BIOSYNTHESIS OF ZINC OXIDE NANOPARTICLE USING FRUIT PULP EXTRACT OF ABELMOSCUS MANIHOT AND ITS ANTIBACTERIAL STUDY.

Avinash Ramesh Thakare[1]*, Mr Rajendra D Godile[2] , Dr P R Padole[3] , Dr G. S Deshmukh[4] , Ku Akansha Ambade[5] , Dr P. D patil[6]

Abstract;- When compared to other traditional physical and chemical processes, the biosynthesis of metallic nanoparticles using plants, enzymes, and microorganisms has been recognised as an environmentally benign option. Scientists and researchers have recently shown a great deal of interest in the biological production of nanoparticles because of its straightforward method, low cost, non-toxicity, and environmental friendliness. Therefore, it was reported in this work that zinc oxide nanoparticles (ZnO-NPs) were synthesised utilising a reduction agent derived from *Abelmoscus Manihot* fruit pulp extract. Using an X-ray diffraction (XRD), UV-visabsorption spectroscopy, and a scanning electron microscope (SEM), the biosynthesized ZnO-NPs were characterised. It was discovered that the nanoparticles' average size fell between 30 and 57 nm. Using the agar diffusion method, ZnO-NPs were tested for their antibacterial effectiveness against harmful microbes. ZnO-NPs that were biosynthesized in this manner were found to have effective antibacterial activity against *S. Typhi* and *E. Coli*. In conclusion, the green ZnO-NPs were synthesised utilising *Abelmoscus Manihot* fruit extract, and remarkably, it was also demonstrated to have an antibacterial impact on strains of *S. Typhi.*

Keywords;- Zinc oxide nanopartical, Abelmoscus Manihot, Antibacterial study.

[1]*Shankarlaal Agrawal Science College Salekasa Dist Gondia Maharashtra
[2]S. P. M Gilani College Gatanji Yavatmal Maharashtra
[3]Shri Shivaji Science College Amravati Maharashtra
[4]Shankarlaal Agrawal Science College Salekasa Dist Gondia Maharashtra
[5] Shankarlaal Agrawal Science College Salekasa Dist Gondia Maharashtra
[6] Shankarlaal Agrawal Science College Salekasa Dist Gondia

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Introduction;-

Understanding and using the special morphological and physicochemical properties of the vast surface area of small particles (average size of 1–100 nm) for a variety of applications in nearly all scientific and technological domains is the goal of nanomaterial research [1, 2]. It is a highly favoured field of study in the fields of engineering, chemistry, physics, biology, material science, and medicine [3]. The study of nanotechnology focuses on different synthesis techniques, changes in nanoparticle structure, and reformulations of nanoparticle sizes. Nanoparticles are materials at the nanoscale, less than 100 nm in size, possessing exceptional thermal stability, a high surface-to-volume ratio, and exceptional electrical, mechanical, optical, and magnetic capabilities [4]. Physical, chemical, and biological approaches can all be used to synthesize the nanostructured materials [5]. The various processes utilized in chemical synthesis, including pyrolysis, micelle, hydrothermal, sol-gel, and precipitation, make it hazardous and dangerous for the environment and ecology [6]. Green synthesis emerges as a lifesaver in reducing this adverse impact on the environment. Microorganisms, algae, and plants can all be used as the biological building blocks for green synthesis [7]. The creation of environmentally friendly processes for creating nanoscale materials has drawn a lot of attention from material scientists lately. In this regard, the green synthesis of nanoparticles—particularly through the use of extracts derived from various plants—is a developing field in green chemistry that is regarded as easy, affordable, and safe [8-10]. Furthermore, because of its stabilizing and lowering potentials, the green synthesis approach of nanoparticle creation has been regarded as safer and more environmentally friendly [11]. In order to create nanoparticles, a wide variety of metals have been employed as reduction and coating agents, such as copper (Cu) [13], gold (Au)[12], silver (Ag)[11], and trace elements [14]. However, reports of the toxicity of Ag, Au, and Cu nanoparticles and the ensuing limit in therapeutic uses have been made [15]. By tackling a number of challenges related to daily living, such as energy sufficiency, climate change, and the beauty, textile, and health industries, including the treatment of fatal diseases like cancer and Alzheimer's, nanotechnology has also raised the standard of living for people [16-17].

Over the past ten years, extensive research on metal oxide nanoparticles has been focused because of their numerous uses in diverse technical domains [18]. As a cheap, safe, and biocompatible substance, zinc oxide (ZnO), a rare inorganic metallic oxide, has drawn a lot of interest. ZnO has received US FDA approval as the safest metal oxide [19]. Energy conservation, textiles, electronics, healthcare, cosmetics, catalysis, semiconductors, and chemical sensing are just a few of the industries that can benefit from the application of ZnO-NPs [20-24]. The NPs exhibit exceptional medicinal applications, including targeted drug delivery [25], wound healing, bioimaging [26, 27] and anticancer, anti-inflammatory, and antibacterial [28, 29] characteristics.

Numerous investigations have shown how to synthesize ZnO nanoparticles utilizing various plant extracts. For instance, *Hibiscus rosasinensi* leaf extract [30] and *Cassia auriculata* flower extract [31] were utilized as reducing agents for zinc nitrate to create ZnO NPs. The size of NPs is also influenced by the type of plant or source species from which the plant extract was derived. For instance, ZnO nanosheets ranging in size from 18 to 30 nm were created using *Olea europea* leaf extract [32]. On the other hand, the average diameters of the ZnO nanoparticles produced by the green synthesis of ZnO nanoparticles were 13.86 nm and 25–40 nm when *Ocimum tenuiforum* [33] and *Aloe barbadensis* [34] were utilized as reducing agents . ZnO NPs have recently been shown to have antibacterial action in a number of papers. ZnO nanoparticles (NPs) were synthesized through the use of leaf extracts from *Passifora caerulea, Scadoxus multiforus,* and *Camellia sinensis*. The results demonstrated strong antimicrobial efficacy against *Staphylococcus aureus*, *Aspergillus spp*., and *Klebsiella pneumonia*, respectively [35-37]. This suggests that the synthesis of ZnO NPs mediated by medicinal plant extracts can be a highly effective method. Well-known for its medicinal properties, *Abelmoschus manihot subsp. tetraphyllus* is primarily utilized as an anti-inflammatory, analgesic, antibacterial, antileishmanial, antidiabetic, antilarvicidal, antioxidant, antiviral and protein kinase inhibitor [38, 39].

We present here the biosynthesis of zinc oxide nanoparticles from plants, utilizing the fruit's aqueous extracts from *Abelmoschus manihot subsp. tetraphyllus*. ZnO-NPs synthesized environmentally and will be used in a variety of biological processes. The metabolites in *Abelmoschus manihot subsp. tetraphyllus* fuit's aqueous extract function as a capping and reducing agent during the synthesis of biogenic ZnO-NPs. Modern methods including Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction

(XRD), and scanning electron microscopy (SEM) used to characterize the green synthesized nanoparticles. The anti-inflammatory, antilarvicidal, and antiviral properties of the NPs will be examined.

2 Materials and Method-

1.1Preparation of *Abelmoschus manihot subsp. tetraphyllus* fruit extract.

First, fresh *Abelmoschus manihot subsp. tetraphyllus fruit* was gathered from the Salekasa (Gondia Maharastra) region lat. 21.30 and long. 80.48. It was then cleaned, cut into small pieces, and added 20 g to 50 mL of distilled water. The mixture was then agitated for two hours at 60 °C. After being filtered using filter paper for the next research studies, the fruit extract was placed in the refrigerator.

1.2Fabrication of zinc oxide nanoparticles.

In order to create the green ZnO nanoparticles, 20 ml of fruit pulp extract from *Abelmoschus manihot subsp. tetraphyllus* was combined with 50 ml of 0.1 mol/L zinc sulphate (Himedia, purity = 99.74%) in a conical flask. The mixture was then stirred magnetically for duration of 1 hour. The mixture was then gradually mixed with 0.1 mol/L sodium hydroxide solution to bring the pH level to 10. For 2 hours, the liquid was magnetically stirred. Following the reaction, the products underwent a 10-minute centrifugation at 2000 rpm. After discarding the supernatant, the precipitates underwent two ethanol washes and were then dried at 70 °C.

2.3. Characterization of zinc oxide nanoparticles.

The following methods were used to characterize green generated ZnONPs.

2.3.1. Ultraviolet spectroscopy;-

To verify the synthesis of ZnONPs, a colour shift in the solution was seen. UV-Vis spectrophotometry was also employed in addition to this. Using a BMS UV-2600 spectrophotometer, the produced sample's absorption spectra was measured in the 200–1100 nm region.

2.3.2. Fourier transform infrared (FTIR) spectroscopy;-

The prepared sample was needed to look at the capping agents on the ZnONPs' surface using FTIR inspection. ZnONPs in dried powder form were needed for this approach. Under the following circumstances, (Shimadzu: IRTracer-100) performed the FTIR analysis of dry powder. decrease in the overall reflection mode, four cm^{-1} resolution, and a spectral range of 4000–400 cm^{-1}.

2.3.3. Scanning electron microscopy (SEM);-

Zinc oxide nanoparticles were morphologically analyzed using an electron scanning microscope (EVO-LS10). The picture and size of ZnONPs were obtained by subjecting the dried sample to an electron beam.

2.3.4.Charecterization of ZnONPs using XRD;-

X-ray diffraction was employed to identify the type of green-synthesised zinc oxide nanoparticles. The X-ray diffraction pattern was obtained using a PANalytica X'pert X-ray diffractometer. To determine the crystallite's size, Scherer's equation is employed [40].

$$D\ k = \lambda\ \beta / \cos\theta$$

where λ is the X-ray wavelength of 1.5421 Å, D is the half peak height of an XRD line caused by a certain crystallographic plane, k is the form factor (0.94), and β and θ stand for FWHM in Bragg's angle and radians, respectively.

2.4. Anti-microbial activity ZnONPs;-

One crucial technique for analyzing a compound's ability to inhibit bacteria is antimicrobial screening [41]. A few laboratory techniques are available to assess a compound's antibacterial activity. The most popular is the disc diffusion or agar dilution method [42]. ZnO NPs (5 µl dosage and 10 µg disc-1 and disc-2) were tested for antimicrobial activity against a range of bacterial species. The agar well diffusion method was employed with bacterial strains, including, *S. Typhi*., and *E. Coli*. Potato dextrose agar medium (HiMedia, India) was employed to cultivate bacterial strains, whereas Mueller-Hinton agar (HiMedia, India) was utilized to create an agar medium for the cultivation of bacteria. As standards, amoxicillin (5 µl dosage and 10 µg disc−1) was used for bacterial strains while Ciprofloxacin (5 µl) was utilized for bacterial strains [43]. The discs were incubated for 24 hours at 37°C for bacteria and 48 hours at 26°C for fungus after the sample was placed in a culture medium. The zone of inhibition (ZOI) was measured in order to ascertain the antibacterial activity.

3. Results and discussion; -

3.1 Ultraviolet—Visible (UV-Vis) Spectroscopy; -

Spectral analyses are employed in the sample to evaluate the performance of elemental components. UV-vis (ultra violet—visible) one observable observation that can be utilized to investigate the electronic configuration of the root structure is spectroscopy analysis. ZnO is reported

to have an absorption edge between 200 and 400 nm UV-vis regions. The spectrum documents the adsorption of the electronic ZnO bandwidth gap and displays the highest absorption at 291 nm fig 1. It shows good concordance other earlier literature value [44].

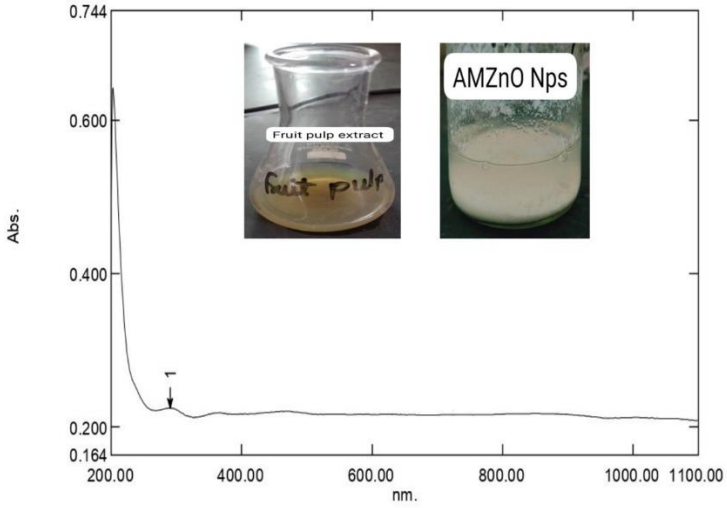

Figure 1. UV Absorption spectra for AMZnO Nanoparticles

3.2. Fourier transform infrared (FTIR) spectroscopy; -

We use FTIR spectroscopy in the 4000–400 cm−1 range to identify functional groups in the aqueous extract and zinc oxide nanoparticles, as illustrated in Figure 1B. Zinc and oxygen bonding vibrations may be the cause of the peak of ZnO seen at 536 cm^{-1} [45] additionally, some additional bands were identified at approximately 1631, 1119, and 885 cm−1. The low absorption peak found at approximately 3420 cm−1 could be assigned to hydroxyl (OH) groups. The peak at 2085 indicating CN stretching, a significant shift in the IR spectra was observed. The presence of carbohydrate (C-O) and (C=C) rings (polysaccharides, pectin, and cellulose) is indicated by a deep absorbance band at 1100. There are plenty of both primary and secondary metabolites in the plant body. The stretching mode of the antisymmetric carbonate species present in the interlayer is responsible for the band area at 1491.04 cm^{-1} and the sharper band region at 1406.17 cm^{-1} was noted for the interlayer region's free CO_3^-ion[46].

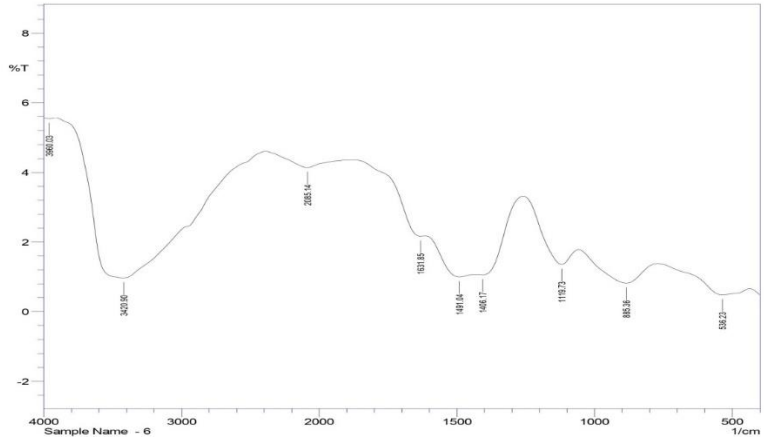

Figure 2. IR spectra of AMZnO nanoparticles

3.3. Scanning electron microscopy (SEM);-

Using SEM examination, the surface morphology of the produced ZnO NPs was examined. Figure 3 illustrates the consistent distribution of flower-shaped ZnO molecules in the SEM image of ZnO NPs. Using ImageJ software, the ZnO NPs' approximately 15 nm particle size was determined from the SEM picture. Bioorganic capping

molecules and NPs have been found to accumulate together due to hydrogen bonding and electrostatic interaction [47]. Additionally, the ZnO NPs' lack of direct contact with one another in the SEM image indicates that the NPs have been stabilized by capping agents [48].

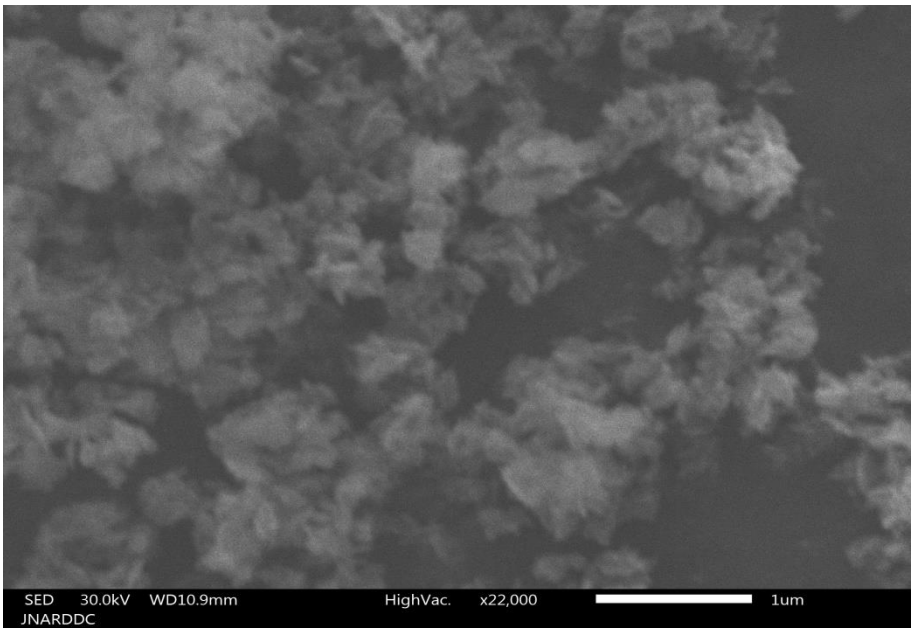

Figure 3 SEM image for AMZnO nanoparticles

3.4. XRD analysis

As seen in Figure 4, the produced particles were in the nanoscale range based on the clear line broadening of the X-ray diffraction peaks in the zinc oxide nanoparticle X-ray diffraction pattern. The spherical to hexagonal phase of ZnO with strong crystallinity [49, 50] has been indexed as the diffraction peaks at 31.77, 34.43, 36.26, 47.55, 56.60, 62.87, and 67.96° (JPCDS card number 36-1451). It was discovered that all of the distinctive peaks belonged to ZnO-NPs and that generated ZnO-NPs do not contain any of these contaminants. Zinc oxide crystals' diameter was determined using the Debye-Scherrer formula. Bragg's diffraction angle (θ) and full width at half maximum (FWHM) of more powerful peaks (β) correspond to 101 planes at position 36.26°; these values indicate that the average crystallite size is 57 nm, while the crystallite size is around 35 nm. The indexation validates ZnO-NPs' typical hexagonal wurtzite structure (JCPDF file no. 00-036-1451), which has been previously documented in various investigations[51].

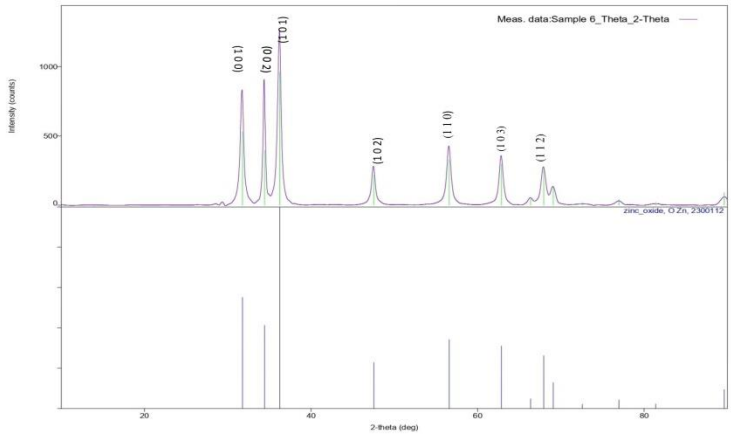

Figure 4. XRD for AMZnO nanoparticles

3.5 Anti-microbial activity ZnONPs;-

The current investigation demonstrated the mild antibacterial efficacy of green produced zinc oxide (AMZnO) Nps against bacterial infections of *S. typhi*, and not against *E. coli*. The fruit pulp extract of A. manihot, which acts as a capping agent in

Nps, reduces particle size, and increases antibacterial activity, may be the cause of the green produced ZnO Nps antimicrobial efficiency [52]. This could be explained simply by noting that smaller particles often have a higher surface to volume ratio, meaning that their antibacterial activities are more effective overall [53]. It is verified in this study that the crystallite sizes of green produced ZnO NPs are 57 nm. The gradual release of Zn^{2+} ions is the cause of ZnO Nps' mild antibacterial potential.

Test organism	Activity of AMZnO	Antibiotics	CLSI Standards
S. typhi	8 ±0.75	Ciprofloxacin	20
E. COLI	1 ±0.2	amoxicillin	20

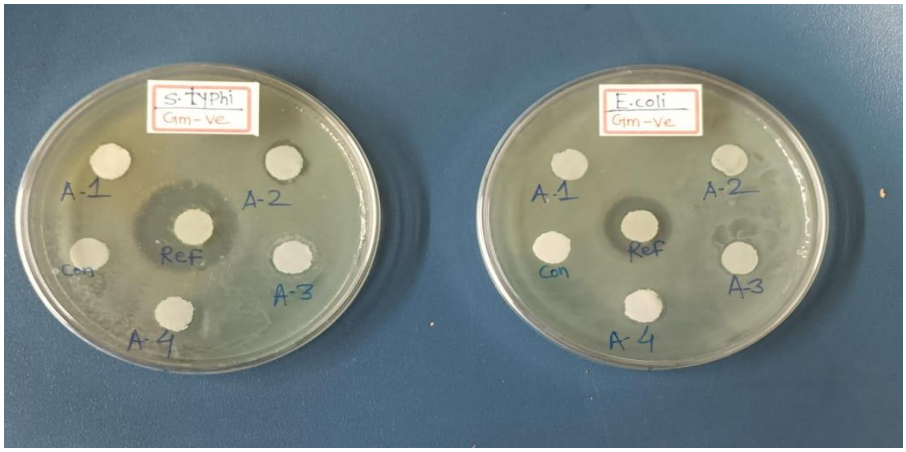

Figure 5 Antibacterial assay for AMZnO nanoparticles

4. Conclusion;-

The main goal of this research is to synthesize biomedically significant ZnO-NPs in an environmentally friendly one-pot process using aqueous fruit pulp extracts from the medicinally significant plant *A. manihot*. The synthetic NPs' crystalline structure has been confirmed by XRD investigation. Analyses using Fourier transform infrared (FTIR) spectroscopy confirmed that phytochemicals were present and contributed to the transfer of metallic ions to NPs. SEM examinations were used to evaluate the morphologies and vibrational modes. ZnO-NPs that have been synthesized have demonstrated moderate antibacterial activity against *S. Typhi*.

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