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PREPARATION, CHARACTERIZATION AND DETERMINATION OF ANTIMICROBIAL ACTIVITIES OF SPINEL ($MgCo_2O_4$) NANOPARTICLE USING CITRIC ACID

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Abstract

The present research deals with a study on the synthesis and characterization of spinel ($MgCo_2O_4$) nanoparticles. The spinel ($MgCo_2O_4$) nanoparticles were synthesized by the sol-gel method using citric acid. Magnesium nitrate and cobalt nitrate were used to obtain a homogeneous gel solution. The gel solution was stirred and heated to obtain gel powder. The gel powder was dried in an oven at a temperature of 105˚C to obtain constant weight. The resulting sample was thermally heated in a muffle furnace at a temperature of 500˚C for four hours to get the required spinel nanoparticles. The prepared nanoparticles were characterized by X-ray diffraction (XRD). The antimicrobial activities of prepared spinel ($MgCo_2O_4$) nanoparticles were also determined by the agar well diffusion method. The average crystallite size of prepared spinel ($MgCo_2O_4$) nanoparticles was 24.492nm. Antimicrobial activities of the prepared spinel ($MgCo_2O_4$) nanoparticles showed high activity in *Escherichia coli.*

***Keywords* :** Spinel nanoparticles, sol-gel method, XRD, agar well diffusion method

Introduction

In current years, researchers from the developed countries have focused on various types of nanoparticles researches. Many questions may be asked about nanoparticles. Among them, two questions should be asked and could be solved about nanoparticles. Firstly, what is a nanoparticle? Secondly, why are researchers from many countries focusing on nanoparticles? The nanoparticle is a small particle that ranges between 1 to 100 nanometers in size. Metal nanoparticles have been used in a wide-ranging application in various fields. Specifically, like shapes, sizes, and composition of metallic nanomaterials are significantly linked to their physical, chemical, and optical properties, technologies based on nanoscale materials have been exploited in a variety of fields from chemistry to medicine (Lee,2019). Efforts have been made to explore their attractive properties and utilize them in practical applications, such as anti-bacterial and anti-cancer therapeutics, diagnostics and photoelectrics, water disinfection, and other clinical applications (Lee,2019). Current nanotechnology is the building device of microscopic or even molecular size, which will potentially be benefiting medicine, environmental protection, energy, and space exploration. In the last few years, the term nanotechnology has been inflated and has almost become synonymous with objects that are innovative and highly promising (Gatoo, and Naseem, 2014). Furthermore, several studies employing a diverse classes of nanoparticles showed that surface area is also a critical factor in displaying toxic manifestations (lung and other epithelial-induced inflammatory responses) in rodents (Holgate, 2010). In this research, the spinel ($MgCo_2O_4$) nanoparticles were synthesized by the sol-gel method. The main aim of this research was to apply the spinel nanoparticles in an electrical field, mechanical technology, and medicinal mode for the benefits of humans.

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Materials and Methods

All chemicals were bought from ACADEMY CHEMICAL GROUP, No-101,28th St, Pabedan T/S, Yangon. Magnesium nitrate hexahydrate [$Mg(NO_3)_2.6H_2O$] and Cobalt nitrate hexahydrate [$Co(NO_3)_2.6H_2O$] were produced by "British Drug House Chemical Ltd, Poole, England" is simply abbreviated as BDH. 90% citric acid ($C_6H_8O_7$) was produced by " Hopkin & Williams Ltd, chemical manufacturers in London". Ethylene glycol was a product from Applichem, Germany. All solutions were prepared by using deionized water during preparation procedures. In this research, various conventional and modern instrumental techniques were used throughout the experimental procedure. Moreover, the prepared nanoparticle was characterized by using X-ray Diffraction (XRD).

Preparation of Spinel ($MgCo_2O_4$) Nanoparticles Using Citric Acid

5.12 g of magnesium nitrate $Mg(NO_3)_2.6H_2O$ was dissolved in 50 mL of deionized water to obtain solution A. 5.8 g of $Co(NO_3)_2.6H_2O$, cobalt nitrate was dissolved in 50 mL of deionized water to obtain solution B. The solution A and B were mixed to obtain solution I. 3.8g of citric acid was dissolved in 100 mL of deionized water to obtain solution II. The solution I and II were mixed with 10 mL of ethylene glycol to obtain a homogeneous mixture solution. The mixture solution was stirred and heated on a magnetic stirrer hot plate at 70-80˚C for 2 hours. The gel powder was obtained. The gel powder was dried in an oven at 105˚C until a constant weight was obtained. The dried prepared powder was calcined in a muffle furnace at 500℃ for 4 hours. The prepared spinel ($MgCo_2O_4$) nanoparticle was obtained.

Characterization of Prepared Spinel ($MgCo_2O_4$) Nanoparticle

The characterizations of prepared spinel ($MgCo_2O_4$) nanoparticle were carried by using x-ray diffraction (XRD).

Determination of Crystallite Size and Interatomic Spacing

The crystallite size of prepared spinel ($MgCo_2O_4$) nanoparticle can be calculated by using Debye-Scherrer' formula,

$$D = \frac{0.9\lambda}{\beta cos\theta}$$

The interatomic spacing "d" can be computed by Bragg's equation,

$$d = \frac{\lambda}{2sin\theta}$$

Where, λ = the wave length of X-rays
(λ = 1.540560A for Cu/K-alpha 1)
θ = the diffraction angle
β = full width at half maximum in radian
D = average crystal size
d = interatomic spacing

Crystallite size of prepared spinel that has (2 2 0) of miller indices $= \frac{0.9 \text{ x } 1.54056Å}{\beta cos\theta}$

$$\beta = \text{FWHM x } \frac{\pi}{180}$$

$$=0.370\text{x}\frac{22}{7 \text{ x } 180}$$

$$= 0.00646031$$

$$\theta = \frac{30.663}{2} = 15.3315$$

$$\text{Cos } \theta = \text{Cos } 15.3315 = 0.96441220$$

Crystallite size of prepared spinel that has (2 2 0) of miller indices = $\frac{1.386504Å}{0.00646031 \times 0.96441220}$

= 222.538457230Å

= 22.2538457230 nm

Similarly, the crystallite sizes of other prepared spinel nanoparticles were calculated by using the above equation. The calculated crystallite sizes of prepared spinel nanoparticles were as follows.

The crystallite size of prepared spinel that has (3 1 1) of miller indices = 17.55 nm

The crystallite size of prepared spinel that has (2 2 2) of miller indices = 26.66 nm

The crystallite size of prepared spinel that has (4 0 0) of miller indices = 21.33 nm

The crystallite size of prepared spinel that has (4 2 2) of miller indices = 34.66 nm, Therefore, average crystallite size of prepared spinel ($MgCo_2O_4$) nanoparticles = 24.492 nm

From the peak ID report, the prepared nanoparticles were spinel $MgCo_2O_4$ nanoparticles. Crystalline structures of spinel nanoparticles were cubics. Therefore, lattice constants of prepared spinel were calculated according to the following equation.

$$\frac{1}{d^2} = \frac{h^2 + k^2 + l^2}{a^2}$$

For lattice constants of prepared spinel that has (2 2 0) of miller indices,

$$\frac{1}{(2.9133Å)^2} = \frac{2^2 + 2^2 + 0^2}{a^2}$$

$$a^2 = 67.89853512Å^2$$

$$a = 8.2400Å$$

Similarly, Lattice constants of other prepared spinel ($MgCo_2O_4$) nanoparticles were calculated by using the above equation. The calculated lattice constants of other prepared spinel nanoparticles were as follows.

Lattice constants of prepared spinel that has (3 1 1) of miller indices = a =b = c = 8.1775Å

Lattice constants of prepared spinel that has (2 2 2) of miller indices = a =b = c = 8.1814Å

Lattice constants of prepared spinel that has (4 0 0) of miller indices = a =b = c = 8.1988Å

Lattice constants of prepared spinel that has (4 2 2) of miller indices = a =b = c = 8.1899Å

Therefore, average lattice constants of the prepared spinel ($MgCo_2O_4$) nanoparticle was

a = b = c = 8.1861Å

Determination of Antimicrobial Activities of Prepared Spinel ($MgCo_2O_4$) Nanoparticles

Screening of antimicrobial activities of the prepared sample were carried out by using agar well diffusion method. In this experiment, glucose, yeast extract, peptone, agar, distilled water, autoclave, an incubator, hot plate, Petri-dishes, measuring cylinder, micropipette and clipper were used. The antimicrobial activities of prepared spinel ($MgCo_2O_4$) nanoparticles were detected in six types of microorganisms by the agar well diffusion method. The organisms were (a) *Bacillus subtiluss* that causes fever to humans, (b) *Candida albicans*, causing fever, (c) *Escherichia coli* causing diarrhea, (d) *Pseudomonas aeruginosa*, causing skin disease, (e) *Staphylococcus aureus* that causes food poisoning to humans and (f) *Salmonella typhi* causing typhoid fever to humans.

Procedure

0.5g of glucose, 0.3g of yeast extract, 0.3g of peptone, 1.7g of agar, and 100mL of distilled water were added in a 250mL sterile conical flask and heated on a hot plate until a boil medium. Then, the mouth of the flask was plugged with a piece of cotton wool. This medium was sterilized in an autoclave at 121℃ for 45 minutes. After 45 minutes, 0.1 mL of test organisms were inoculated into 20 mL of agar medium at about 40℃ and were poured into the sterile Petri-dishes at aseptic condition. After the agar became solid, cock borer was used to make the wells (8mm in diameter). Then extract sample (20 µL) was introduced into the well and they were incubated at room temperature for 24-48 hours. After 24-48 hours of incubation, the clear zones were measured. The clear zone surrounding the wells indicated the presence of the antimicrobial active compound in the extracts which inhibit the growth of the test organisms Collins, et.al.,(1998).

Results and Discussion

This section contains two portions of the discussion. Firstly, the characterization of prepared spinel ($MgCo_2O_4$) nanoparticles by XRD was discussed. In the second portion, the determination of antimicrobial activities of the prepared spinel ($MgCo_2O_4$) nanoparticles was described. The prepared spinel ($MgCo_2O_4$) nanoparticle was characterized by using XRD. The crystal structure and phase analysis were performed by X-ray diffraction (XRD) using Rigaku, D-Max 2200, Japan in the Department of Chemistry, Yangon University.

Figure 1 showed the XRD diffractogram of prepared spinel ($MgCo_2O_4$) nanoparticles. In this diffractogram, standard values of Miller indices (h k l) of spinel $MgCo_2O_4$ were identical to values of (h k l) of diffractogram of prepared spinel $MgCo_2O_4$ nanoparticles. The crystallite sizes and phase ID of prepared spinel ($MgCo_2O_4$) nanoparticles were shown in table 1.The lattice constants of prepared spinel ($MgCo_2O_4$) nanoparticles were shown in table 2.

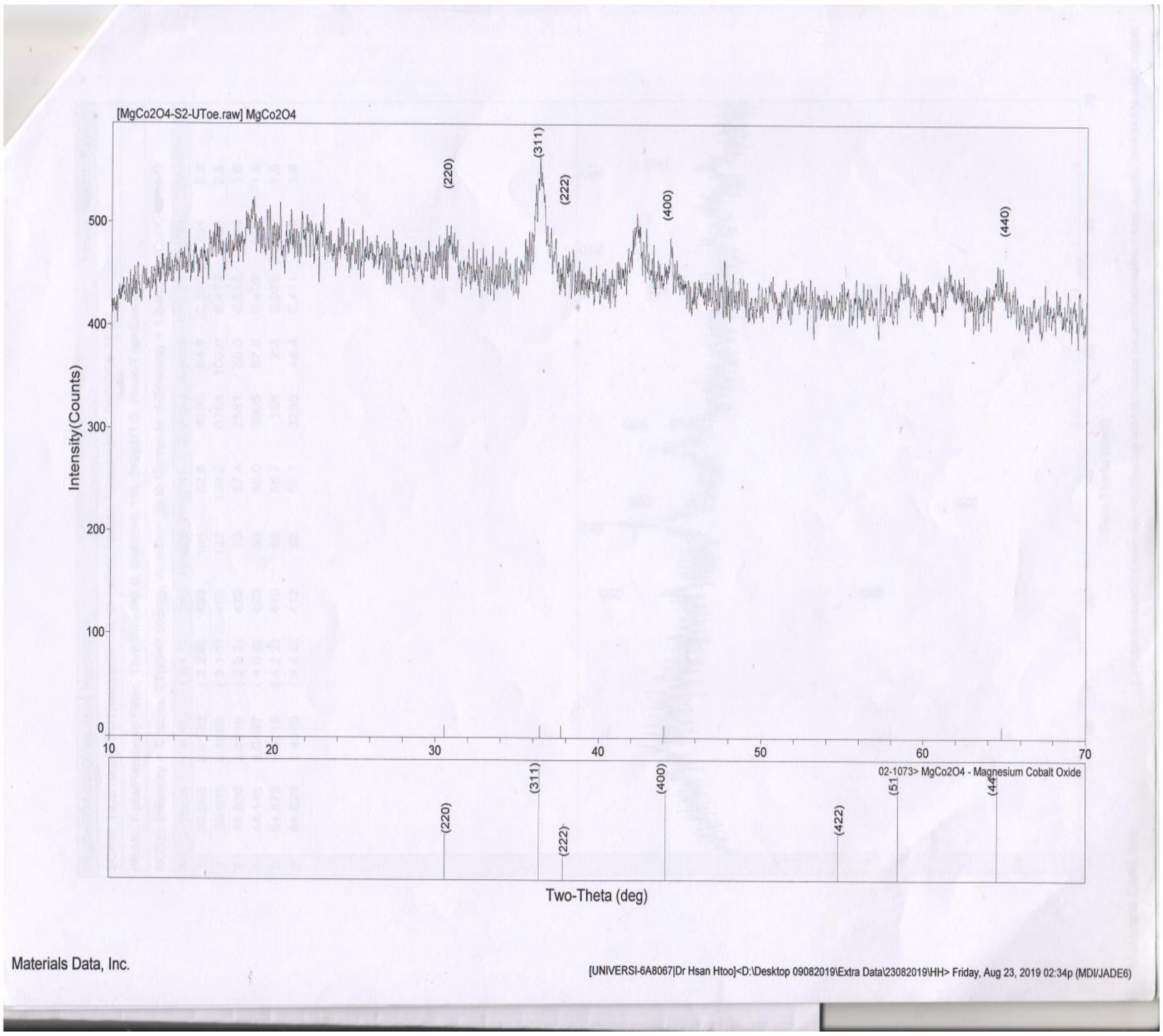


Figure 1. XRD diffractogram of prepared spinel ($MgCo_2O_4$) nanoparticles

Table 1 Crystallite sizes and Phase ID Report of Prepared Spinel ($MgCo_2O_4$) Nanoparticles

Bragg angles (2θ)	Miller indices (h k l)	FWHM of β radian	Crystallite sizes D (nm)	Phase ID
30.663	2 2 0	0.370	22.26	$MgCo_2O_4$
36.409	3 1 1	0.473	17.55	$MgCo_2O_4$
38.070	2 2 2	0.313	26.66	$MgCo_2O_4$
44.148	4 0 0	0.400	21.33	$MgCo_2O_4$
54.873	4 2 2	0.020	34.66	$MgCo_2O_4$
Average crystallite size			24.492	$MgCo_2O_4$

Table 2 Calculated Lattice Constants from Miller Indices and Interplaner Spacing

Interplaner spacing d(Å)	Miller indices (h k l)	a-axis (Å)	b-axis (Å)	c-axis (Å)
2.9133	2 2 0	8.2400	8.2400	8.2400
2.4656	3 1 1`	8.1775	8.1775	8.1775
2.3618	2 2 2	8.1814	8.1814	8.1814
2.0497	4 0 0	8.1988	8.1988	8.1988
1.6718	4 2 2	8.1899	8.1899	8.1899
Average lattice constants a = b = c = 8.1861Å				

Antimicrobial activities of prepared spinel ($MgCo_2O_4$) nanoparticle were screening by agar well diffusion method.

In this section, figure 2(a) showed a screening of antimicrobial activity of prepared spinel ($MgCo_2O_4$) nanoparticle to *Bacillus subtilus*. In the figure, the right dish indicated the activity of blank solution (only distilled water). The hole on the left dish showed the activity of prepared spinel ($MgCo_2O_4$) nanoparticle to *B.subtilus.* Its diameter was below 9 mm. Therefore prepared spinel ($MgCo_2O_4$) nanoparticle did not against *B.subtilus*.

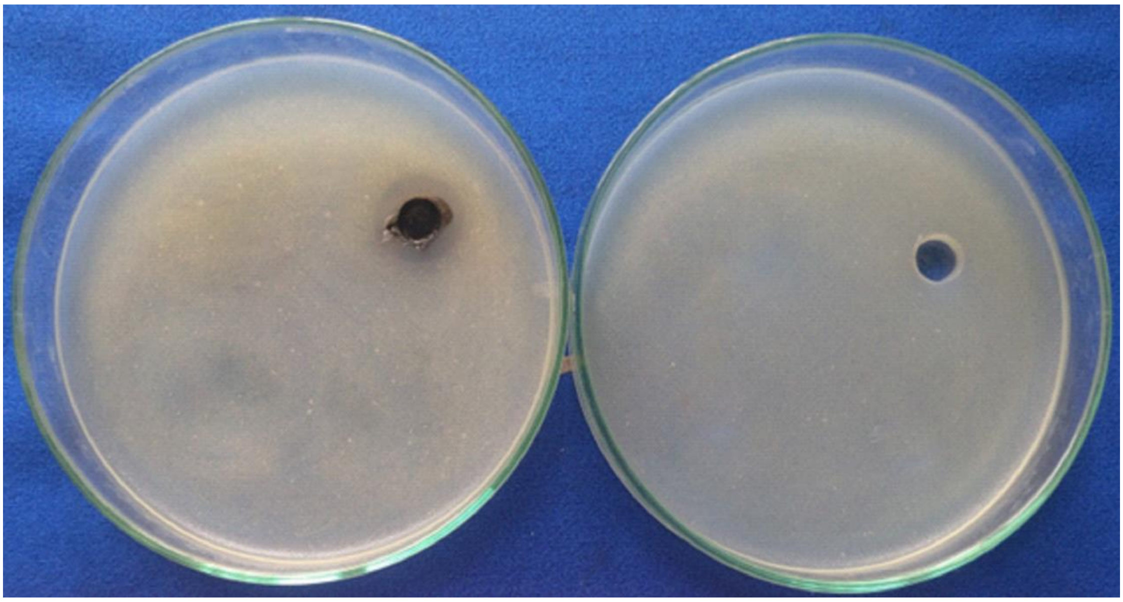
Figure 2(b) showed a screening of antimicrobial activity of prepared spinel ($MgCo_2O_4$) nanoparticle to *Candida albican*. In the figure, the right dish indicated the activity of blank solution (only distilled water). The hole on the left dish showed the activity of prepared spinel ($MgCo_2O_4$) nanoparticle to *Candida albican*. Its diameter was below 9 mm. Therefore, prepared spinel ($MgCo_2O_4$) nanoparticle did not against *Candida albican.*

Figure 2(c) showed a screening of antimicrobial activity of prepared spinel ($MgCo_2O_4$) nanoparticle to *Escherichia coli*. In the figure, the right dish indicated the activity of blank solution (only distilled water). The hole on the left dish showed the activity of prepared spinel ($MgCo_2O_4$) nanoparticle to *Escherichia coli*. Its diameter was 26.34 mm. Therefore, prepared spinel ($MgCo_2O_4$) nanoparticle against *Escherichia coli* in high activity.

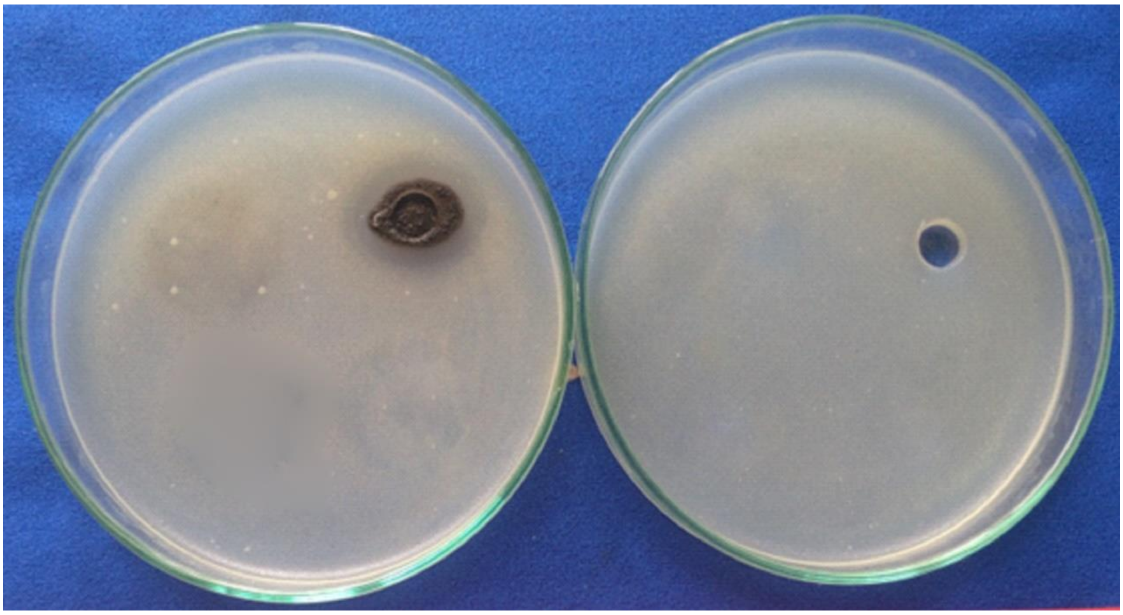
Figure 2(d) showed a screening of antimicrobial activity of prepared spinel ($MgCo_2O_4$) nanoparticle to *Pseudomonas aeruginosa*. In the figure, the right dish indicated the activity of blank solution (only distilled water).The hole on the left dish showed the activity of prepared spinel ($MgCo_2O_4$) nanoparticle to *Pseudomonas aeruginosa*. Its diameter was below 9 mm. Therefore, prepared spinel ($MgCo_2O_4$) nanoparticle did not against *Pseudomonas aeruginosa*.

Figure 2(e) showed a screening of antimicrobial activity of prepared spinel ($MgCo_2O_4$) nanoparticle to *Salmonella typhi*. In the figure, the right dish indicated the activity of blank solution (only distilled water). The hole on the left dish showed the activity of prepared spinel ($MgCo_2O_4$) nanoparticle to *Salmonella typhi*. Its diameter was below 9 mm. Therefore, prepared spinel ($MgCo_2O_4$) nanoparticle did not against *Salmonella typhi*.

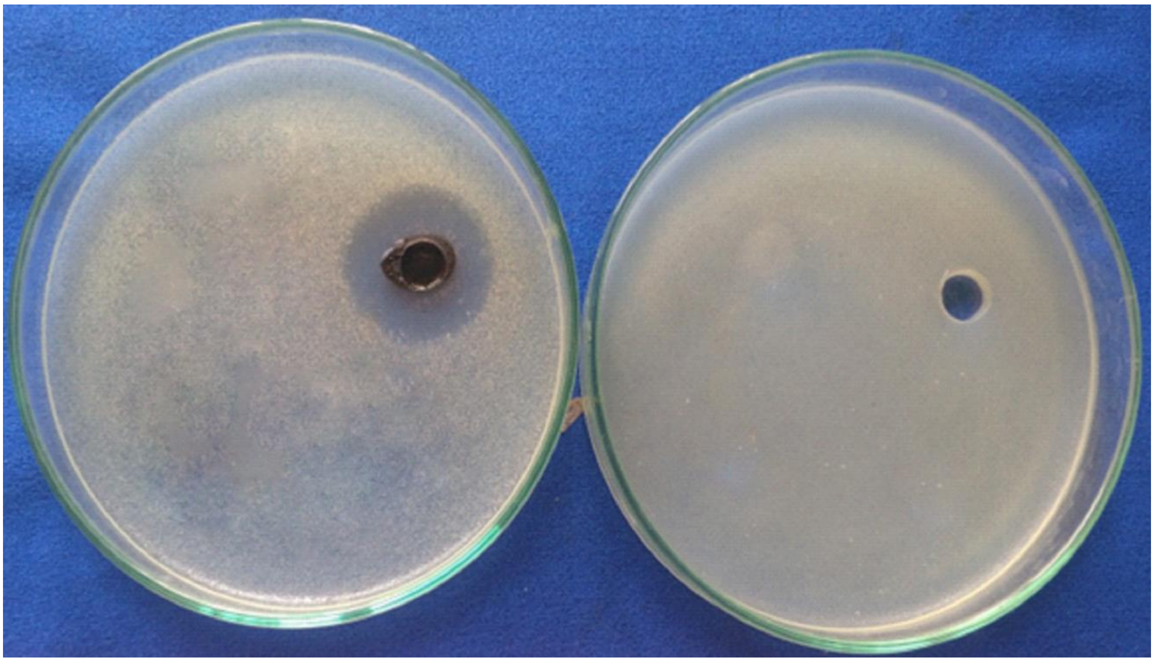
Figure 2(f) showed a screening of antimicrobial activity of prepared spinel ($MgCo_2O_4$) nanoparticle to *Staphylococcus aureus*. In the figure, the right dish indicated the activity of blank solution (only distilled water).The hole on the left dish showed the activity of prepared spinel ($MgCo_2O_4$) nanoparticle to *Staphylococcus aureus*. Its diameter was 16.80 mm. Therefore, prepared spinel ($MgCo_2O_4$) nanoparticle againsted *Staphylococcus aureus* in medium activity. The analytical results were shown in table 3. According to the results, prepared spinel ($MgCo_2O_4$) showed no activity in *Bacillus subtilus*, *Candida albican*, *Pseudomonas aeruginosa*, and *Salmonella typhi* as well as high activity in *Escherichia coli* but medium activity in *Staphylococcus aureus*.



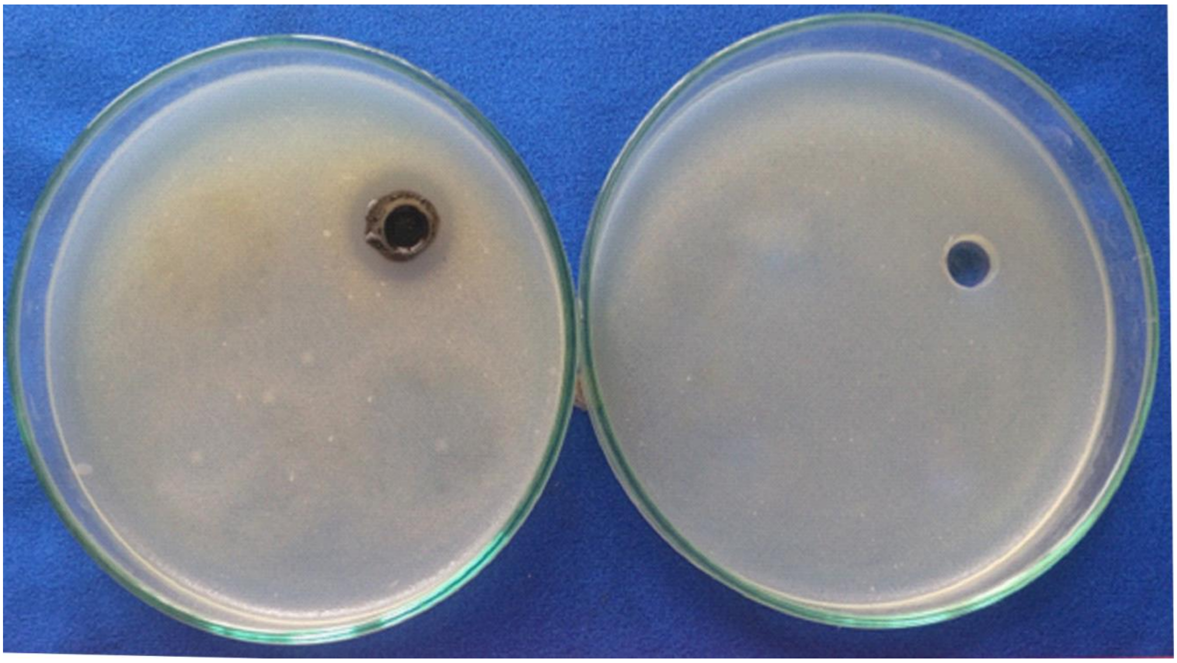
(a) *B. subtilus*



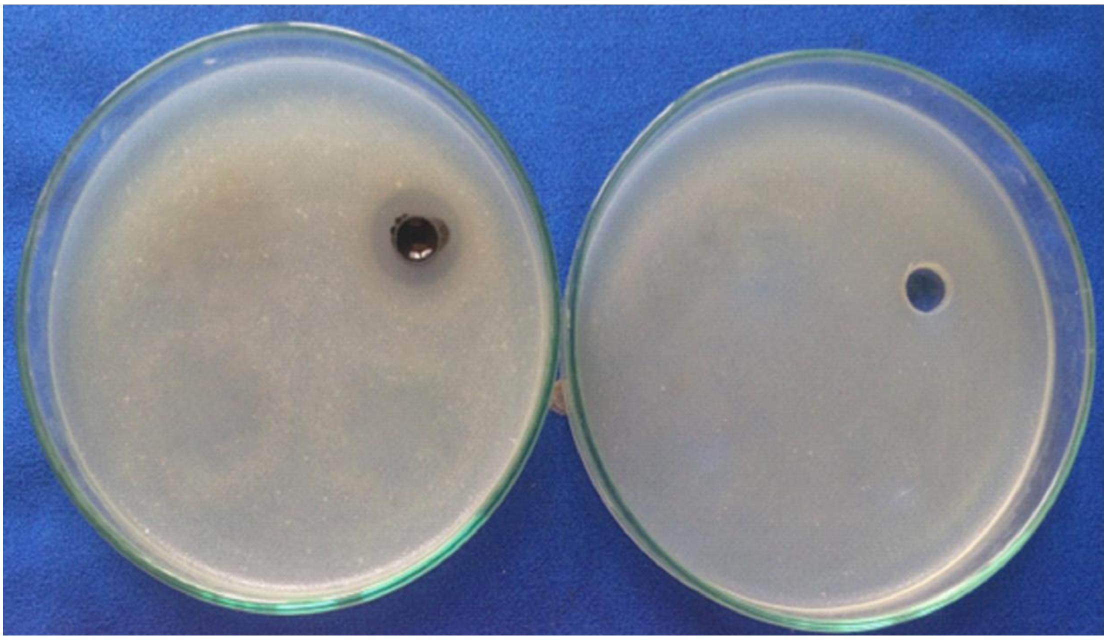
(b) *Candida albicans*



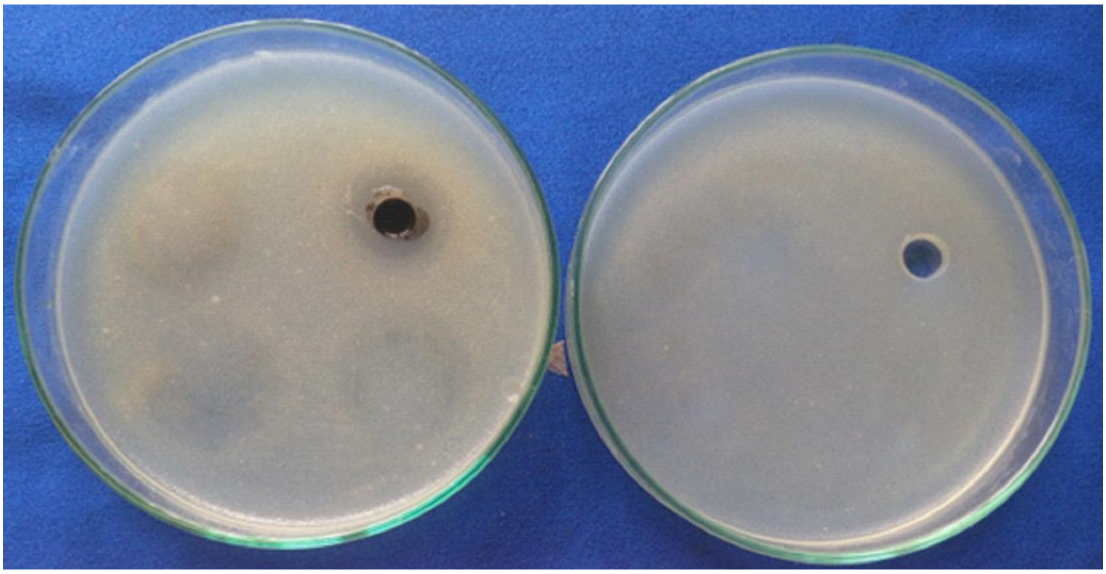
(c) *E. coli*



(d) *Pseudomonas aeruginosa*



(e) *Salmonella typhi*



(f) *Staphylococcus. aureus*

Figure 2. Screening of antimicrobial activities of prepared spinel ($MgCo_2O_4$) nanoparticles
(a) *B. subtilus,* (b) *Candida Albican,* (c) *E.coli*
(d) *Pseudomonas aeruginosa,* (e) *Salmonella typhi*, (f) *Staphylococcus aureus*

Table 3. Results of Screening of Antimicrobial Activities of Prepared Spinel ($MgCo_2O_4$) Nanoparticles

No	Microorganisms	Inhibition zone diameter of sample($MgCo_2O_4$) (mm)
1	*Bacillus subtilus* IFO 90571	-
2	*Candida albican* NITE09542	-
3	*Escherichia coli* AUH 5436	26.34(+++)
4	*Pseudomonas aeruginosa*	-
5	*Salmonella typhi* AUH 7943	-
6	*Staphylococcus aureus* AUH 8465	16.80(++)

(-) No activity
(+) 9-14mm, Low activity
(++) 15-20mm, Medium activity
(+++) 21mm-above, High activity

Conclusion

In this research, spinel ($MgCo_2O_4$) nanoparticle was synthesized from magnesium nitrate and cobalt nitrate by sol-gel method using citric acid. The prepared spinel nanoparticle was characterized by modern sophisticated methods such as XRD. From the XRD results, the average crystallite size of the prepared spinel nanoparticle was 24.492 nm. Average lattice constants was a = b = c = 8.1861Å. The crystal structures of prepared spinel ($MgCo_2O_4$) nanoparticles were cubics. The antimicrobial activities of prepared spinel ($MgCo_2O_4$) nanoparticles were carried out by Agar Well Diffusion Method (Collins1998). According to the screening of antimicrobial activities of the crude extract by using agar well diffusion method, prepared spinel ($MgCo_2O_4$) nanoparticles showed no activity in *Bacillus subtilus*, *Candida albican*, *Pseudomonas aeruginosa*, and *Salmonella typhi* as well as high activity in *Escherichia coli* but medium activity in *Staphylococcus aureus*. According to the analytical results, the prepared particles are nanoparticles since these were in the range of 1-100nm scale and the prepared spinel particles have medicinal effect to cure diarrhea because the particles against *Escherichia coli* are in high activity.

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