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SYNTHESIS AND CHARACTERIZATION OF COCCINIA GRANDIS LEAF EXTRACT CAPPED ZINC OXIDE NANOPARTICLES AND DEVELOPMENT OF ANTIMICROBIAL ACTIVITIES USING CO-PRECIPITATION METHOD

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Abstract
Nanotechnology is relatively a new branch of science that has found a wide range of applications which ranges from energy production to biomedical applications. Zinc Oxide Nanoparticles are known to be one of the most multifunctional inorganic Nanoparticles with its various applications. Additionally, ZnO Nanoparticles show charismatic antibacterial properties because of its surface reactivity. ZnO Nanoparticles is a toxic material with photo catalysis and photo-oxidising properties on biological species. In present study, Zinc Oxide (ZnO) Nanoparticles are synthesized using fresh leaf extract Coccinia grandis by Co-precipitation method. The characterization techniques are XRD, SEM, FTIR, EDX, UV-Visible, and Antimicrobial activity. X-ray diffraction (XRD) Spectroscopy reveals the particle size of ZnO Nanoparticles which are crystalline in nature. The surface morphologies of ZnO Nanoparticles are observed under Scanning Electron Microscope (SEM).The Fourier Transform Infrared Spectroscopy (FTIR) confirms the presence of biomolecules and metal oxides of Nanoparticles. Energy Dispersive X-ray (EDX) analysis confirms the elemental composition which is present in ZnO Nanoparticles. UV-Visible (UV-Vis) Spectroscopy is used to determine band gap energy of ZnO Nanoparticles. The inhibition zone of ZnO Nanoparticles of various bacteria and fungi is determined by Antimicrobial activity. Green synthesis of ZnO Nanoparticles is gaining importance due to its cost-effectiveness, reduction of toxic chemicals and extensive antimicrobial activity. Therefore, the study reveals an efficient, eco-friendly and simple method for the synthesizing ZnO Nanoparticles.

1. Introduction

Nanotechnology literally means any technology on a nanoscale that has applications in the real world. Nanotechnology encompasses the production and application of physical, chemical and biological systems at scales ranging from individual atoms or molecules to submicron dimensions, as well as the integration of the resulting nanostructures into larger systems. Nanotechnology refers to the contrived ability to construct items from the bottom up, using tools and techniques that are being defined to make high performance products. Modern science based on the unifying features of nature at the Nano scale contributes a new foundation for innovation, knowledge and integration of technology [1]. In essence, nanotechnology refers to the production, manipulation and use of materials at the scale of100 nanometres or less. At this scale, materials behave unexpectedly, exhibiting properties that differ physically, chemically and biologically from their counterparts. Many scientist and policy-makers see nanotechnology as the wave of the future, and as a result, investment in nanotechnology has continued to increase [2]. In the Bottom-up method, materials are prepared by atom-by-atom or molecule-by-molecule to make large amount of materials. This method is more frequently used for producing most of the nanomaterials. This method has an ability to produce a uniform size, shape, and well- distributed nanomaterials. This method plays an important role in the production and processing of nanomaterial's with better particle size distribution and better morphology. Another important feature is that it's an environment friendly and economical process for the nanoparticle production. There are many

approaches for synthesizing nanomaterials like hydrothermal, combustion synthesis, gas-phase methods, microwave synthesis, and sol-gel processing [3].

Ligand or antibody conjugated nanoformulation, bifunctional and multifunctional nanoparticles are the newer research approaches through which detection and treatment of cancerous cells can be achieved. Nanomachines are also largely in the research-and-development phase, but some primitive molecular machines have been tested. An example is nanorobot which is capable of penetrating the various biological barriers of human body to identify the cancer cells. Thus, nanodrug delivery systems have a leading role to play in nanomedicine in near future [4]. In a timeframe of approximately half a century, nanotechnology has become the foundation for remarkable industrial applications and exponential growth. Nanotechnology had a profound impact on medical devices such as diagnostic biosensors, drug delivery systems, and imaging probes. In the food and cosmetics industries, the use of nanomaterials has increased dramatically for improvements in production, packaging, shelf life, and bioavailability. Zinc oxide quantum dot nanoparticles show antimicrobial activity against food-borne bacteria, and nanoparticles are now used as food sensors for detecting the food quality and safety [5]. There is the possibility that the future of Nanotechnology is very bright, that this will be the one science of the future. The future of Nanotechnology could improve the outlook for medical patients with serious illnesses or injuries. Physicians could theoretically study Nano surgery and be able to attack illness and injury at the molecular level. This, of course, could eradicate cancer as the surgical procedures would be done on the cellular base [6].

Zinc oxide is an inorganic compound with the formula ZnO. ZnO is a white powder that is insoluble in water. It is used as an additive in numerous materials and products including cosmetics, food supplements, rubbers, plastics, ceramics, glass, cement, lubricants, paints, ointments, adhesives, sealants, pigments, foods, batteries, ferrites, fire retardants, and first-aid tapes. Although it occurs naturally as the mineral zincite, most zinc oxide is produced synthetically [7].

Zinc oxide nanoparticles can enhance the antibacterial activity of ciprofloxacin. It has been shown that nano ZnO that has an average size between 20 nm and 45 nm can enhance the antibacterial activity of ciprofloxacin against Staphylococcus aureus and Escherichia coli in vitro. The enhancing effect of this nanomaterial is concentration dependent against all test strains. This effect may be due to two reasons. First, zinc oxide nanoparticles can interfere with NorA protein, which is developed for conferring resistance in bacteria and has pumping activity that mediate the effluxing of hydrophilic fluoroquinolones from a cell. Second, zinc oxide nanoparticles can interfere with Omf protein, which is responsible for the permeation of quinolone antibiotics into the cell [8].

Zinc oxide (ZnO), as a material with attractive properties, has attracted great interest worldwide, particularly owing to the implementation of the synthesis of nano-sized particles. High luminescent efficiency, a wide band gap (3.36 eV), and a large exciton binding energy (60 meV) has triggered intense research on the production of nanoparticles using different synthesis methods and on their future applications. The increasing focus on nano zinc oxide resulted in the invention and development of methods of nanoparticles synthesis [9]. Many researchers have employed green synthesis process for preparation of metal/metal oxide nanoparticles via plant leaf extracts to further explore their various applications [10].

Our key purpose is to highlight the biological synthesis of nanoparticles, because of the ease of its rapid synthesis and eco-friendly. Also the toxicity and size characterization can be controlled. The variety of natural sources is therefore nanoparticles synthesis, together with plants, fungi, bacteria, etc., additionally, the unicellular and multicultural organism are able to synthesize intracellular and extra cellular nanoparticles. These biosynthesized metallic nanoparticles have a range of unlimited pharmaceutical applications including delivery of drugs or genes, detection of pathogens or proteins, and tissue engineering. The effective delivery of drugs and tissue engineering through the use of nanotechnology exhibited vital contributions in translational research related to the pharmaceutical products and their applications [11]. In the present investigation report the synthesis and characterization of Zinc Oxide nanoparticles using Coccinia grandis leaf extract and was characterized by XRD, SEM, FTIR, EDX, UV-Visible.

2. Experimental Procedure

Fresh leaves of Coccinia grandis (Kovai) were collected from Dharmapuri district, Tamil Nadu, India. The leaves were thoroughly washed with tap water and de-ionized water. Then the 30 grams of cleaned leaves were taken and finely grinded into powder with the help of mortor and dispensed with 50 ml of distilled water and it is stirred in magnetic stirrer for 30 minutes. Then, the extract of the leaves were collected in beakers by standard filtration method using whatman no 1 filter paper and then it is used for nanoparticles synthesis.Zinc acetate dihydrate solution was prepared by dissolving 0.4 Mole of Zinc acetate dihydrate in 50 ml of deionized water and stirred using magnetic stirrer for 30 minutes. The collected leaves extract was added to the Zinc acetate dihydrate and stirred for 30 minutes. The pH of the solution is maintained to 12 by adding Sodium hydroxide pellets and stirring was continued for another two hours till nanoparticles were formed. The observed colour changes from green to pale white. The coloured Zinc Oxide nanoparticle solution was dried at hot air oven of 100˚C for 2 hours. Then the particle is kept in muffle furnance at 400˚C for 2 hours and is grinded using mortar to get fine Zinc Oxide nanoparticles. Zinc oxide nanoparticles were approved by Food and Drug Administration (FDA) as a new and potent anticancer therapy. Zinc oxide nanoparticles can produce selective cytotoxicity towards cancer cells via the induction of disequilibrium of zinc-dependent protein activity, in addition to the production of reactive oxygen species. zinc oxide nanoparticles are considered potent therapy for many cancers [12].

3. Result and Discussion

Coccinia grandis leaf extract capped ZnO nanoparticles have been synthesized using Co-Precipitation method. The particle size, surface morphology, presence of biomolecules and metal Oxides, elemental composition and band gap energy were characterized by XRD, SEM, FTIR, EDX and UV-Visible techniques. The obtained results were presented and discussed in this chapter.

3.1 X-RAY Diffraction Analysis (Xrd)

XRD analysis was carried to confirm the crystalline nature of biologically synthesized Zinc Oxide nanoparticles. Fig. 1 represents the X-ray diffraction pattern of ZnO nanoparticles. A definite line broadening of the XRD peaks indicates that the prepared material consist of particles in nanoscale range. From this XRD patterns analysis, we determined peak intensity, position width, full-width at half-maximum (FWHM) data. The average particle size was determined by Debye Scherrer's formula [13].

$$D = \frac{k\lambda}{\beta \cos\theta} nm$$

Where,
k = Scherrer's constant (0.89)
λ = wavelength of X-rays (1.54×10^{-10})
θ = Bragg diffraction angle
β = full width at half-maximum (FWHM) of the diffraction peak.

XRD pattern of the Zinc Oxide nanoparticles using Coccinia grandis leaf extract is shown in Fig 1. Typical XRD pattern of Zinc Oxide was found by Bragg reflections corresponding to (100), (002), (101), (102), (110), (103), (200) sets of lattice planes. The peaks that were obtained correspond to the pure hexagonal wurtzite phase of ZnO (JCPDS – Joint Committee for Powder Diffraction Standards, card number: 79-2205) and further it also confirms the synthesized Nano powder was free of impurities as it does not contain any characteristic XRD peaks other than ZnO peaks. The present study clearly indicated the X-Ray diffraction pattern of biologically synthesized Zinc Oxide nanoparticles which are crystalline in nature. The mean size calculated from Debye Scherrer formula range is 25.9697nm which is derived from the FWHM of more intense peak corresponding to 101 planes located at 36.26˚.The crystalline size hkl values are depicted in Table 1.

Table 1 Crystalline size and HKL value of observed crystalline peaks

S.No	2θ Degree	d A˚	FWHM (deg)	hkl	Crystalline size (nm)	Average crystal size (nm)
1.	36.2569	2.47567	0.54390	101	26.11745	25.9697
2.	31.7819	2.81329	0.53100	100	25.8072	
3.	34.4231	2.60324	0.49270	002	25.9697	

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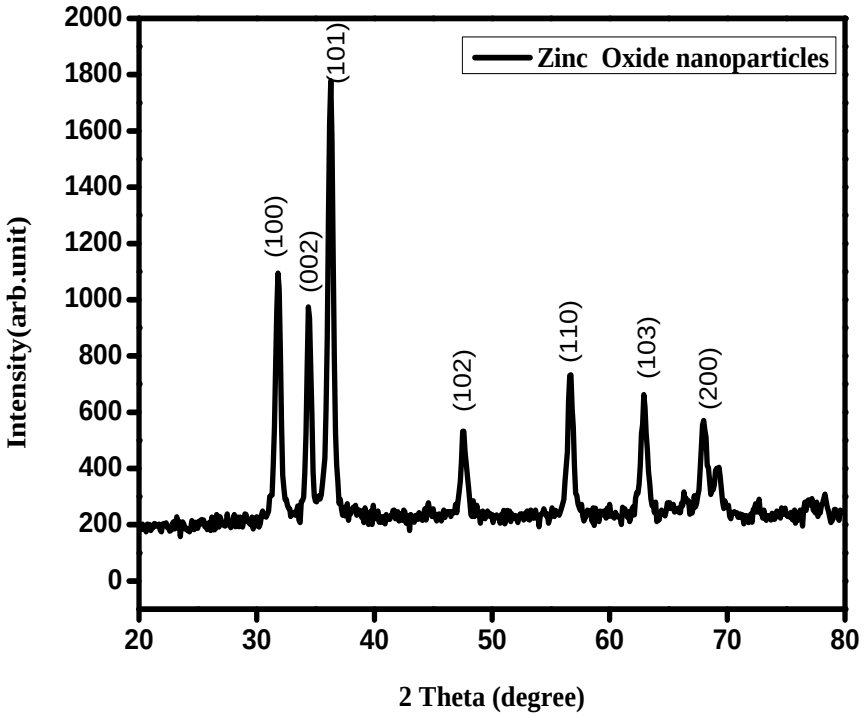
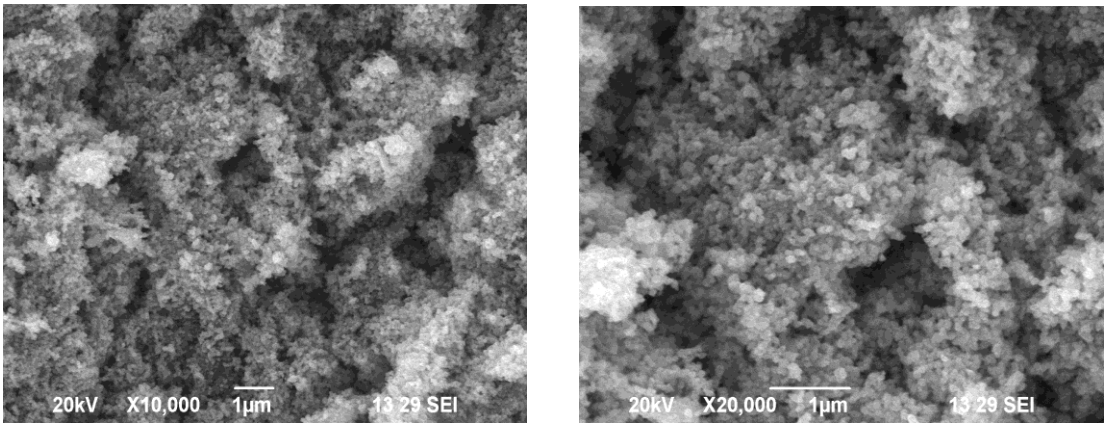


Fig 1 XRD Pattern of Green synthesized ZnO nanoparticles using Coccinia grandis leaf extract

3.2 Scanning Electron Microscope (SEM)

The surface morphology, size and shape of Zinc Oxide nanoparticles were analysed by Scanning Electron Microscope [14]. The Fig 2 shows the morphology of synthesized Zinc Oxide nanoparticles of Coccinia grandis leaf extract. Predominantly the shapes of the particle are Spherical and aggregated into large particles. A closer look shows the presence of several nanoparticles aggregates and some individual crystals are clearly visible.



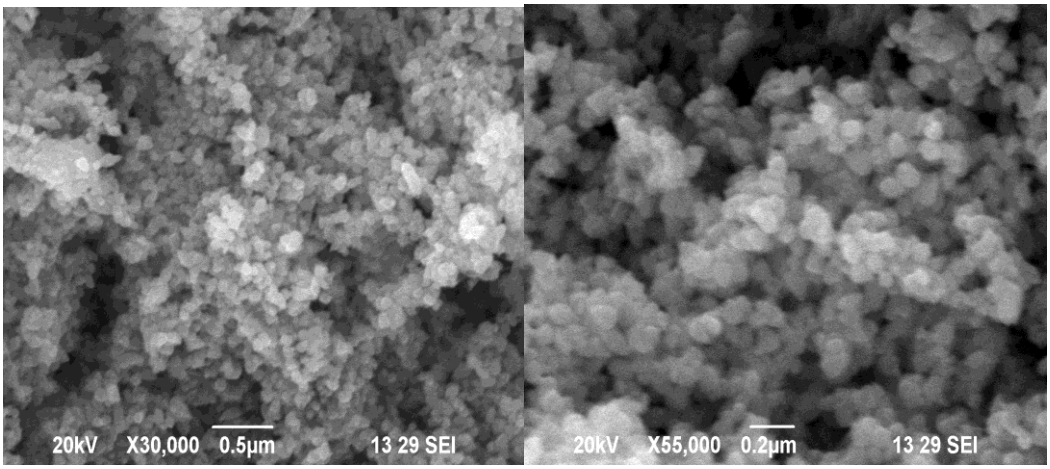


Fig 2 SEM images of Zinc Oxide nanoparticles using Coccinia grandis leaf extract

3.3 Fourier Transform Infrared Analysis (FTIR)

Fourier Transform Infrared Analysis was performed to identify the possible biomolecules for capping and for proficient stabilization of the Zinc Oxide nanoparticles synthesized by Coccinia grandis leaf extract. The FTIR spectrum of ZnO nanoparticles in the wavenumber ranges from 500 to 4000 cm^{-1}. Fig 3 shows FTIR spectrum of ZnO nanoparticles using Coccinia grandis leaf extract.

The FTIR spectrum of the leaf extract shows strong peaks at 3429.42 cm^{-1}, 2922.31 cm^{-1}, 2342.31 cm^{-1}, 1547.80 cm^{-1}. The O-H stretching appears in the spectrum as a very broad band extending from 3429.42cm^{-1}- 3000cm^{-1}. Prominent levels of doublet absorption observed at 2922.03cm^{-1}-2852.49cm^{-1}reveal the presence of C-H stretching vibrations of an aromatic aldehyde. The absorption peak at 1547.80cm^{-1}shows stretching of C-C alkanes.The peak at 874.99 $cm^{-1}$is attributed to the presence of C-H bending Vibration [15].The peak at 431.12 cm^{-1} confirms the presence of Zinc Oxide nanoparticles.

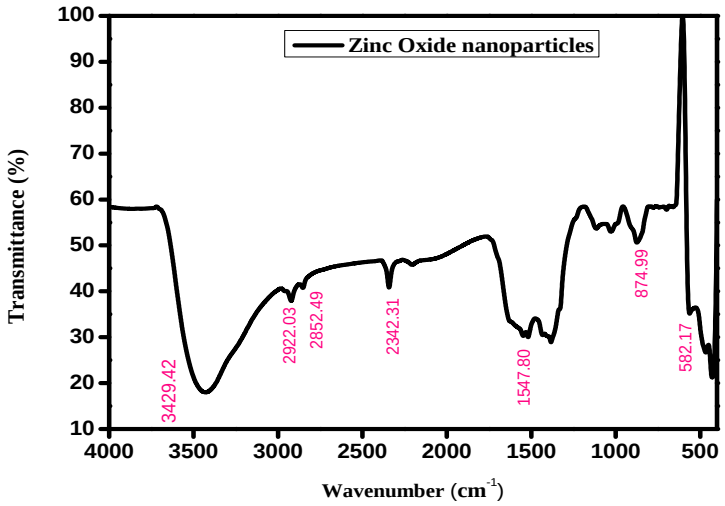


Fig 3 FTIR spectrum of ZnO nanoparticles using Coccinia grandis leaf extract

3.4 Energy Dispersive X- Ray Analysis (EDX)

Energy dispersive X-Ray Spectrum (EDX) is mainly used to determine the elemental composition of a sample [16-18]. In this, study it is used to confirm that the nanoparticle suspension contains nothing but Zinc and Oxygen. The percentage of Zinc and Oxygen are 77.01and 22.99. The EDX spectrum is spherical in shape with high aggregation of Zinc Oxide nanoparticles. The surface of the cell is prepared based on the bio reduction method by using Coccinia grandis leaf extract. Fig 4 shows the compositional analysis of Zinc Oxide nanoparticles using Coccinia grandis leaf extract. At the time of scanning the binding energies, the presence of bio organic compound is witnessed which acts as an additional peak value. Thus, the EDX analysis helps to find the existence of strong signals in the region of Zinc Oxide and it helps to run to a conclusion regarding the formation of Zinc Oxide nanoparticles by using the biological source [19]. The obtained percentage of ZnO elements are shown in Table 2.

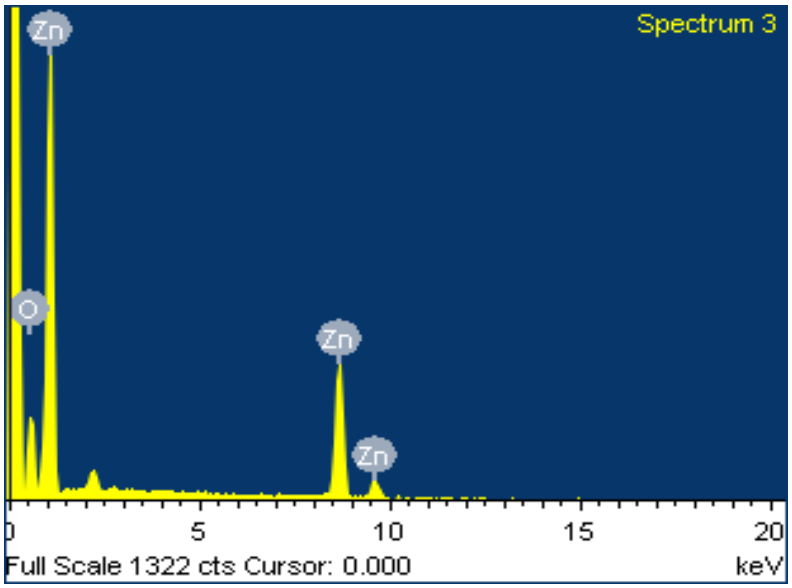


Fig 4 Compositional analysis of Zinc Oxide nanoparticles using Coccinia grandis leaf extract

Table 2 EDX analysis for Zinc Oxide nanoparticles using Coccinia grandis leaf extract

S.No	Sample	Wt. %of Zinc	Wt. % of Oxide
1.	Zinc Oxide Nano particles	77.01	22.99

3.5 UV-Visible Spectral Analysis

The formation and stability of Zinc Oxide nanoparticles in aqueous solution were confirmed by using UV-Visible spectral analysis in the range from 300 to 800nm [20]. The band gap energy due to the energy transfer spectra was calculated using formula

$$E = \frac{hc}{\lambda} eV$$

Where,
h = Planck's constant (Joule sec)
C = Velocity of light (m/sec)
λ = Wavelength of light (nm)

The plant extract was mixed with aqueous solution of Zinc acetate dihydrate and stirred in a magnetic stirrer. The solution started to change colour from greenish to pale white colour. This confined the formation of Zinc Oxide nanoparticles. Fig 4 shows the UV-Visible spectrum of ZnO nanoparticles using Coccinia grandis leaf extract.

The UV-Visible Spectrum was recorded for Zinc Oxide nanoparticles using Coccinia grandis leaf extract. The result showed optical absorbance peak within the range around 375.20 nm and the band gap energy is 3.31eV which are given in Table 3.

Table.3 UV –Absorbance Spectrum of Zinc Oxide nanoparticles using Coccinia grandis leaf extract

Sample	h (Js)	C (m/sec)	λ (nm)	Band gap (eV)
Biosynthesized Zinc Oxide nanoparticles using Coccinia grandis leaf extract	6.626×10^{-34}	3×10^{8}	375.20	3.31

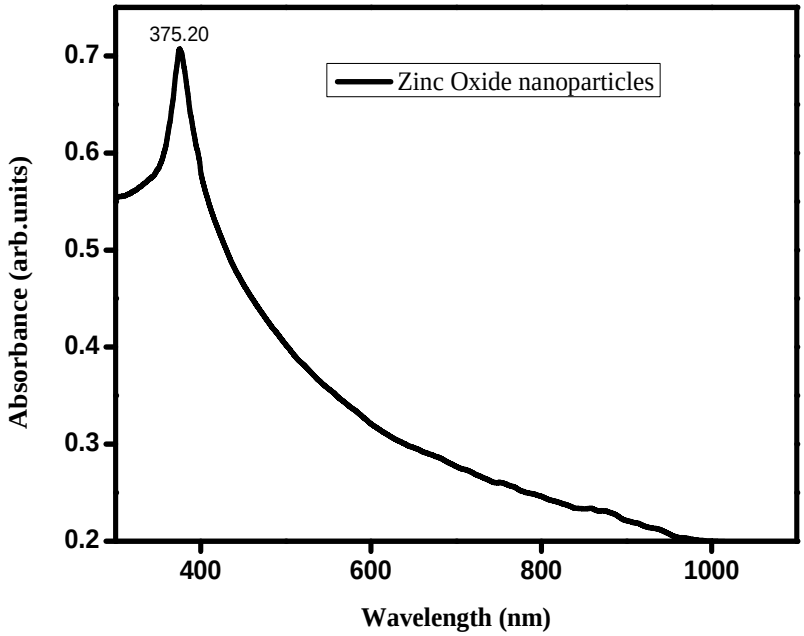


Fig 5 UV-Visible absorption spectra of Zinc Oxide nanoparticle using Coccinia grandis leaf extract.

3.6 Antimicrobial Activity

Microbes are considered as eco-friendly factories of nanoparticles synthesis. Interactions between metals and microbes have been exploited for various biological applications. It is one of the most sustainable, eco-friendly techniques so far in spite of its few limitations. Both prokaryotes and eukaryotes are used for the synthesis of metal/metal oxide especially ZnO. Further, the synthesis may be intracellular or extracellular. Fungi are decomposers as well as parasite in nature. In intracellular

synthesis, fungal biomass is incubated for a particular time period in dark along with a zinc salt solution, while in extracellular synthesis fungal filtrates are treated with the precursor solution and synthesis is assessed.

3.6.1 Antibacterial and Antifungal activity

The bacterial activity of the biosynthesized Zinc Oxide nanoparticles using leaf extract of Coccinia grandis has potential antibacterial activity against both Gram positive and Gram negative bacteria on human pathogens. ZnO nanoparticles displayed antibacterial activity against Gram positive and Gram negative bacteria, with varying degrees as suggested by the diameter of inhibition zone. The results of antibacterial and different concentrations of Zinc Oxide nanoparticles were shown against two gram negative bacteria (E.Coli and Proteus Vulgaris), two gram positive bacteria (Streptococcus oralis and Staphylococcus aureus).

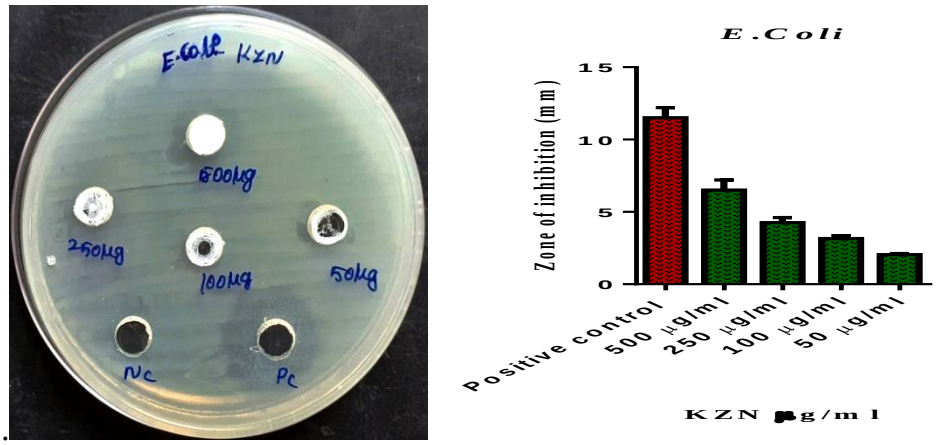


Fig 6 Antibacterial activity of ZnO nanoparticle using Coccinia grandis leaf extract against gram negative bacteria E.coli

Fig 6 shows the antibacterial activity of biosynthesized ZnO nanoparticles from leaf extract of Coccinia grandis against E.coli. The maximum zone of inhibition was observed for 2.1mm for 50µg/ml, 3.3mm for 100µg/ml, 4.5mm for 250µg/ml, 7mm for 500µg/ml concentrations. Fig 7 shows the antibacterial activity of biosynthesized ZnO nanoparticles from plant extract of Coccinia grandis against Proteus vulgaris. The maximum zone of inhibition was observed for 3.4mm for 50µg/ml, 4.6mm for 100µg/ml, 7.7mm for 250µg/ml, 10mm for 500µg/ml concentrations. By comparing the gram negative bacteria, the zone of inhibition is higher in Proteus vulgaris than in E.coli.

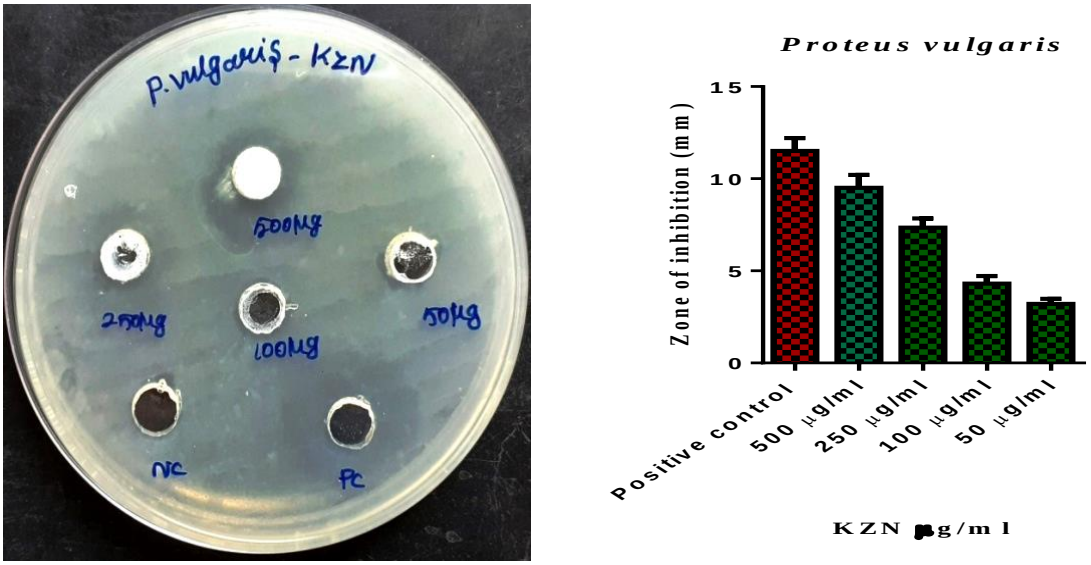


Fig 7 Antibacterial activity of ZnO nanoparticle using Coccinia grandis leaf extract against gram negative bacteria Proteus vulgaris

Fig 8 shows the antibacterial activity of biosynthesized ZnO nanoparticles from plant extract of Coccinia grandis against Staphylococcus aureus. The maximum zone of inhibition was observed for 0mm for 50µg/ml, 0mm for 100µg/ml, 4.5mm for 250µg/ml and 7mm for 500µg/ml concentrations. Fig 9 shows the antibacterial activity of biosynthesized ZnO nanoparticles from plant extract of Coccinia grandis against Streptococcus oralis. The maximum zone of inhibition was observed for 2.2mm for 50µg/ml, 3.3mm for 100µg/ml, 5.5mm for 250µg/ml, 12.5mm for 500µg/ml concentrations.

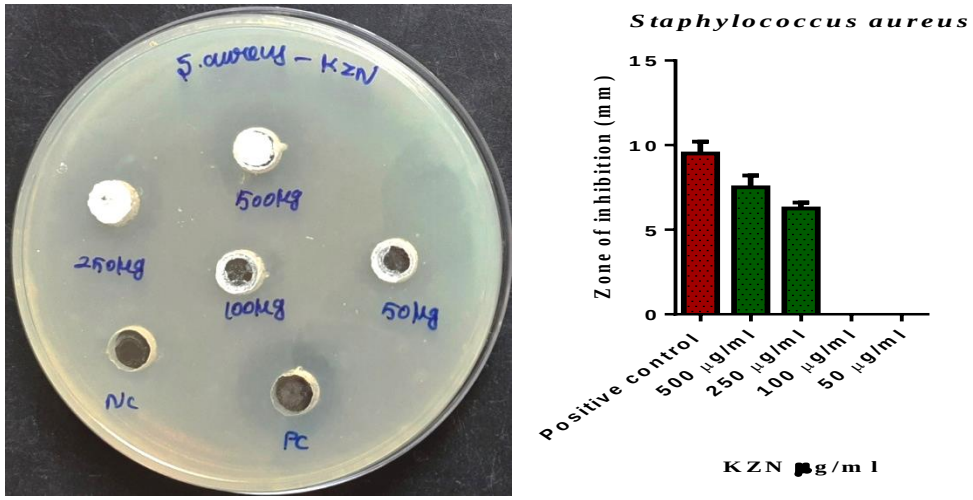


Fig 8 Antibacterial activity of ZnO nanoparticle using Coccinia grandis leaf extract against gram Positive bacteria Staphylococcus aureus

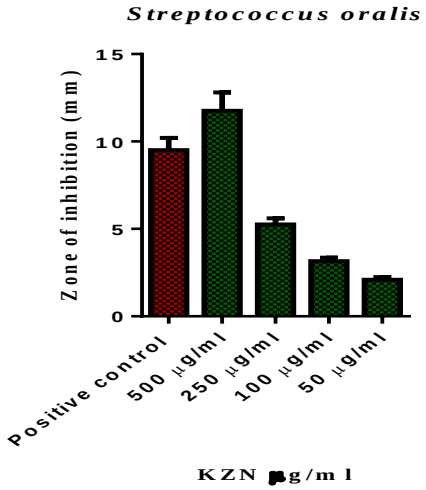


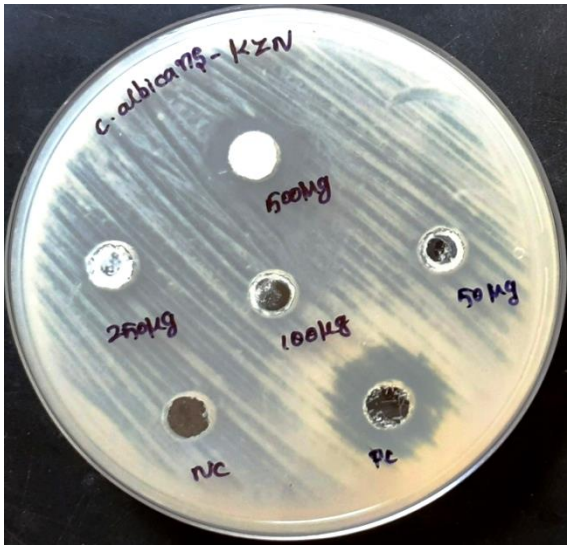
Fig 9 Antibacterial activity of ZnO nanoparticle using Coccinia grandis leaf extract against gram Positive bacteria Streptococcus oralis

By comparing gram Positive bacteria the zone of inhibition is higher in Streptococcus oralis than in Staphylococcus aureus. The SD± Mean of inhibition obtained by ZnO nanoparticles are shown in Table 4

Table 4 SD± Mean of Zone of inhibition obtained by ZnO nanoparticles using Coccinia grandis leaf extract against the pathogens of two gram negative bacteria (E.Coli and Proteus Vulgaris), two gram positive bacteria (Streptoccoccus oralis and Staphylococcus aureus).

S.No	Name of the test organism	Zone of inhibition (mm) SD ± Mean				
		500µg/ml	250µg/ml	100µg/ml	50µg/ml	AB
1.	E.coli	7±1.0	4.5±0.5	3.3±0.3	2.1±0.1	10±1.0
2.	Proteus vulgaris	10±1.0	7.7 ±0.7	4.6±0.6	3.4±0.4	12±1.0
3.	Staphylococcus aureus	7±1.0	6.5±0.5	0	0	10±1.0
4.	Streptococcus oralis	0.5±1.5	5.5±0.5	3.3±0.3	2.2±0.2	10±1.0

SD – Standard Deviation,*Significance – $p<0.05$



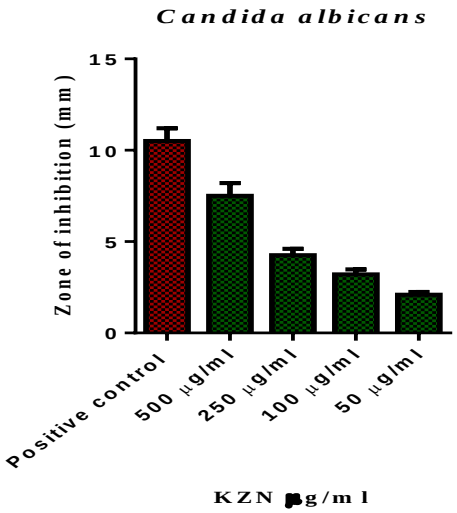


Fig 10 Antifungal activity of ZnO nanoparticle using Coccinia grandis leaf extract against Candida albicans

Fig 9 shows the antifungal activity of biosynthesized ZnO nanoparticles from plant extract of Coccinia grandis against Candida albicans. The maximum zone of inhibition was observed for 2.2mm for 50µg/ml, 3.4mm for 100µg/ml, 4.5mm for 250µg/ml, 8mm for 500µg/ml concentrations. In Candida albicans the zone of inhibition increases when concentration increases which are shown in Table 5

Table 5 SD± Mean of Zone of inhibition obtained by ZnOnanoparticles using Coccinia grandis leaf extract against Candida albicans.

S.No	Name of the test organism	Zone of inhibition (mm) SD ± Mean				
		500µg/ml	250µg/ml	100µg/ml	50µg/ml	AB
1.	Candida albicans	8±1.0	4.5±0.5	3.4±0.3	2.2±0.2	11±1.0

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the World's population. In the present work, the leaf extract obtained from Coccinia grandis shows strong activity against most of the tested bacterial and fungal strains. The results are compared with standard antibiotic drugs. In this screening work, extracts of Coccinia grandis are found to be not inactive against any organism, such as Gram-positive, Gram-negative, and fungal strains are resistant to all the extracts of Coccinia grandis

The above results show that the activity of leaf extract of Coccinia grandis shows significant antibacterial and antifungal activities. This study also shows the presence of different phytochemicals with biological activity that can be of valuable therapeutic index. The result of phytochemicals in the

present investigation showed that the plant contains more or less same components like saponin, triterpenoids, steroids, glycosides, anthraquinone, flavonoids, proteins, and amino acids. Result shows that plant rich in tannin and phenolic compounds have been shown to possess antimicrobial activities against a number of microorganisms.

4. Conclusion

In this study, ZnO nanoparticles were successfully synthesized from leaf extract of Coccinia grandis for the first time through a simple, cost-effective, eco-friendly, and green approach. This showed Coccinia grandis could potentially be used as an effective reducing and capping agent for biological synthesis of ZnO nanoparticles. The biosynthesized ZnO nanoparticles were characterized using the techniques such as XRD, SEM,FTIR, EDX,and UV-Visible. The XRD analysis proved the crystalline nature of the ZnO nanoparticle and the size of the nanoparticle is 25.49 nm.The SEM analysis demonstrated the size of ZnO nanoparticle as spherical shape.Organic functional groups (e.g. carbonyl, hydroxyls)attached to the surface of nanoparticles and the other surface chemicals residues are detected using FTIR.The EDX analysis confirmed the presence of zinc and oxide ions in the nanoparticles.ZnO nanoparticle was measured using the UV-Visible spectroscopy and the Bandgap energy is 3.31 eV. The biosynthesized ZnO nanoparticles exhibited strong levels of antibacterial activity against Proteus vulgaris (gram negative bacteria) and Streptococcus oralis (gram positive bacteria) and a stronger antifungal behaviour exhibited in Candida albicans. Biosynthesized ZnO nanoparticles were found to protect against bacterial and fungal pathogens, suggesting that they may be used as effective antimicrobial and anticancer agents for commercial and biomedical applications.

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