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Green synthesis of zinc oxide nanoparticles using *Hibiscus rosa-sinensis* leaf extract and their antibacterial activities

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Abstract

Nanotechnology is emerging as a quickly growing field with its application in science and technology for the motivation of manufacturing new materials at the nanoscale level. The development of new biocidal agent and capable to produce highly ordered nanoparticles size and any shape is the latest advance in the field of nanotechnology. Nanomaterials are called "a wonder of modern medicine". Therefore, in order to minimize the impact on the environment, green synthesis processes have been used to synthesize ZnO nanoparticles (ZnO NPs). Green synthesis is a method to produce nanoparticles using microorganisms and plants with biomedical applications. This method has many advantages, such as environmental friendliness, cost effectiveness, biocompatibility, and safety.

Keywords: *Hibiscus Rosa-sinesis leaf , Green synthesis, ZnO, Antibacterial activity*

1 Introduction

Nanotechnology systems manufacture at the molecular level, is a multidisciplinary scientific field undergoing volatile development. A nanoparticle is a small particle that ranges between 1 to 100 nm in size [1].

The material properties change as their size approaches the atomic scale [2]. This is due to the surface area to volume ratio increasing, resulting in the material's surface atoms dominating the material performance. Owing to their very small size, nanoparticles have a very large surface area to volume ratio when compared to bulk material, such as powders, plate and sheet. This feature enables nanoparticles to possess unexpected optical, physical and chemical properties, as they are small enough to confine their electrons and produce quantum effects [3].

The metal and metal oxide nano particles possess individual features such as UV absorption, specific colour absorption in the visible region, dichroism, and photoluminescence [4]. Biological interfaces that depend on colloidal forces as well as dynamic bio physicochemical interactions. This interaction forms control size shape, surface chemistry, roughness and surface coatings [5].
Biological systems like bacteria, fungi and yeast have been used for the synthesis of nanoparticles. Synthesis of nanoparticles using microorganism involves elaborate process of maintaining cell cultures, intracellular using "green" methods in the synthesis of Zinc oxide nanoparticles has became the topic of interest racial chemical compound [6].

Hibiscus rosa-sinensis, known colloquially as Chinese hibiscus, China rose, Hawaiian hibiscus, rose mallow and shoeblack plant, is a species of tropical hibiscus, a flowering plant in the Hibisceae tribe of the family Malvaceae. It is widely cultivated as an ornamental plant in the tropics and subtropics, but its native range is Vanuatu. The specific epithet rosa-sinensis literally means "rose of China", though it is not closely related to the true roses. Hibiscus rosa-sinensis was first described in 1753 by Carl Linnaeus in Species Plantarum [7].

The flowers of Hibiscus rosa-sinensis are edible and are used in salads in the Pacific Islands. The flower is additionally used in hair care as a preparation. It can also be used as a pH indicator. When used, the flower turns acidic solutions to a dark pink or magenta color and basic solutions to green. The plant may have some potential in cosmetic skin care; for example, an extract from the flowers of Hibiscus rosa-sinensis has been shown to function as an anti-solar agent by absorbing ultraviolet radiation [8].

The biological approach of Hibiscus rosa-sinensis leaf extract used as a reducing agent and stabilizing agent for the synthesis Zno nanoparticles. The structure, phase, and morphology of synthesized product were investigated by the standard characterization techniques [9].

Zinc Oxide have many and very impressive properties like large binding energy, wide band gap, high piezoelectric property etc. It is used in large number of applications like laser devices, optoelectronic devices, electromagnetic coupled sensor, surface acoustic wave device. Zinc oxide nanoparticles have been used to eliminate sulphur, arsenic from water because bulk ZnO cannot remove arsenic because nanoparticle have great

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surface area than bulk material. Zinc oxide have amazing application in diagnostics, biomolecular detection, micro electronic [10].

Nanoparticles have widely emerged as an anti-bacterial agent in the last decade. The nanoparticle can be used as a personalized medicine because it shows specific targeting and minimum toxicity. They have proven useful for inhibiting antibiotic-resistant bacteria particularly. Nanoparticles exhibit their bacteriostatic or bactericidal effect by either blocking their food source or by disrupting their cell membrane.

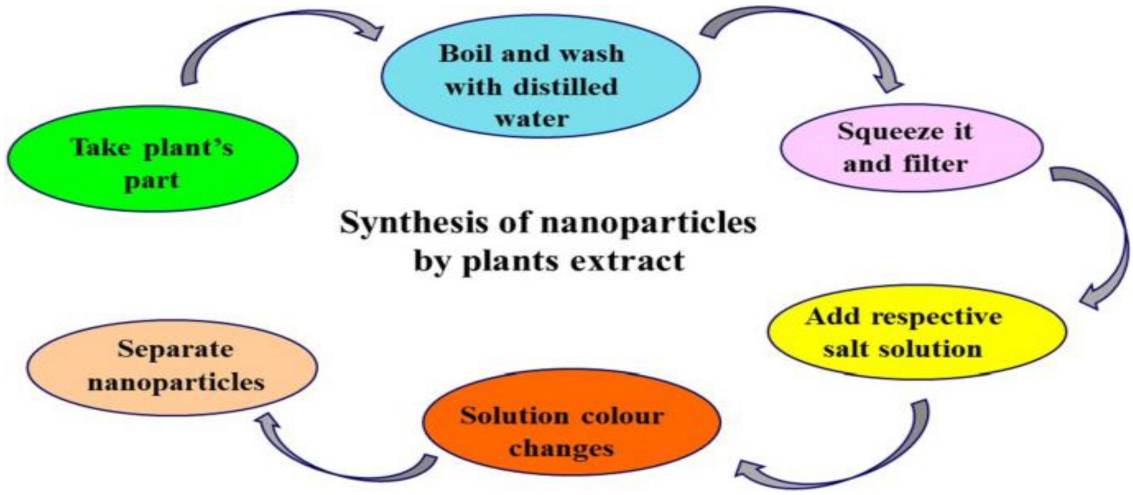

Fig 1. Green Synthesis Of ZnO using various plants and Biological sources

The nanoparticle can accommodate a large number of ligands for better targeting of pathogenic microbes owing to its large surface area to volume ratio. Several types of metal and metal oxide nanoparticles have been already reported to possess anti-microbial property like Silver, gold, copper. Iron Zinc Oxide, titanium oxide, copper oxide, Iron oxide nanoparticles. ZnO NP has gained considerable attention out of all other nanoparticles because of its unique electronic, optical and medicinal properties. Zinc oxide nanoparticle is highly biocompatible and its electron transport kinetics rate is fast so, it's suitable to use it as a biological membrane or for other biological applications [11,12].

They afford numerous advantages over the other techniques, including being clean, cost-effective, and having single-step protocols Furthermore, green fabricated NPs reveal distinguishing proper-ties such as their optical, photo-electrical, and chemical characteristics, which give them the competence to be used for a wide range of applications, including agricultural purposes.

In this context, the biological system will fulfil the function of biological laboratories for synthesizing metal oxide nanoparticles via a biomimetic approach. Several plant extracts are being used in the green synthesis of nanoparticles because they contain several important metabolites and biomolecules that function as reducing stabilizing, and capping agents for synthesizing NPs, including ZnO NPs which have improved the field of nanoscience [13].

2 Experimental Sections

2.1. Materials and methods:

A magnetic stirrer-heater was used to boil 50ml of Hibiscus rosa-sinensis leaves extract to 60-80 degrees Celsius for the creation of ZnO nanoparticles. 5 grams of zinc nitrate are added when the solution reaches 60 degrees Celsius. After that, the mixture is reduced to a dark yellow paste by boiling it. This paste was then placed in a crucible and heated for 2 hours at 200 degrees Celsius in an air-heated furnace. A light yellow powder was obtained, which was carefully collected and neatly packaged for characterization and antibacterial activity testing.

Fig 2. Work flow of green Synthesis Of NPs using *Hibiscus Rosa-sinesis* leaf

2.2 Materials Characterization:

2.2.1. X-Ray Diffraction (XRD) Analysis

A 2θ X Ray Diffractometer was used to validate the presence of ZnO and to analyse the structure of the powered sample XRD works by irradiating a material with incident X-rays and then measuring the intensities and scattering angles of the X-rays that leave the material. XRD is a technique employed to determine the underlying crystal structure of a material; it enables verification of the crystallinity and structure of a sample but gives no information of a chemical nature [14, 15 &16].

2.2.2. UV spectroscopy Analysis

UV spectroscopy or UV–visible spectrophotometry (UV–Vis or UV/Vis) refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible regions of the electromagnetic spectrum [17]. This means it uses light in the visible and adjacent ranges. The absorption or reflectance in the visible range directly affects the perceived colour of the chemicals involved. In this region of the spectrum, atoms and molecules undergo electronic transitions. Absorption spectroscopy is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions of electrons from the excited state to the ground state, while absorption measures transitions from the ground state to the excited state.

2.2.3. Infrared Spectroscopy Analysis

IR Spectroscopy measures the vibrations of atoms, and based on this it is possible to determine the functional groups. Infrared spectroscopy (IR spectroscopy or vibrational spectroscopy) is the measurement of the interaction of infrared radiation with matter by absorption, emission, or reflection. It is used to study and identify chemical substances or functional groups in solid, liquid, or gaseous forms [18]. It can be used to characterize new materials or identify and verify known and unknown samples. The method or technique of infrared spectroscopy is conducted with an instrument called an infrared spectrometer (or spectrophotometer) which produces an infrared spectrum [19,20].

2.2.4. SEM-EDAX Analysis

The most well-known and widely used surface analysis technique is scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX). Energy Dispersive X-Ray Analyzer (EDX or EDA) is also used to provide elemental identification and quantitative compositional information [21].

3.Results and Discussion

3.1. X-Ray Diffraction (XRD) Analysis

XRD can be done on a many number of different kinds of sample. The ideal sample will be a crystalline powder that has been presented into the sample holder, have smooth surface and hold in the sample at an angle of 45 degrees. Solid samples, small volumes of sample taped on microscope slide glass or thin films deposited on a substrate can also be used, but will have varying degrees of effectiveness. The more crystalline the sample, the better the result will be. The peaks appeared at 2θ value of fig 3. corresponds to JCPDS 36-1451 confirming pure sample. The presence of zinc oxide particles was confirmed. The peaks appeared at 2θ value ranging from 31.73°, 34.38°, 36.22°, 47.50°, 56.56°, 62.81°, 66.34°, 67.91°,69.02°, 72.6° and 76.90° values corresponds to pure ZnO.

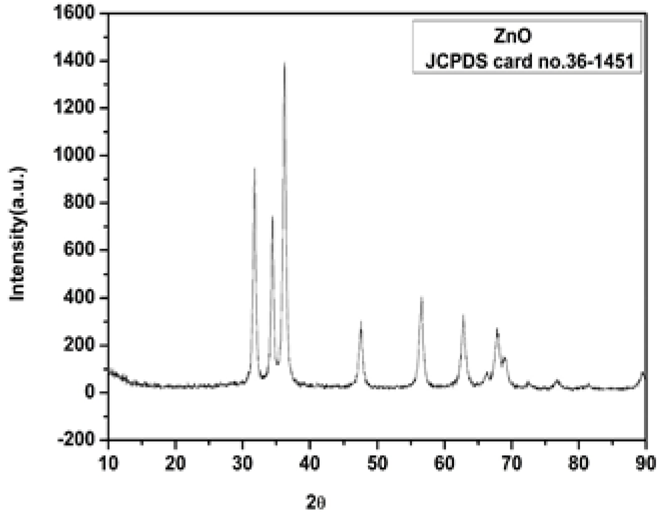

Fig 3. XRD image of ZnO nanoparticle.

3.2 SEM- EDAX Analysis

Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM-EDAX grid were allowed to dry by putting it under a mercury lamp for 5 min. SEM-EDAX image in fig 4. has showed individual zinc particles as well as a number of aggregates. The SEM-EDAX analysis was used to determine the structure of the reaction products were formed. The visual displays of size and shape of synthesized zinc oxide nanoparticles as detected by SEM-EDAX images as shown in the figure. All the possible irregular shapes such as hexagonal, cylindrical, triangular and prismatic shapes of zinc oxide nanoparticles with varying particles size were found in the images. The size of the particles was calculated by SEM-EDAX analysis was found to be in the range of 2 to 0.5μm with an average particular diameter displayed. The number of aggregates nano particles and some of them show the undefined shape nano particles.

3.3 Analysis of UV Spectroscopy:

The band at 250 and 390 cm^{-1} in fig 5. are due to stretching vibrations of Zn-O bond in ZnO NPs. Two bands observed at 321 and 402 cm^{-1} are attributed to the metal oxygen, stretching vibrations of ZnO NPs. The bands observed at 718 and 800 cm^{-1} are represented to stretching vibrations of moisture content on the surface of ZnO NPs and tabulated in table 1.

3.4 IR Spectroscopy Analysis

Fig 6. Shows IR Spectrum of ZnO nanoparticles. Solids run in solution, solids may be dissolved in non-aqueous inert solvent and a drop of this solution is placed on an alkali metal disc and solvent is allowed to evaporate, leaving a thin film of solute or the entire solution is placed in a liquid sample cell which is then mounted in spectrometer. Add about 1 to 2%of the sample, mix and grind to a fine powder. The sample must be very finely ground as in the mulling technique to reduce scattering losses and absorption band distortions.

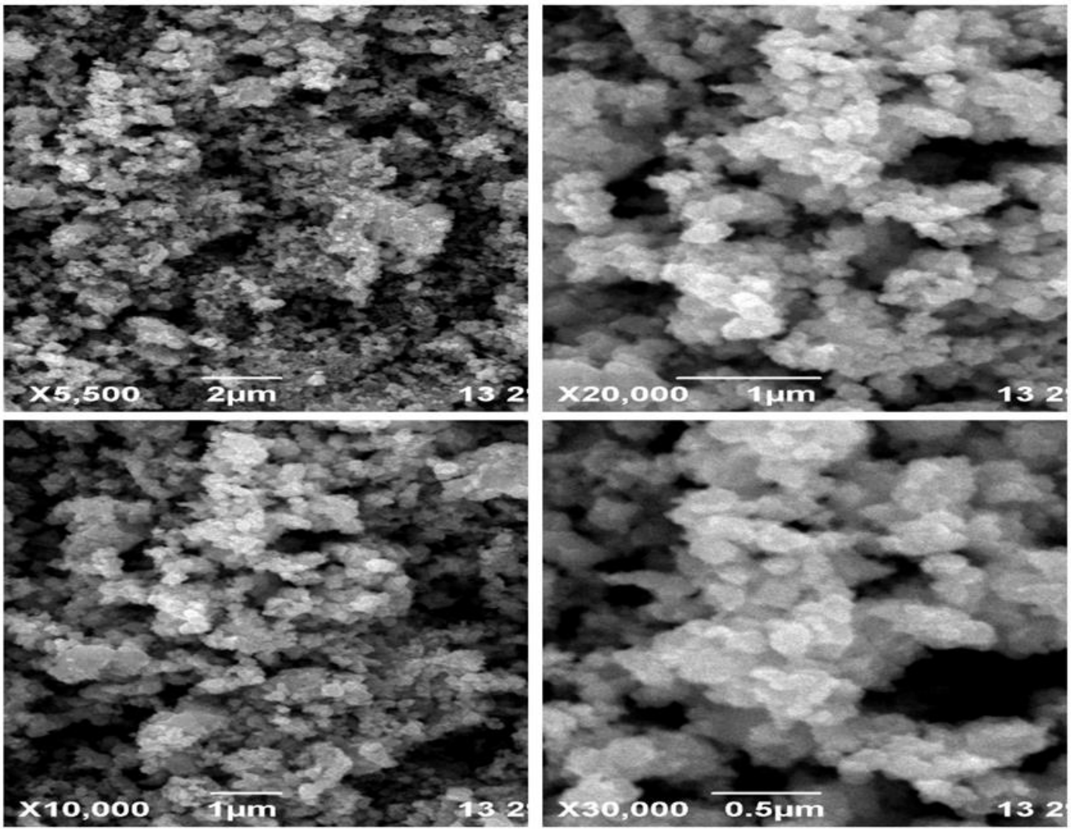

Fig 4. SEM-EDAX images of ZnO nanoparticles.

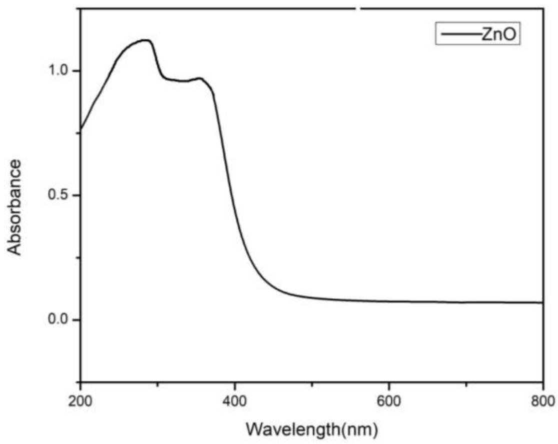

Fig 5. UV-Vis image of ZnO nanoparticles

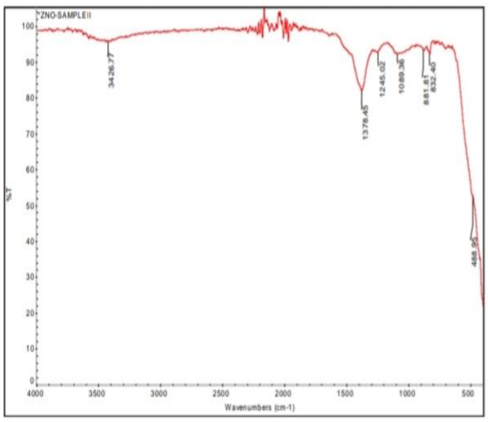

Fig 6. IR Spectrum of ZnO nanoparticles

Making a sandwich to prepare a liquid sample to IR analysis, firstly pace a drop of the liquid on the face of a highly polished salt plate, then place a second plate on top of the first plate on top of the first plate so as to spread the liquid in a thin layer between the plates, and clamps the plates together. The FTIR spectrum of ZnO nanoparticles showed peaks at 3426.77, 1378.45, 1245.02, 1089.36, 881.81, 832.40 and 488.95 are shown in the figure. From those indicated peaks, the broad absorption peaks of ZnO nanoparticles observed at 3426.77 cm^{-1} represents the presence of higher concentration of alcohols with O-H stretches. The medium O-H stretching at 1378 cm^{-1}. The strong C-O stretching at 1089.36 cm^{-1}.

The absorption peaks are 881.81 and 832.40 represents the C=C bending which is alkene and mentioned in the table 1. Therefore it appears more likely that the synthesis of ZnO nanoparticles is the responsibility of many functional groups. Thus it is obvious that bands are assigning to the alcohol, alkane and alkene vice versa stretch.

Table 1. Wavenumber assignment from IR spectroscopy

Frequency cm^{-1}	Types of Vibration	Bond present
3426	Alcohol	O-H stretching
1378	Alcohol	O-H stretching
1089	Alkane	C-O stretching
881	Alkene	C=C stretching
832	Alkene	C=C stretching

4.Antibacterial activity:

4.1. Antibacterial Activity of Hibiscus rosa-sinensis of zinc oxide agar disc method.

The antibacterial activity of the ZnO nanoparticles was tested against gram negative bacteria Salmonella typhi and E.coli and gram positive bacteria Streptococcus sp. The results for the antibacterial activity of ZnO nanoparticles was given in the table below. The antibacterial properties of the zinc oxide nanoparticle are due to their small size and large surface area and involves in electrostatic interactions between nanoparticles and microbial cell surfaces. By increasing the concentration the antibacterial activity is highest in streptococcus sp. compared to E.coli and Salmonella typhi. The darker zones in the plates represents the Antibacterial activity against the bacteria. The antibacterial activity of nanoparticles can be attributed to the presents of bioactive compounds on the surface of nanoparticles as capping and stabilizing agents. ZnO nanoparticles have been shown to have a wide range of antibacterial activities against both Gram-Positive and Gram-Negative bacteria. The zinc oxide nanoparticles inhibit the growth of microorganism by permeating into the cell membrane. Antibacterial activity of Hibiscus rosa-sinensis stabilized ZnO NPs were examined against three pathogenic bacteria such as *Escherichia coli, Streptococcus sp, and Salmonella typhi.* The standard drug used for the comparison was Ciprofloxacin (1 mg/ml). The optimized concentration of ZnO NPs fixed was fixed at 100 µg/ml, which tested against all the bacteria. The ZnO NPs sample synthesized with 1000 ml Hibiscus rosa-sinensis exhibit zone of inhibition against *Escherichia coli,* Streptococcus sp, *and Salmonella typhii* were 17, 17, 17 and 15 mm.

The ZnO NPs sample synthesized with 750 ml Hibiscus rosa-sinensis exhibit zone of inhibition against *Escherichia coli,* Streptococcus sp, *and Salmonella typhii* were 17, 17, 17 and 13 mm.

Similarly, The ZnO NPs sample synthesized with 500 ml Hibiscus rosa-sinensis exhibit zone of inhibition against *Escherichia coli,* Streptococcus sp, *and Salmonella typhii* were 17, 17, 17 and 15 mm.

The highest Antibiotic value is 15nm recorded against E-coli and streptococcus sp. The lowest Antibiotic value is 13 nm recorded against Salmonella typhi. The wide zone of inhibition of zinc oxide nanoparticles against bacteria confirms their great potential as a remedy for infectious disease. It has a great potential as a safe antibacterial drug which may replace antibiotics in future.

Table 2. antibacterial activity of ZnO nanoparticles

Organisms	Zone of Inhibition (mm)			Antibiotic (1mg/ml)
	Sample (1mg/ml)			
	1000	750	500	
Streptococcus sp.	7	7	7	15
Salmonella typhi	7	7	7	13
Escherichia coli	7	7	7	15

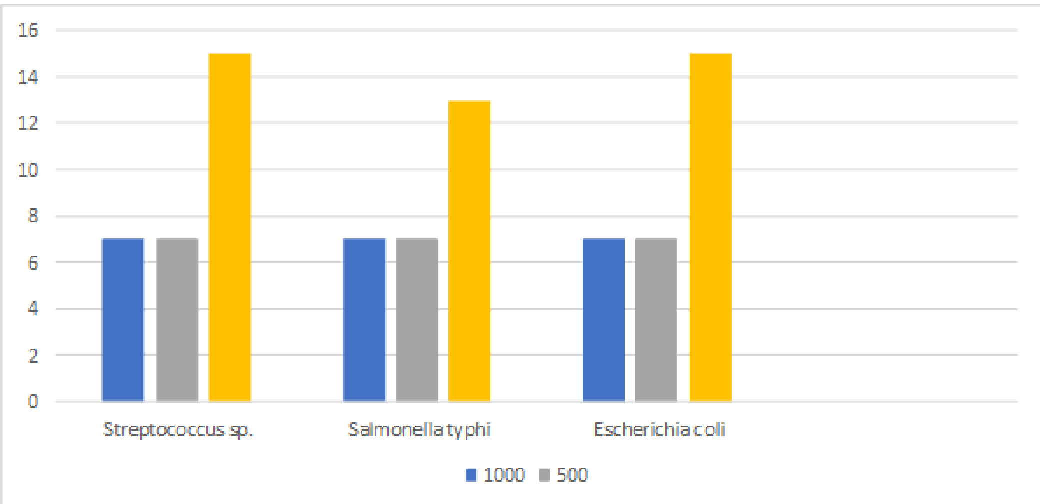

Fig 7. Graph of antibacterial activity of ZnO nanoparticles

Fig 5. Antibacterial activity of ZnO nanoparticles

5. Conclusion:

ZnO nanoparticles are successfully synthesised via green method using Hibiscus rosa- sinensis leaf extract. The biological production of metal nanoparticles becoming a very important field in chemistry, biology and material science. Metal nanoparticles have been produced chemically and physically for a long time however their biological production has only been investigated very recently. The rapid biological synthesis of zinc oxide nanoparticles using leaf extract of hibiscus rosa-sinensis provides and environmental friendly, simple and efficient route for synthesis of nanoparticles. The structure, morphology and size of the prepared ZnO nanoparticles were characterized by XRD, FT-IR, SEM-EDAX and UV-Vis analysis. UV-Vis studies confirmed that the presence of ZnO by absorption peak 250-390 nm. The FT-IR spectra revealed that functional groups of stretching bands for zinc oxide nanoparticles were found around 3426-832 cm^{-1}. The SEM-EDAX analysis shows the size of the zinc oxide nanoparticles from 2-0.5µm. The XRD 2θ value shows the presence of ZnO nanoparticles. The synthesised zinc oxide nanoparticles was studied for antibacterial activities against bacteria like Salmonella typhi, E.Coli and streptococcus sp. Streptococcus sp. and E.Coli has same range of inhibition 15nm against pathogens. Salmonella typhi has inhibition of range is 13nm. The use of plant extracts avoids the usage of harmful and toxic reducing and stabilizing agents. Finally the present study is so helpful and useful to synthesise zinc oxide nanoparticles are inexpensive, stable and eco-friendly without side effect of human being.

Conflict of Interest

The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript

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