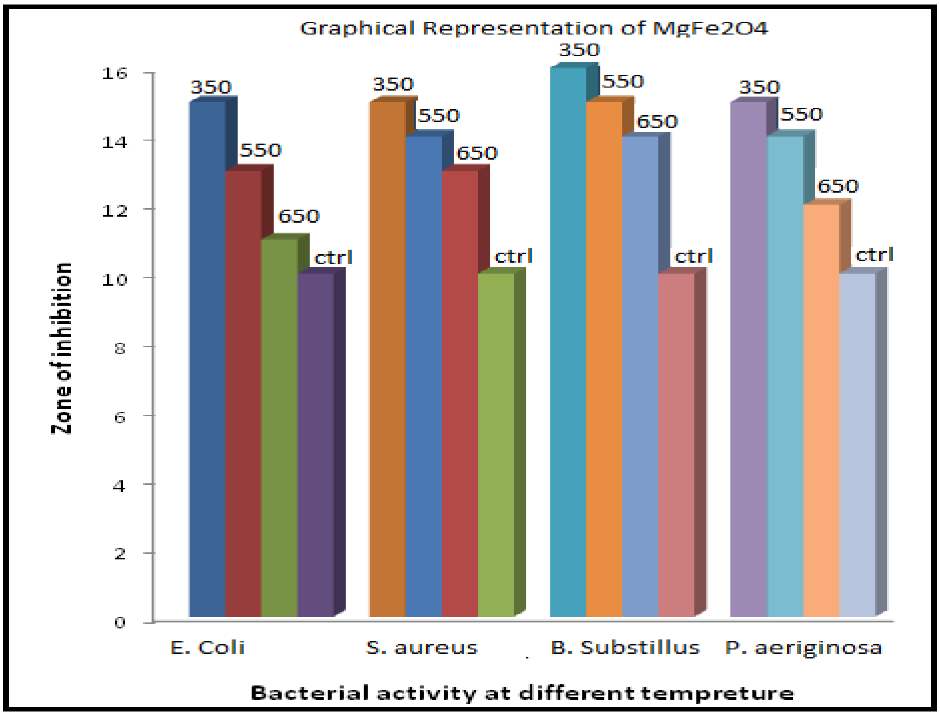



**Fig. 5 Graphical representation of $MgFe_2O_4$**

## IV. Conclusion

Magnesium ferrite nanoparticles are prepared by the Sol- gel method and calcinied at different temperature $350^0$C, $550^0$C and $650^0$C. The FTIR Spectra show main absorption bands around 595 cm-1 and 565 cm-1 corresponding to the vibration modes of the spinel compounds. XRD pattern reveals that the synthesized ferrites consist of nano crystalline particles with size in the range from 10to 20nm and the crystalline nature of the sample is increased as the sintering temperature increases. It is also confirmed by the TEM micrographs. The antibacterial efficacy was tested against gram negative and gram positive bacterial strains Antibacterial activity towards bacteria E. Coli, S. aureus, B. Substillus, P. aureginosa and B. Streptothermophillus of magnesium ferrite at different calcinations temperature. The results show that at lower calcinations temperature antibacterial activity is maximum as increased the calcinations temperature there was an enhancement in the antibacterial behaviour is observed. That means the antibacterial activity is depends on the size of the nanoparticles. The confluence of magnetic and antibacterial properties can make this material important for application in biomedicine. Therefore, in the present study, attempts have been carried out to synthesized magnesium ferrite nanoparticles and calcinied at lower temperature hoping to achieve improvement in the antibacterial activity of magnesium ferrite. Thus the Magnesium ferrite nanoparticles with good magnetic and antibacterial properties can offer great promises in biomedical and pharmaceutical applications and also wide application in the health and hygiene will constitute the basis for the next generation drug development.

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