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BIOSYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF ZINC OXIDE NANOPARTICLE

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ABSTRACT

Bio-nanotechnology is the most emerging area of research these days due to its immense potential and applications and is making an impact in all spheres of human life. Hence the present investigation was carried out to synthesize Zinc oxide nanoparticle using bacteria, *Escherichia coli,* to study the characterization and antibacterial activity of synthesized Zinc oxide nanoparticle. Biosynthesis of Zinc oxide nanoparticle using bacteria, *E.coli* and Zinc acetate dehydrate solution with ≥ 99.0% purity was carried out. Characterization of nanoparticle was carried out by FTIR analysis, (SEM) and (TEM) and antibacterial activity of biosynthesized Zinc oxide nanoparticle was carried out by well diffusion method. The results of characterization of Zinc oxide nanoparticle by FTIR analysis of the biosynthesized nanoparticle revealed the presence of biomolecules in the nanoparticle that are responsible for the reduction and stabilization process for the biosynthesis of nanoparticle. The results of the SEM studies showed rectangular and cuboidal shaped nano stuctures and Zinc oxide nanoparticle without any aggregation were observed in the TEM image. The results of the antibacterial activity of biosynthesized Zinc oxide nanoparticle using *E.coli,* against the test

organisms (*Bacillus* sp., *Staphylococcus epidermidis and Staphylococcus saprophyticus*) carried out by well diffusion method revealed that Zinc oxide nanoparticle had significant inhibitory effect against all the test bacterial species but slightly higher rate of inhibition was recorded in *Staphylococcus saprophyticus* (29 mm) followed by *Bacillus* sp. (27mm) and *Staphylococcus epidermidis* (23mm). Thus from the results of the present study, it can be concluded that biosynthesis of Zinc oxide nanoparticle using supernatants of *E.coli* has several advantages such as simple, cost effective, less time consuming, safe and ecofriendly compared to physical and chemical methods of nanoparticle synthesis and biosynthesized Zinc oxide nanoparticle has a potent antibacterial activity as evidenced in the present study.

Keywords: Biosynthesis, *E.coli,* Zinc oxide nanoparticle, characterization, antibacterial activity, well diffusion method

INTRODUCTION

Nanotechnology is the production and use of materials at the smallest possible scale **[1]**. Nanoparticles due to their small size and large surface to volume ratio display a wide variety of chemical and physical properties. Thus, by modifying and controlling the size and shape at nano metric level, nanoparticles exhibit interesting properties like bio sensing, catalytic activity, optical activity, antimicrobial activity etc and balanced fusion of nanotechnology, inorganic chemistry and microbiology can design a novel antimicrobial agents. It is evident that the metal based nanoparticles constitute an effective antimicrobial agent against common pathogenic microorganisms. Therefore, some of the nanoparticles such as silver, titanium dioxide and Zinc oxide are receiving considerable attention as antimicrobial aspects and additives in consumer, health-related and industrial products **[2]**. Zinc oxide is an inorganic compound with the formula ZnO. It usually appears as a white powder, nearly insoluble in water. Most Zinc oxide used commercially is produced synthetically **[2]**. Zinc oxide is nontoxic and is compatible with human skin making it a suitable additive for textiles and surfaces that come in contact with human body **[2]**. The vast applications of nanoparticles in medical sciences are drug delivery, imaging and diagnosis **[3]**. Synthesis of nanoparticles employing microorganisms such as bacteria, yeasts etc. has attracted much attention due to their higher production yields and with low expenses. Based on their enormous biotechnological applications, microorganisms such as bacteria, fungi and yeast are regarded as possible ecofriendly "nano-factories" **[4]**. Bacteria such as *Bacillus licheniformis, Corynebacterium* sp., *Staphylococcus*

aureus, Proteus mirabilis, Brevibacterium casei were used to synthesize small sized nanoparticles **[5]**. *E.coli* is a gram negative bacteria, known for its reduction activity as reported in bioremediation studies **[4]** and it synthesize nanoparticles within short duration of time. Hence, this study is an investigation to synthesize Zinc oxide nanoparticle using bacteria, *E.coli* to study its characterization and antibacterial activity of Zinc oxide nanoparticle against pathogenic bacterial species like *Bacillus* sp., *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* using well diffusion method.

MATERIALS AND METHODS

Materials:

Preparation of Zinc acetate dehydrate solution for synthesis of Zinc oxide Nanoparticle

Zinc acetate dihydrate with ≥ 99.0% purity was obtained from Sigma Aldrich and distilled water was used throughout the experiments for the synthesis of Zinc oxide nanoparticle.

Collection of *E.coli* for Biosynthesis of Zinc oxide Nanoparticle

The subculture of *E.coli* was obtained from Microbiology laboratory, Chandigarh, India and was revived in LB broth (Himedia). The bacteria was then cultured in 250 ml flasks using LB broth media. The culture flasks were incubated for 36 h at 37ºC with shaking at 1500 rpm. After an incubation period of 36 hours, the cultures were centrifuged at 5,000 rpm for 10 minutes and the supernatant was collected, which was used for the synthesis of nanoparticle.

Collection of bacterial isolates for antibacterial activity of Zinc oxide nanoparticle (Plate 4)

Clinical isolates like*Bacillus* sp., *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* were collected from a tertiary care hospital. Samples were transported to the laboratory for further processing in an ice box.

Methods:

Biosynthesis of Zinc oxide nanoparticle using Bacteria, *E.coli*

Biosynthesis of Zinc oxide nanoparticle using bacteria, *E.coli* was carried out **[5]**. 0.1 g of Zinc acetate (≥ 99.0% purity, Sigma Aldrich) was added to de-ionized water followed by 1% supernatant of *E.coli* culture. The biosynthesis of Zinc oxide nanoparticle was carried out in Erlenmeyer flask containing de-ionized water treated with Zinc acetate and was incubated at 37ºC for agitation (2000 rpm) for 24-48 hours until the deposition appears at the bottom of the flask.

Characterization of biosynthesized Zinc oxide nanoparticle

FTIR Analysis:

The FT-IR spectra of biosynthesized Zinc oxide nanoparticle was recorded in

SHIMADZU-8400 spectrometer using KBr pellet method.

Scanning Electron Microscope

In the present work, Scanning Electron Microscope (SEM) was employed to study the morphology of biosynthesized Zinc oxide nanoparticle. The experiment was performed at an accelerating voltage of 20 kV. The slide was coated with platinum and after the platinum coating, the SEM image was taken.

Transmission Electron Microscope

Transmission Electron Microscope (TEM) analysis was done using Philips (Technai 10). Thin films of sample were prepared on a carbon coated copper grid by just dropping a very small amount of sample on the grid, extra solution was removed using a blotting paper and then the film on the TEM grid were allowed to dry by putting it under incubator. The images were obtained by Technai, Twin 200KV and a bias voltage of 200kV was used to analyse samples.

Antibacterial activity of biosynthesized Zinc oxide nanoparticle against Bacterial isolates.

Antibacterial activity of biosynthesized Zinc oxide nanoparticle against bacterial isolates was carried out using well diffusion method **[6].**

Preparation of culture inoculums:

The test organisms (*Bacillus* sp., *Staphylococcus epidermidis and Staphylococcus saprophyticus*) were inoculated on to nutrient agar (Himedia) and was incubated for 24hrs. The cultures were stored at 4°C as stock cultures. Active cultures of the isolates for experiments was prepared by transferring a loop full of isolate culture from the stock culture to tube containing nutrient broth which was incubated at 37°C for 24 hrs.

Agar Well Diffusion Method:

The effects of Zinc oxide nanoparticle on the test organisms was assayed by agar well diffusion method. Muller-Hinton agar was poured into the Petri plates aseptically and was allowed to solidify. The lawn culture of the test bacterial strain was made with the help of sterile cotton swab. Wells were made with the help of sterile cork borer (6mm) and the cuts agar discs were removed aseptically with sterile forceps. 100µl of biosynthesized Zinc oxide nanoparticle was added into the well. The test plates were incubated aerobically at 37°C for 24hrs in the incubator. After incubation, the results were recorded as the presence or absence of inhibition zone. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well.

STATISTICAL ANALYSIS

The data obtained from the experiments was analyzed and expressed as mean,standard deviation and Chi- square test.

- **S.D. =** $\sqrt{\frac{1}{N-1}\sum x^2 - \frac{(\sum x^2)}{N}}$

Where,

N = Number of individual observation

$\sum x2$ = Sum of square of individual observation

$(\sum x2)$ = Square of the total individual observation

- **Chi-square test =** $\chi^2 = \Sigma \frac{(O-E)^2}{E}$

O = Observed values

E = Expected values

RESULTS AND DISCUSSION

Biosynthesis of Zinc oxide nanoparticle using bacteria, *E.coli*

Different types of physical and chemical methods are employed for the synthesis of nanoparticle. The use of these synthetic methods requires both strong and weak chemical reducing agents and protective agents like sodium borohydride, sodium citrate and alcohols. These agents are mostly toxic, flammable, cannot be easily disposed off due to environmental issues and also show a low production rate **[7-9]**. It leads to in search of alternatives which could be ecofriendly and does not cause any harm to human and domestic animals health. One such methods were the use of microbes and plants either as reducing agents or protective agents. Many biological organisms, both unicellular and multicellular are known to produce inorganic materials either intra or extra cellular, often of nanoscale dimensions. The biosynthesis of nanoparticle employs biological agents like bacteria, fungi, actinomycetes, yeast, algae and plants **[8, 10]**. The rate of reduction of metal ions using biological agents is found to be much faster and also at ambient temperature and pressure conditions.

Use of biological organisms such as microorganisms, plant extract or plant biomass could be an alternative to chemical and physical methods for the production of an eco-friendly manner of nanoparticle **[11]**.

Hence, based upon the literature cited above, the present investigation was aimed to synthesize Zinc oxide nanoparticle using bacteria, *E.coli,* to characterize the biosynthesized Zinc oxide nanoparticle and to evaluate its antibacterial efficiency against pathogenic bacterial isolates such as *Bacillus* sp*., Staphylococcus epidermidis* and *Staphylococcus saprophyticus.*

The results of biosynthesis of Zinc oxide nanoparticle using bacteria, *E.coli* was shown in **Plate 1**. Zinc oxide nanoparticle was synthesized successfully by the biological method using Bacteria, *E.coli* supernatant. During exposure to supernatant of *E.coli,* reduction of zinc ions into zinc nanoparticle was monitored, as a result, pale white colour was formed, which occurred due to the Surface Plasmon Resonance phenomenon. The metal nanoparticles have free electrons, which

helps in the formation of the Surface Plasmon Resonance absorption band. It occurs due to the united vibration of the electrons of metal nanoparticle in resonance with light wave.

Characterization of biosynthesized Zinc oxide nanoparticle

FTIR Analysis

The Fourier Transform Infrared Spectroscopy of the sample has been carried out to identify the possible biomolecules in the samples that are responsible for the reduction of ions and also for capping agents responsible for the stability of the biosynthesized nanoparticle.

FTIR measurements of Zinc oxide nanoparticle

The result of FTIR analysis of Zinc oxide nanoparticle synthesized using *E.coli* was presented in **Figure 1**. The results of the study revealed the formation of peaks at 516.98 cm-1 and 479.33 which may be attributed to the stretching vibration of Zinc oxide nanoparticle. Peaks formed at 614.36 and 696.33 cm-1 were due to the presence of C-C groups. Peak at 1034.85 cm-1 represent the amide group of linkage. Peak at 1550.83 cm-1 was due to the stretching vibration of N-H group present in amide linkage of protein. Band formation at 2543-2631 cm-1 was due to the presence of carbonyl groups. Formation of bands at 2547-2945.43 cm-1 was attributed to C-H group of stretching of aromatic ring. Band at 3012-3423 cm-1 was due to stretching of OH group of phenols.

Certain membrane bound proteins act as a reducing agent for the synthesis of nanoparticle. The ionic reduction in bacteria takes place due to certain proteins present along the lipopolysaccharides / cell wall which reduces the metallic ions in its vicinity of the bacterial cell, thereby producing stable nanoparticle indicated by the formation of the bands.

The results of FTIR analysis of the biosynthesized nanoparticle revealed the presence of biomolecules in the nano-particle that are responsible for the reduction and stabilization process of the biosynthesis of nanoparticle which were identified by FTIR analysis. Fourier Transform Infrared measurements confirm the role of phenolic and alcoholic compounds in the reduction of zinc oxide and proteins as the stabilizing material for the generated nanoparticle. From the results of IR spectrum, it can be observed that the Zinc oxide nanoparticle are rich in different functional groups such as carbonyl, phenols, proteins, amides etc, the enzymes like nitrate reductase etc and presence of large amount of polyphenols, alkaloids and flavonoids. The involvement of these biomolecules in the reduction and stabilization (capping actions) are clearly evident from the results of IR spectrum of

the biosynthesized Zinc oxide nanoparticle **[12]**.

SEM

The results of the SEM studies on Zinc oxide nanoparticle was depicted in **Plate 2**. Rectangular and cuboidal shaped nanostuctures were formed. The size of the Zinc oxide nanoparticle synthesized using Bacteria, *E.coli* ranges from 46.18nm - 86.92nm in diameter. SEM can achieve resolution better than 1 nanometer. SEM provided further insight into the morphology and size details of the Zinc oxide nanoparticle **[12]**.

TEM

Transmission Electron microscope is another type of microscope where a beam of electrons is transmitted through an Ultra-thin specimen, interacting with the specimen as it passes through it. An image is formed from the interaction of the electrons transmitted through the specimen. The image is magnified and focused on to an imaging device.

The result of TEM micrographs of biosynthesized Zinc oxide nanoparticle was shown in **Plate 3**. TEM micrographs provides a clear idea on the shape of nanoparticle. Nanoflowers were observed which were in dispersed form with the size of 50nm diameter. This nanoparticle was well distributed without any aggregations were observed in the TEM image which could be due to capping by proteins **[12].**

Antibacterial activity of Zinc oxide nanoparticle against the bacterial isolates

The results of antibacterial activity of Zinc oxide nanoparticle against the bacterial isolates **(Plate 4)** were presented in **Plate 5, Table 1a and 1b and Figure 1**. The results of the study showed that Zinc oxide nanoparticle gave a zone size ranging from 19mm ± 1 to 29mm ±1 in diameter. The maximum zone of 29mm $\pm$ 1 in diameter was exhibited towards *Staphylococcus saprophyticus* followed by *Bacillus* sp. 27mm $\pm$ 1 and *Staphylococcus epidermis* 23mm $\pm$ 1. The significant level was 0.5%. The result of antibacterial activity of biosynthesized nanoparticle against bacterial species showed that though biosynthesized Zinc oxide nanoparticle was found to be efficient in inhibiting the growth of the bacterial isolates, which may be due to the presence of different functional groups, proteins, amides etc. The electrostatic interaction between bacterial cell surface and nanoparticle for inhibiting the growth and also production of hydrogen per oxide from zinc oxide nanoparticle leads to the entry of particles into bacterial cell membrane cause injury and finally the death of the bacterium has occurred **[13]**. Based upon the above possible phenomena, the present study reports the inhibition of the growth of bacteria by damaging the cell

membrane that occurred by penetration of zinc oxide nanoparticle **[14]**.

Statistical Analysis

The results of the statistical analysis of the data obtained from the above experiments revealed that the probability was found to be less and equal to 5. The data were significant at 0.5% level.

Table 1a: Agar well diffusion method of biosynthesized Zinc oxide nanoparticle

S. No.	Name of the test Organism	Zinc oxide nanoparticle (zone size in mm)
1.	*Bacillus* sp.	27 mm
2.	*Staphylococcus epidermidis*	23 mm
3.	*Staphylococcus saprophyticus*	29 mm

Table 1b: Statistical Data of Agar well diffusion method of biosynthesized zinc oxide nanoparticle

S. No.	Name of the Organisms	Mean	S.D	Chi Square
1	*Bacillus* sp.	26.6	1.5	0.5
2	*Staphylococcus epidermidis*	23.3	1.5	
3	*Staphylococcus saprophyticus*	28.6	1.5	

The values are significant at 0.5% level

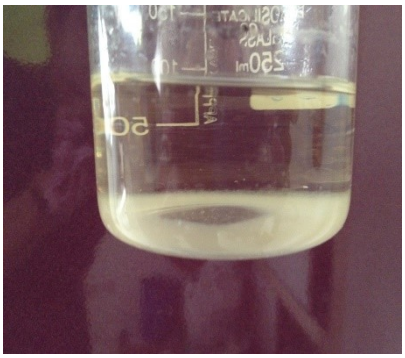


Plate 1: Biosynthesis of Zinc oxide nanoparticle

Characterization of biosynthesized Zinc oxide nanoparticle

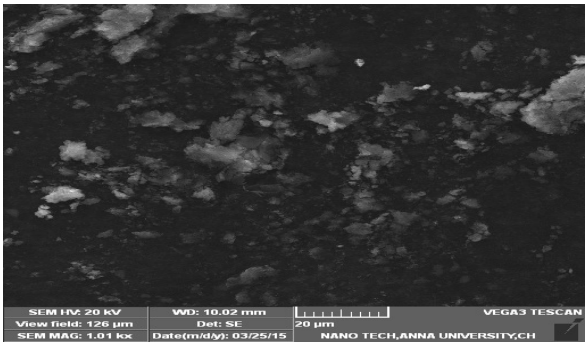


Plate 2: SEM of Zinc oxide nanoparticle

Plate 3: TEM of Zinc oxide nanoparticle

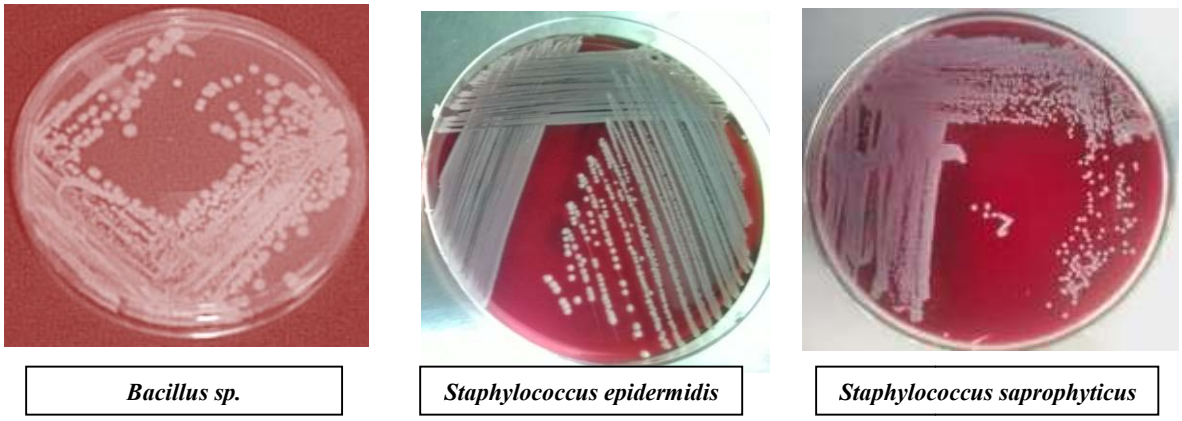


Plate 4: Bacterial isolates for antibacterial activity of Zinc oxide nanoparticle

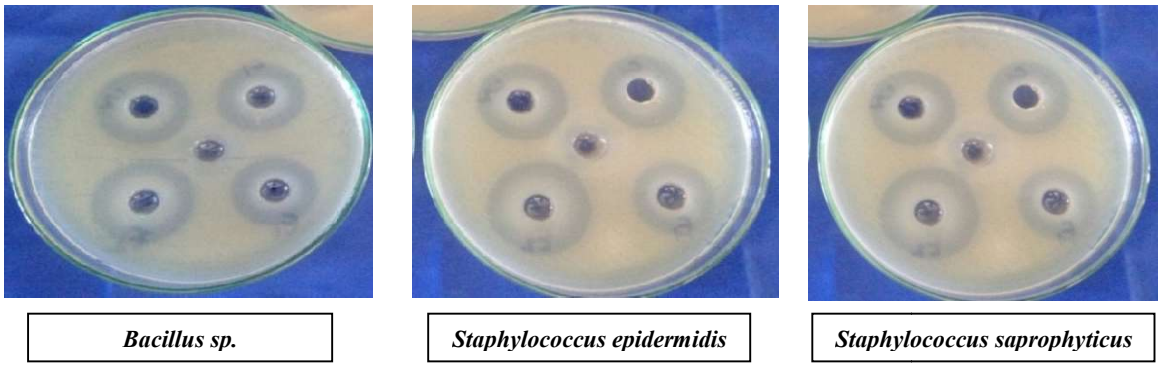
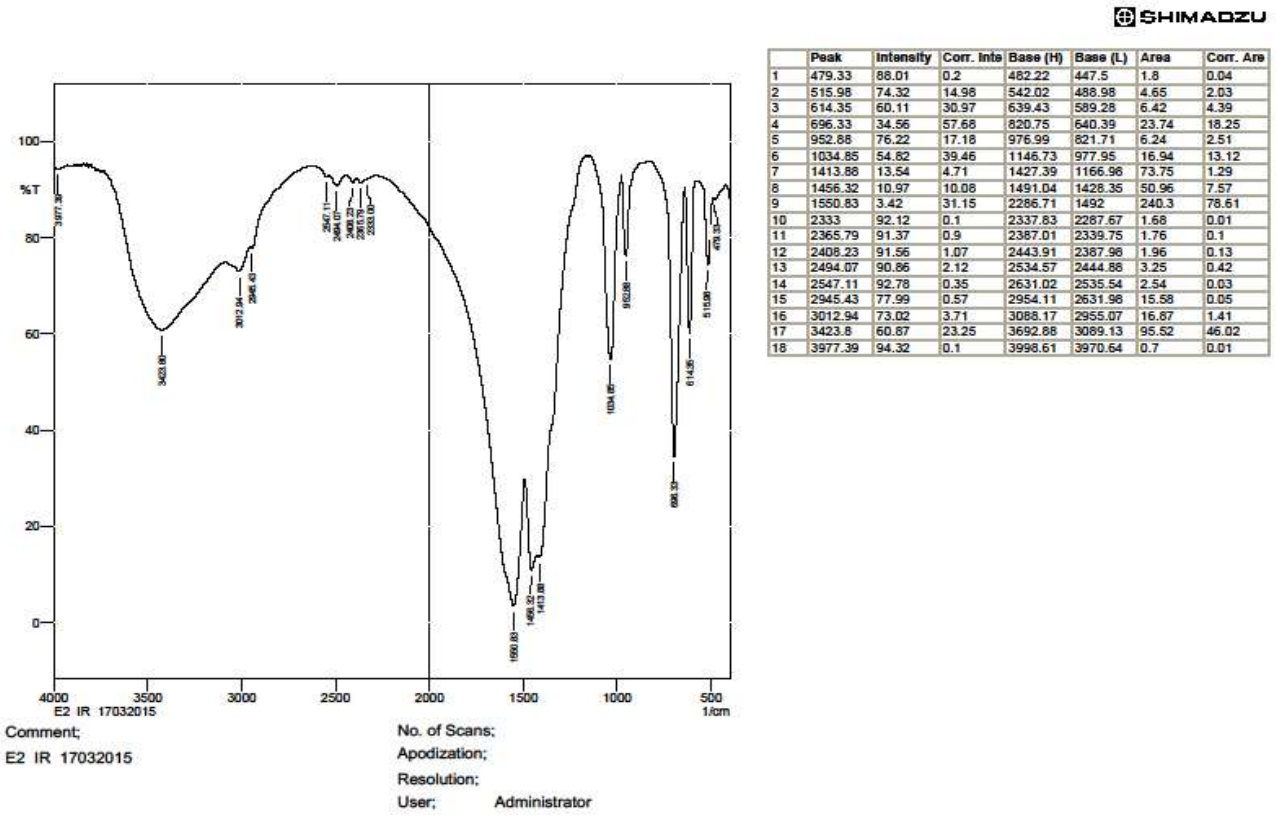


Plate 5: Antibacterial activity of Zinc oxide nanoparticle against the bacterial isolates Zone of inhibition



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	479.33	88.01	0.2	482.22	447.5	1.8	0.04
2	515.98	74.32	14.98	542.02	488.98	4.65	2.03
3	614.35	60.11	30.97	639.43	589.28	6.42	4.39
4	696.33	34.56	57.68	820.75	640.39	23.74	18.25
5	952.88	76.22	17.18	976.99	821.71	6.24	2.51
6	1034.85	54.82	39.46	1146.73	977.95	16.94	13.12
7	1413.88	13.54	4.71	1427.39	1166.98	73.75	1.29
8	1456.32	10.97	10.08	1491.04	1428.35	50.96	7.57
9	1550.83	3.42	31.15	2286.71	1492	240.3	78.61
10	2333	92.12	0.1	2337.83	2287.67	1.68	0.01
11	2365.79	91.37	0.9	2387.01	2339.75	1.76	0.1
12	2408.23	91.56	1.07	2443.91	2387.96	1.96	0.13
13	2494.07	90.86	2.12	2534.57	2444.86	3.25	0.42
14	2547.11	92.78	0.35	2631.02	2535.54	2.54	0.03
15	2945.43	77.99	0.57	2954.11	2631.98	15.58	0.05
16	3012.94	73.02	3.71	3088.17	2955.07	16.87	1.41
17	3423.8	60.87	23.25	3692.88	3089.13	95.52	46.02
18	3977.39	94.32	0.1	3998.61	3970.64	0.7	0.01

Figure 1: FTIR analysis of biosynthesized zinc oxide nanoparticle

CONCLUSION

Thus from the results of the present study, it can be concluded that bacteria, *E.coli* can be used to synthesize the nanoparticle. From the result of antibacterial activity of Zinc oxide nanoparticle, it is clear to know that the zinc oxide nanoparticle has the ability to inhibit the growth of bacteria (*Bacillus* sp., *Staphylococcus epidermidis and Staphylococcus saprophyticus*). Thus from the results of the present study, it can be inferred that biosynthesis of zinc oxide nanoparticle using supernatant of *E.coli* has several advantages such as simple, cost effective, time consuming, safe and ecofriendly compared to physical and chemical methods of nanoparticle synthesis as evidenced in the present study.

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