GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING AQUEOUS SEED EXTRACT OF BLACK PEPPER AND THEIR BIOLOGICAL APPLICATIONS

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Abstract

In the production of nanoparticles utilizing biological products, bioinspired or green technology gains significance every day owing to its ecofriendly and cost-effective nature. In the current study, Zinc nitrate was used to synthesize zinc oxide nanoparticles (ZnO-NPs), Zinc oxide nanoparticles were stabilized and reduced by bioactive molecules of black pepper seed extract. Further characterization of ZnO-NPs was carried out using UV-Vis spectroscopy, SEM, EDAX, X-ray, DLS, TEM diffractograms, and infrared spectroscopy (FTIR). It was found that zinc nanoparticles can be best synthesized at 60ºC, metal salt (zinc oxide) concentration of 4.0 mg, and reaction time of 60 minutes. From antibacterial bioassay, it was observed that ZnO-NPs showed significance antimicrobial activity against *Klebsiella pneumoniae* (17±2.64 mm), by *Pseudomonas aerugenosa* (19±2.54), *Proteus vulgaris* (16±9.61) and *Staphylococcus aureus* (19±7.32) at 100 microliters of extract. Furthermore, ZnO-NPs exhibited significant Red Blood Cells haemocompatibility and antioxidant activity, the highest TAC and ABTS free radical scavenging activity at 400 µg/mL was noted as (42.24±0.65) and (42.12±0.83) for ZnO-NPs respectively. The particles exhibited excellent hemocompatibility as even at the highest concentration of 400 µg/mL (3.67±0.13%) hemolytic activity was observed respectively. It was concluded that Black Pepper extract is useful for the reduction and capping of ZnO-NPs. The bioengineered nanoparticles were biocompatible and have strong antioxidant, antibacterial and In-vitro biocompatibility capability against pathogenic bacterial isolates, therefore by utilizing ZnO-NPs in combination with commercially available antibiotics may provide an alternate therapy for the potential treatment of bacterial infections.

Keywords: *Black Pepper* ZnO-NPs, Antibacterial activity, Anti-oxidant assay, Biocompatibility assay.

I. INTRODUCTION

Nanoparticles made from transition metal oxides are of great interest as a next generation technology. One of the best metal oxides is ZnOnps that may be employed at the nanoscale. Having optical and electrical properties there are numerous applications of ZnONps such as in field of optoelectronics, cosmetics and medical industries. Besides these zinc oxide nanoparticles are also useful in photo catalysts, solar energy cells and gas sensors. ZnO-NPs have been determined to be particularly hazardous between metal oxides tested for antibacterial action. It's popular in the agricultural and food sectors because of its low toxicity and ability to withstand rigorous processing conditions [1]. The mineral zincite is found naturally in the

earth's crust, however the bulk of zinc oxide (ZnO) used commercially is synthesized. ZnO is nontoxic and friendly with human skin since it is a good to use it as colorant in textiles and other surfaces that touches skin. Some of other applications of ZnOnps are that they are also used in the devices for UV emissions and in gas sensors and cosmetics etc. Because of features such as non-toxicity, self-cleaning compatibility with skin, antibacterial, and skin related degree, UV-blockers are utilized in suntan lotion and a range of health specialized uses. Numerous investigations have discovered that CaO, MgO, and ZnO exhibit substantial antibacterial drug action, which has been accredited to the production of reactive oxygen species (ROS) on their surfaces. Regardless of these advantages, ZnO is biocompatible and bio safe because ZnO are structure dependent, electrical, and property of thermal transport [2]. Zinc oxide (ZnO) is becoming increasingly commonly used in biomedical uses like drug administration and bioimaging. Due to the rapid dissolution of ZnO nanoparticles in water and acidic solutions, their surfaces must be adapted to safeguard them in biological systems. To employ ZnO nanoparticles for fluorescence in imaging, the nanoparticles must be tamper with since the ZnO bandwidth is in the UV region, which cannot enter tissue and is also damaging to cells and tissue [3]. Due to ROS facilitated damage of biological macromolecules diseases comprising neurodegenerative disease, Alzheimer's cancer, Parkinson's disease, atherosclerosis, inflammation and reperfusion injury are caused. It is caused by a physiological imbalance between the systems that scavenge and those that produce free radicals. Atomic or molecular which have one or more unpaired electrons are called free radicals [4]. Food stabilizers which are antioxidant help to prevent the free radicals' productions which are very harmful to human cells and tissues. Oxidation-induced rancidity is the second cause of food degradation. With a broad band gap and as well as having high excitation binding energy ZnO are of many utilities. One of the materials that have been dogged to be safe for humans are ZnOnps [5]. In addition, ZnO NPs have antibacterial characteristics that are effective against a wide range of microbes. They inhibit many bacterial enzymes resulting in substantial antibacterial action [6]. Against Escherichia coli, oxidative stress resulting in membrane damage has been proven as the mechanism underpinning ZnO NPs antibacterial activities. They have the ability to penetrate the skin's layer. And by penetrating skins layer it protects skin from free radicals' production and also boosts cellular soothing and patch-up [7]. Biosynthesized ZnO-NPs have been proven to have higher antibacterial activity when compared to chemically generated nanoparticles. Because of their non-toxic nature, zinc oxide nanoparticles (ZnO-NPs) have been broadly working in biological applications, and they are also designated as generally recognized as harmless [8]. Because they may be employed in optical, piezoelectric, magnetic, and gas sensing applications, zinc oxide nanoparticles are one of the most fascinating metal oxide nanoparticles. Apart from these characteristics, ZnO nanostructures have a high catalytic efficiency and a strong adsorption, and they're employed in sunscreens, ceramics, and rubber processing, as well as wastewater treatment and fungicides. In fact, because ZnO absorbs both UV-A and UV-B rays whereas nTiO2 just blocks UVB, it may beat nano-titanium dioxide (nTiO2) in terms of UV protection and opaqueness [9]. To kill human cancer cells at low dosages nanomaterials, such as metal oxide nanoparticles, have been discovered in a growing number of studies. Although their larger micrometer-sized equivalents are essentially not harmful.

Nanoparticles have a strong affinity for cancer cells while being quite harmless to normal cells. Nanomaterials are also being researched for their possible application in intracellular delivery of DNA, RNA, proteins, peptides, and small medications to trigger cancer cell death, as contrast agents for cancer imaging, and as platforms for targeted gene and chemotherapeutic administration to tumor sites. The ability of ZnO nanoparticles to inhibit mast cell degranulation suggests that they might be used to control allergic reactions. Biodegradable ZnO nanowires have been discovered to breakdown into ions that may be absorbed by the body and become part of the body's environment. Recently ZnO nanoparticles have shown potential as, dietary modulators for hydrolase activity in diabetes, cholesterol biosensors and hyperlipidemia, and cell imaging [10].

Black pepper's botanical name is *Piper nigrum*. *Piper nigrum* belongs to the order Piperales, family Piperaceae, and genus Piper in the plant kingdom. Pepper is derived from the Old English pipor, the Latin piper, and the Sanskrit pippali, all of which imply "long pepper." Black pepper is planted for the peppercorn-like fruit, which is dried and used as a spice and taste. Since ancient times peppercorns have been used for flavor and traditional medicine. Black pepper is the most frequently traded spice on the earth, as well as one of the most widely used spices in cuisines throughout the globe. The spiciness is due to the chemical component piperine, which is a different type of hot than the capsaicin found in chili peppers [11]. Pepper spirit may be found in a wide range of pharmaceutical and cosmetic items. Pepper oil is also utilized in a variety of cosmetic and herbal therapies, as well as an Ayurvedic massage oil. Peppercorns, dubbed "black gold" and utilized as commodity money, were a highly prized trading item [12]. According to the Buddhist pepper is one of the limited drugs that a monastic is permitted to carry. Black pepper (or maybe long pepper) was supposed to help with pain in tooth, sleeping disorders sunburns and constipation, and many other problems. Many 5th century authors suggested using pepper to treat eye problems, often by smearing pepper salves directly to the eye. Despite the fact that existing health research has yet to reveal any therapeutic benefit for people, some advantages have been demonstrated in animal modelling experiments. Pepper phytochemicals such amides, piperidines, pyrrolidines, and trace amounts of safrole have been reported to cause cancer in experimental rats. Piperine is being studied for its capacity to improve the absorption of beta-carotene, selenium, vitamin B12, and curcumin, among other things [13]. Piperine, a bioactive phytochemical found in black pepper (*Piper nigrum*), has antioxidant, anti-inflammatory, anticancer, anti-apoptotic, depressive, anti-HVB, and gastroprotective activities. Piperine also helps medications and phytochemicals absorb better (such as curcumin). Free radicals, reactive oxygen species, and lipid peroxidation are all inhibited by piperine's flavonoids and phenolic content. Black pepper's antioxidant activity was measured using glutathione peroxidase, catalase, superoxide dismutase, including reduced glutathione content and the amount of malondialdehyde, and protein carbonyl levels in the hippocampus [14].

The objectives of the current study were the synthesis of zinc oxide (ZnO) nanoparticles using black pepper seed extract, characterization i.e., EDX, XRD, SEM, TEM, DLS and evaluation of biological application i.e., anti-bacterial activity, antioxidant, biocompatibility of the synthesized NPs.

II. MATERIALS AND METHODS

Plant material processing

The black pepper seeds were purchased from local market. To manufacture a plant extract of Black pepper, the plant components were smashed using an electric grinder. Fifty gram of black pepper powder was mixed with 500 ml distilled water in a 500 ml flask, which was boiled at 150°C for 20 minutes. The mixture was incubated for 24 hrs at 37°C for maximal extraction. The extract was then filtered using Whatman filter paper and kept at 4°C until needed.

Preparation of ZnO NPs

Black Pepper seed extract (100 mL) was combined with 6.0 g zinc nitrate to create zinc oxide nanoparticles [NO_3. 6H2O] precursor salt and heated for 2 hours at 100°C. The white precipitate was washed three times with deionized water before centrifuging for 10 minutes at 1000 rpm. After that, the supernatant was discarded, and the product was stored at 100 °C in an oven. To create extremely crystalline ZnO NPs, the dried sample was annealed at a high temperature (500 °C) for 2 hours.

Characterization of Biosynthesized ZnO NPs

Fourier transform infrared spectroscopy (FTIR), UV spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy dispersive x-ray (EDX) analysis, Dynamic light scattering, are the characterization techniques used for examination of the physicochemical properties black pepper ZnOnps. UV-Visible range of 200 to 700 nm was observed for the extract and zinc nitrate solution. The crystalline state of biosynthesized NPs was evaluated by XRD technique. With PanAlytical X'Pert the X-ray diffractometer XRD pattern was studied. By means of Scherer's equation 15, the crystallite size was investigated

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

D stands for size of crystal, k for factor form (0.94), 1.5421 for wavelength of X-ray and FWHM and Bragg's angle for FWHM and Bragg's angle, respectively. To identify the functional groups related with nps FTIR spectroscopy in the 400 cm -1 to 4000 cm $^{-1}$ spectral area was used. For morphology and physical dimensions, studies SEM (JSM-7600F, Japan) and TEM (JEM-2100F, Japan) were employed while elemental studies were carried out using EDS (JSM-7600F, Japan) (EDX). For the determination the zeta potential and particle size distribution (PSD the Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK) was used. At room temperature 1 mL of the sample (tenth fold dilution) was mixed with dH_2O in a nutshell and examined. To capture, the Helmholtz–Smoluchowski equation (PSD) 16 was used in combination with built-in software.

Biological applications

Antibacterial activity of ZnO NPs. against Tested Strains

Collection of bacterial species

Four multidrug-resistant bacteria i.e., *Escherichia. coli*, *Pseudomonas aeruginosa*, *Klebsiella p neumonae* and *Staphylococcus aureus* were recovered from UTI (urinary tract infection) patients. These isolates were earlier detected using biochemical testing and a molecular technique at the Hayat Abad Medical Complex in Peshawar, Pakistan (16s rRNA) [15].

Agar Well Diffusion Assay for ZnO NPs.

In the medium of Nutrient Agar 8 mm wide wells were pierced which had been lawned with bacteria. A 100µl suspension of ZnOnps was pumped into each well. Then the petri dishes remained incubated for 24 hours at 37 degrees Celsius in an incubator. Against the MDR bacterial strains the efficacy of ZnO

NPs studied was measured in millimeters immediately after incubation.

Antioxidant Assays

Total Antioxidant Capacity Determination (TAC)

The over-all antioxidant ability of the sample was measured. In the observation, a micropipette was used to pipette 100µlof material into the Eppendorf tubes. The TAC reagent was then added to the Eppendorf tubes containing the samples (28 mM sodium phosphate, 0.6 M sulphuric acid, and 4 mM ammonium molybdate in 50 ml dH20). In a microplate reader absorbance of samples was measured at 630 nm after two hours of incubation at 90 degrees Celsius in a water bath. TAC was computed three times in grammes of ascorbic acid equivalent per milligrams of sample. [16].

Total Reducing Power Determination (TRP)

To estimate the sample's total reduction power, the Aziz et al. approach was employed. In Eppendorf tubes, the test sample (100 µl), 400 µlof phosphate buffer (pH 6.6 and 0.2M), and potassium ferric cyanide (1 percent w/v) were mixed and incubated for 30 minutes at 55 degrees Centigrade in a water bath. Individually Eppendorf tubes were then filled with 400 mL of 10% w/v trichloroacetic acid and centrifuged for 10 minutes at 3000 rpm. Each combination's supernatant (140 µl) was placed in 96-well plate wells previously filled with 60 µlferric cyanide solution (0.1 percent w/v). The samples absorbance was measured by means of a microplate reader at 630 nm. Same procedure was used on both negative and positive controls [17].

Total Free radical scavenging assay (FRSA)

With minor alterations, the protocol initially reported by was implemented. To examine if ZnO NPs might scavenge free radicals, the DPPH reagent was employed to assess their antioxidant capability at doses ranging from 12.5µl to 400µl. With minor alterations, the protocol initially reported by was implemented. To examine if ZnO NPs might scavenge free radicals, the DPPH reagent was employed to assess their antioxidant capability at doses ranging from 12.5µl to 400µl. The tested chemicals were placed in each well (10 µl) of a 96-well plate. After that, each well holding the sample received 190µl of DPPH reagent. Then incubation was carried out for one hour in dark at 37°C. Ascorbic acid was taken as a positive control. DMSO was used negative control. The rate of absorbance at 515nm were also measured by using a microplate photometer. The biosynthesized Ag-NPs' FRS potential was calculated in percentages.

$$(\%)\ \text{FRSA} = \left(1 - \frac{Abs}{Abc}\right) \times 100$$

Abc is absorbance of the negative control while abs is sample absorbance.

Total Trolox Antioxidant Assay (ABTS)

A modified ABTS test was used to measure the antioxidant capacity of the biosynthesized assay. In equal amounts, (2.45 mM) potassium persulphate and 7 mM ABTS salt were mixed and incubated at room temperature overnight. After incubation, samples were added to the mixture and kept at room temperature in the dark for 15 minutes. At 734 nm using the BioTek ELX800 the absorbance of the sample in the reaction mixture was calculated. The positive control was trolox reagent, whereas the DMSO was negative control. The results of a triplet test were given the abbreviation TEAC (antioxidant potential).

Biocompatibility Studies

ZnO NPs were examined for biocompatibility against freshly formed human red blood cells. After healthy volunteers provided their permission, in an EDTA tube 1ml of blood was collected and deposited. To isolate red blood cells, For 7 minutes blood samples were centrifuged 12,000 rpm. The particle was recovered after three PBS washes, and the supernatant was discarded. 200 liters of erythrocyte were mixed with 9.8 litres of PBS to make the PBS-erythrocyte suspension (pH: 7.2). Erythrocytes suspension and varied concentrations of biosynthesized NPs were mixed in Eppendorf tubes and incubated at 35 degrees Celsius for 1 hour. 200 l of the supernatant was transferred to a 96-well plate after centrifuging the Rge mixture at 1000 rpm for five minutes, and hemoglobin release absorption spectra at 540 nm were recorded. A positive control of 0.5 percent Triton X-100 was employed, while a negative control of DMSO was used. The following formula was used to compute the percentage of haemolysis:

$$(\%)\ \text{Haemolysis} = \left(\frac{sample\ Ab - negative\ control\ Ab}{Positive\ control\ Ab - Negative\ control\ Ab}\right) \times 100$$

While Ab denotes the reported absorbance of the samples.

III. RESULTS

4.1 Biosynthesis of ZnO-NPs

For the synthesis of ZnONps, aqueous seed extract of black pepper was employed as a capping, reducing and stabilising agent. According to significant research, inside the dry biomass of black pepper volatile oils are the major constituents. Only a handful of the numerous substances that contribute to the dry weight include D-pinene, myristin, myristic acid, and its esters. Furthermore, one-half of the dry biomass is made up of myristicin, fatty acids, and mesin. At the nanoscale, all of these main components might have aided in the creation and stability of stable elemental zinc (Zno). The production of ZnO-NPs was noticed during the reaction by the color shift of the combination ($Zn(CH_3COO)2.2H_2O$ + black pepper) from dark black to light brown (Fig. 1). The physicochemical and morphological properties of ZnO-NPs are thought to be highly dependent on the kind and species of plant utilized, as well as reaction circumstances such as pH, temperature, and synthesis medium.

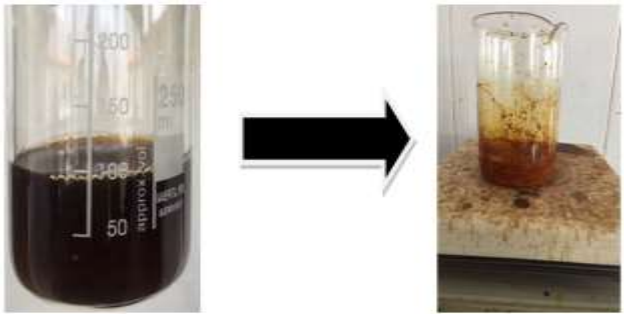

Fig: 1 Preparation of ZnONps (Indicated by change in color from dark brown to light brown)

4.2 UV-Vis study

The UV-Vis absorption research is a straightforward and widely used approach for determining the optical characteristics of nanoparticles. UV-Vis absorption tests of ZnO nanoparticles at wavelengths of 200–800 nm are shown in Fig. 2. Figure 2 shows that ZnO has a broad absorption range and a significant absorption peak at 270 nm. Vimala et al., [18] reported on 370 nm absorbance spectra of ZnOnps [19].

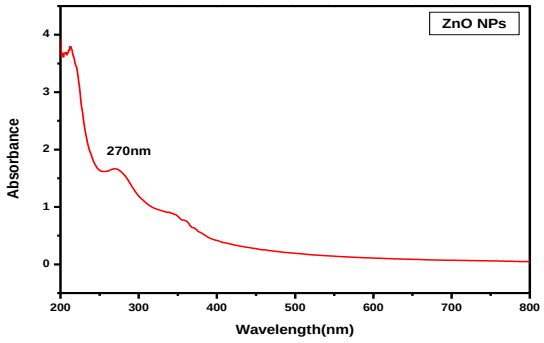

Fig. 2 UV-Vis spectroscopy of ZnO nanoparticles

4.3 Fourier transform infra-red spectroscopy (FT-IR)

The absorption measurement of IR radiations was validated using Fourier Transform Infrared Spectroscopy in terms of sample plot vs wavelength (FTIR). In the IR spectrum, the link between sample compounds and absorption bands was studied. As seen in Figure 3, FTIR was employed to classify the functional group of extract and ZnO-NPs in the 4000–400 cm^{-1} range. At 502 cm^{-1} evidence of ZnO was found due to zinc and oxygen bonding vibrations. The slight absorption peak detected about 3200 Cm^{-1} can be attributed to the hydroxyl group (OH). Alkane (C-H), alkane di-substituted (C=C), and alcohol (C-OH) have been assigned to the other bands at 1622, 1227, and 960Cm^{-1}, respectively. ZnO Nanoparticles have been created as a result of this research, the OH group was drastically reduced. The FTIR measurements in the graph below show that the produced ZnO-NP was extremely pure. [20].

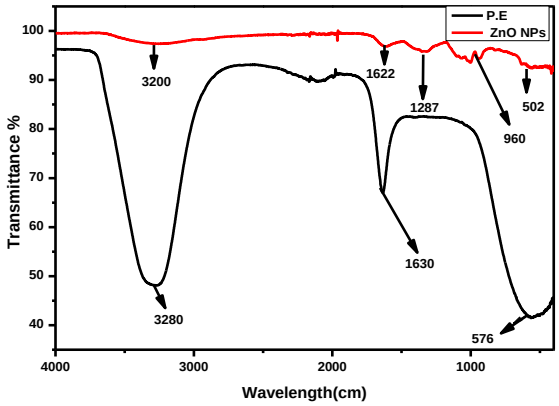

Fig: 3 FT-IR spectra of biosynthesized ZnO nanoparticles and plant extract.

4.4 X-ray diffraction analysis (XRD)

Nanoparticles were investigated by means of X-ray Diffraction (XRD) analysis in the scanning angle (2) from 100 to 80° with Cu K radiation (=1.5418A) as the X-ray source at 40kV and 30mA to assess purity and crystallinity. The main peaks (100), (002), (101), (110), (103), (112), (201), and (202) corresponding to the diffraction planes (100), (002), (101), (110), (103), (112), (201), and (202) revealed that ZnO-NPs are crystalline. The tiny and strong diffraction peaks in Fig.4 also indicate the crystallinity of the ZnO-NPs. By the XRD diffraction patterns of ZnO-NPs, as evidenced by the Joint Committee Powder Diffraction Standard Data (JCPDS card no.008, 82–1042 and 50, 664) the crystalline sphere-like shape of nanoparticles was validated.

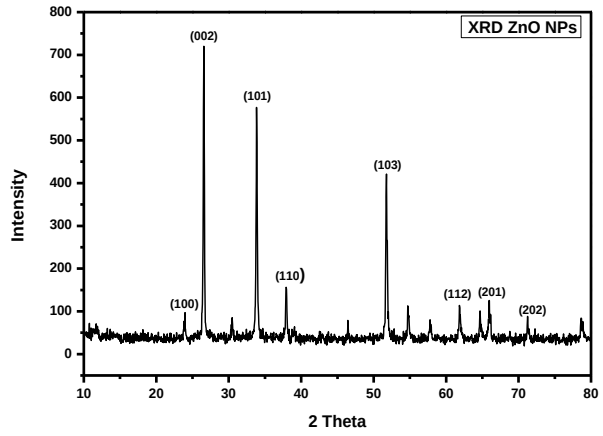

Fig:4 XRD spectra of biosynthesized ZnO nanoparticles.

4.5 Energy dispersive x-ray analysis (EDX)

In nanoparticles EDX spectra the creation of Zinc oxide nanoparticles is clearly visible. The Oxygen atomic mass was 28.73 percent, while its weight was 9.81 percent. The atomic mass of zinc was 55.95 percent, while its weight was 78.02 percent. The other minor constituents present in the ZnO nanoparticles had an atomic weight of 55.95 percent and a weight of 78.02 percent as shown in fig 5.

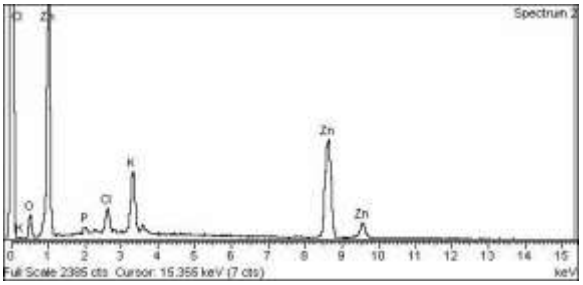

Fig 5: EDX spectra of biosynthesized ZnO nanoparticles.

4.6 Scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

To study the morphology of green-produced zinc oxide nanoparticles SEM was used. As seen in Fig. 6 (A), the particles exhibit an uneven crystalline structure and are heavily agglomerated. This obviously proves that the particles are present in a homogenous state, which is crucial for the varied activities of nanoparticles. The particles ranged in size from 42.5 nanometers to 90.4 nanometers. TEM micrographs were employed to examine the shape and particle size of pure ZnO nanoparticles, as revealed in fig.6 (B). The shape of particles was spherical. The detected diameter of particles was 39.3 nm. The particle size predicted by the XRD study is quite similar to the particle size seen in the TEM picture.

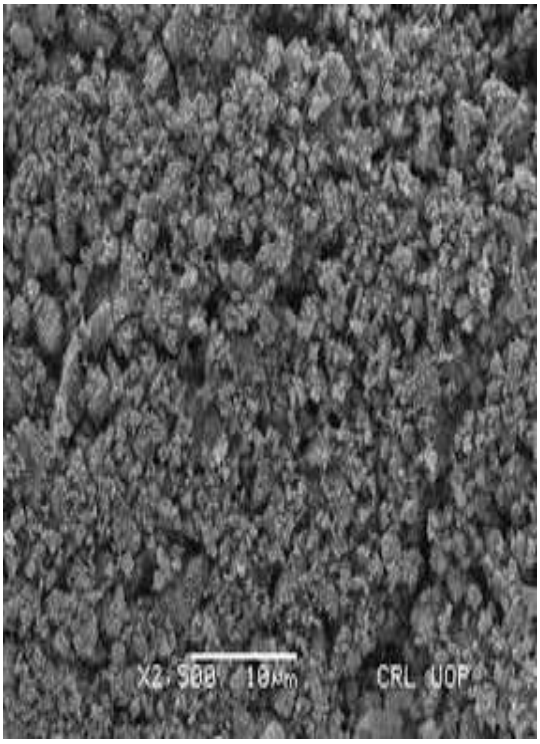

Fig: 6 (A) SEM image ZnONps

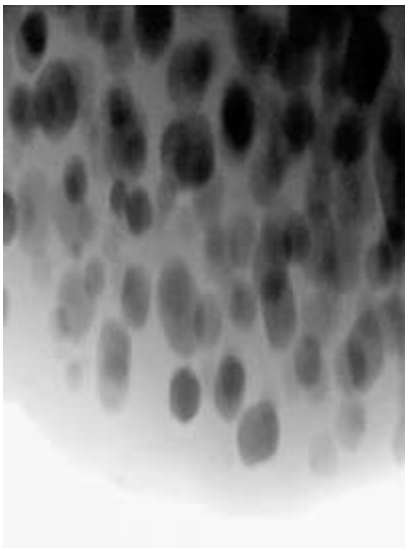

Fig: 6 (B) TEM image of ZnONps

4.7 Dynamic light scattering (DLS)

The dynamic light scattering method is based on light's interaction with particles. The hydrodynamic size of zinc nanoparticles produced in aqueous solution was determined using DLS and found to be 32 nm. (Fig. 7).

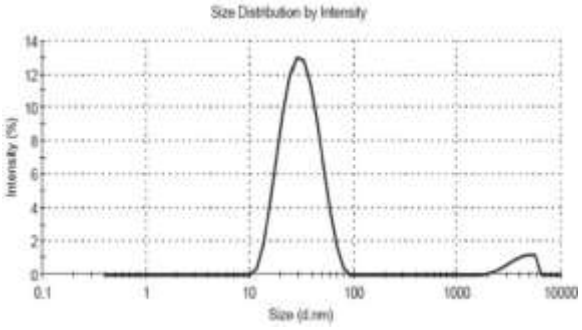

Fig: 7 DLS Spectra of Biosynthesized ZnO Nanoparticles.

4.8 Biological activity

4.8.1 Antibacterial assay

A huge percentage of the world is infected due to resistance of bacteria towards antibiotics and that continues to creating havoc on the healthcare systems of both poor and wealthy republics. The development and extent of multidrug-resistant diseases has a substantial influence on antibiotic therapy. Plant intervened nanomaterials are included in the inspection of new antimicrobials, which contain a variety of bioactive compounds with well-established medicinal benefits. In today's world, environmentally friendly methods of creating metallic nanoparticles have shown to be advantageous. Phytochemicals from extracts of plants which serve as both reducing and covering agents, have evolved into a unique nanoparticles production technique. In this effort, we synthesized zinc oxide nanoparticles from a popular pharmaceutical plant and investigated their antibacterial activity against pathogenic bacterial strains. Fig 7 (a) comprehensive profile of ZnO-NPs antibacterial activity against test organisms was shown. At 100 microliter, 1 percent ZnO-NPs solution (1mg/mL DMSO solution) showed the greatest inhibition zone against *K. pneumoniae* (172.64 mm*)*, *P. auregonisa* (192.54), *P. vulgaris*(169.61), and *S. aureus* (197.32) at 100µL, amoxicillin were used as a standard drugs according to the previous study [21].

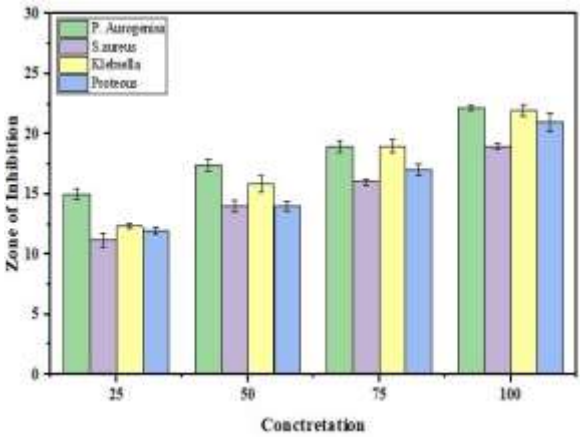

Fig: 7 (a) Antibacterial activity of ZnO-NPs against K. pneumoniae, P. auregonisa, P. vulgaris and S. aureus.

4.8.2 Antioxidant assays

In plant metabolic pathways reactive oxygen species are produced which in turn

causes destruction to lipids, plant cells, DNA, and proteins of membrane. Flavonoids, terpenoids, and oxidative stress response phenolics are among the metabolic components produced by plants that work as shielding mechanisms, and these phytochemicals are mainly implicated in the stability and coating of nanoparticles. Total antioxidant capacity (TAC), total reduction power (TRP), ABTS, and DPPH-free radical scavenging assays were used to evaluate the in vitro antioxidant activity of plant-derived NPs (FRSA). The results are summarized in the table below. At 400 g/mL, ZnOnps had the best TAC and ABTS free radical scavenging activities, with 42.240.65 and 42.120.83, individually. Altogether of these tests were carried out three times, and from the average of the triplicates the results were calculated.

Table No. 1: Antioxidant Activity of ZnONps

Conc (µg/mL)	TAC (µg AAE/mg)	TRP (µg AAE/mg)	ABTS (TEAC)	DPPH (% FRSA)
400	42.24±0.65	33.25±0.27	42.12±0.83	31.4±0.82
200	30.57±0.61	23.51±0.67	26.57±0.78	18.53±0.61
100	21.81±0.63	19.22±0.51	21.59±0.54	15.58±0.63
50	13.5±0.52	15.56±0.74	19.52±0.72	9.16±0.51
25	8.28±0.71	10.58±0.52	7.52±0.42	7.52±0.31

4.8.3 *In-vitro* biocompatibility studies

To establish if nanoparticles had any toxicological potential, they were experienced for hemolysis in contradiction of human red blood cells (hRBCs). Freshly separated hRBCs were co-incubated with various NP formulations (25g/mL to 400g/mL) in a buffer solution that mimicked an extracellular environment in the experiment. The concept is based on RBC rupturing, which releases hemoglobin into the media, which can be detected using a spectrophotometer at 405 nm. If the tested material possesses cell rupturing potential this can only be induced then. The investigation's findings are summarized in Table 3. Substances with hemolysis of less than 2% are non-hemolytic, those with hemolysis of 2–5% are slightly hemolytic, and those with hemolysis of more than 5% are hemolytic, according to ASTM (American Society for Testing and Materials) guidelines. There are other elements to consider, as shown in Table 3. There was no noticeable hemolytic activity even at the highest stock concentration, suggesting excellent hemocompatibility. In order to be used in biomedical applications, engineered nanomaterials (ENMs) must be biocompatible. The particles demonstrated good hemocompatibility, with 3.670.13 percent hemolytic activity, even at the maximum concentration of 400 g/mL. As a result, our findings corroborate the particles' biosafety and pave the way for therapeutic applications of NPs produced by Black Pepper Seed.

Table No.2. % Hemolysis

S.NO	Concentration (µg/mL)	% Hemolysis
1	400	3.67±0.13
2	200	2.32±0.41
3	100	0.89±0.43
4	50	0.52±0.21

IV. DISCUSSION

Due to its simplicity, nontoxicity, speed, and cost effectiveness, as well as its feasibility for large-scale manufacturing, the

green nanoparticle synthesis technique has gained a lot of support in the scientific community in recent years. The current study looked at the antibacterial, antioxidant, and biocompatibility properties of ZnO-NPs made from Black Pepper extract utilising a green synthesis approach. A number of analytical methods were used to characterize ZnO-NPs, including UV–visible spectroscopy, FTIR, SEM, EDAX, DLS, TEM, and XRD. The sample absorbed energy at 451 nm, which is a typical peak value for ZnO-NPs, according to UV–visible spectroscopy. The same findings were discovered using X-ray spectroscopy [22]. Apart from that, the nanoparticles' outstanding purity was demonstrated by an absorption peak at 451 nm with no other peak. A strong absorption peak of ZnO Nanoparticles was discovered below 450 nm wavelength in several experiments, which was attributed to the samples' red shift at 500 and 700 degrees Celsius. They also stated that as an electron gains energy, it transitions from a lower to a higher energy level in materials transitions. [23,24]. Stretching vibration peaks of ZnO-NPs were observed in FTIR analysis of Black Pepper Seed Zinc Oxide nanoparticles, demonstrating interrelation of different functional groups and vibrations of functional groups such as alkanes, phenol, alcohols, aromatics, alkenes, alkyl-halids, and aliphatic amines. Furthermore, they discovered that the peaks in carboxylic acid, polysaccharide, and amino acid were traceable to –C = O–, C–O–C, and C–O stretching vibrations, respectively, when they used red apple [25]. The generated ZnO-NPs' spherical form was validated by SEM micrographs, with particle sizes ranging from 42.5nm to 90.4nm. Both Nano-measurer and Image analysis have verified this. [26]. Furthermore, the shape of the synthesized ZnO-NPs was hexagonal, which is consistent with our findings; however, the size of the nanoparticle was larger in this study than in, which could be due to differences in synthesis conditions such as temperature, incubation time, plant extract nature, and handling applications. [27,28]. In the test sample, EDAX analysis revealed pure ZnO-NPs, as well as phases and a significant peak at 2.5 keV in the EDAX spectrum, suggesting pure silver. The EDAX spectra of ZnO-NPs were obtained using a simple precipitation process that used silver nitrate as the starting material. Pure ZnO-NPs with prominent peaks were successfully generated, according to the EDAX spectrum. Additional peaks in the spectrum, however, were detected, indicating that algal biomolecules were involved in nanoparticle synthesis. In their research found the same EDAX pattern of ZnO-NPs with high purity [29]. EDAX analysis was used to assess the purity of ZnO-NPs. Pure silver and extra peaks were visible in the spectrum, showing that the substance was pure. The size and crystallinity of biosynthesized Zinc Oxide nanoparticles were determined by XRD analysis. The planar orientation and crystalline structure of ZnO-NPs were verified by the XRD spectrum. The primary XRD peaks at 2Theta were (100), (002), (101), (110), (103), (112), (201), and (202), (202). [30]. The definite XRD reflection planes correspond with the International Center of Diffraction Data card (JCPDS-36–1451), proving the synthesis of crystalline hexagonal structure, which accord with the result of [31], validate the crystalized sphere-like structure of nanoparticles. K. pneumoniae had the largest inhibitory zones (172.64 mm) at 100 microliter, followed by *P. auregonisa*(192.54), *P. vulgaris* (169.61), and *S. aureus* (197.32). Zinc oxide nanoparticles have been shown to interact with bacteria's cell membrane and cause harm by leaking internal components into the environment [32]. Zinc Oxide nanoparticles have a bactericidal effect due to their inter

linkage with and inhibition of bacterial cell membranes [33]. *Pseudomonas aeruginosa* showed the highest damage at 45 mg/ml ZnO-NPs. Nanoparticles' antibacterial, antifungal, and other biological effects are highly reliant on their size and concentration; small sizes easily breach bacterial protective barriers and cause injury; large sizes easily breach bacterial defensive barriers and cause harm. [34]. Antioxidants are chemicals that aid in the prevention or reduction of cell damage caused by free radicals, which are unstable molecules created by cells in reaction to environmental and other stresses. "Free-radical scavengers" is another name for them. Antioxidants, both natural and synthetic, are available. Antioxidant properties of zinc oxide nanoparticles. [35]. At 400 g/mL, the maximum TAC and ABTS free radical scavenging activity for Zinc Oxide nanoparticles (ZnO-NPs) was 42.240.65 and 42.120.83, respectively. ZnO-NPs have the potential to effectively scavenge DPPH free radicals, making them a suitable antioxidant test candidate. 3.670.13 percent hemolytic activity was detected at the highest dose of 400 g/mL, demonstrating excellent hemocompatibility. The findings of the current study will be helpful in terms of the particles' biosafety and therapeutic applications of NPs produced by Black Pepper Seed. Our findings back up the bio-safety of Zinc Oxide nanoparticles, revealing that Black Pepper Seed-mediated Zinc Oxide nanoparticles are secure *in vivo* and can be used for therapeutic applications. [36].

V. CONCLUSION

The results from the present study concluded that, bio-assisted nanoparticle production provides a safer alternative to conventional physical and chemical methods. 270 nm was found in the UV-Visible spectroscopy, which verified the synthesis of ZnO-NPs to be within the expected range. The crystalline nature of the nanoparticle is confirmed by XRD. One research used FTIR technology to validate the metal ions' transition into nanoparticles. The morphological characteristics as well as the elemental composition were discovered using SEM, TEM, DLS and EDX. The results showed that nanoparticles were efficient against a variety of bacterial types. In addition, it was shown that ZnO-NPs significance antibacterial activity against K. pneumoniae, P. auregonisa, P. vulgaris and S. aureus. In biological tests, NPs of a smaller size play an essential role, and it has been shown that smaller NPs perform better. Our studies have shown that nanoparticles (NPs) may be utilized in a wide range of biological application such as Anti-oxidant, Anti-Bacterial and biocompatibility assay. It was also suggested that ZnO-NPs synthesized from black pepper extract could be of great medical importance in pharmaceutical and medical science for their biological activities as it is found biocompatible in nature. However, more research is needed to fully exploit the innovative and remarkable capability of NPs in Nanomedicines.

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