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GREEN APPROACH ON ACHIEVING ZINC OXIDE NANOPARTICLES AND ITS POTENTIAL BACTERICIDAL AS WELL AS ANTIOXIDANT ACTIVITY

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Keywords:

Zinc oxide nanoparticles, Lemon peel, Antioxidant, Antibacterial, Green approach

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ABSTRACT: Biosynthesis of nanoparticles by biological means especially plant extract plays a major role in the field of nanotechnology. In the present study, the aqueous extract of lemon peel (*Citrus limon*) was used for the green synthesis of ZnO nanoparticles using 0.1 M $Zn(NO_3)_2$ as a precursor in alkaline condition using NaOH with vigorous stirring for 3 h. ZnO NPs obtained were characterized by UV-vis spectroscopy in which the surface Plasmon resonance was 310 nm, followed by XRD, FTIR that showed appropriate speaks for various chemical groups present including ZnO NPs and AFM results shows monodispersed in nature. Further, the antibacterial property of ZnO NPs synthesized by the green method was revalued by the well diffusion method. The zone of inhibition was found to be 9 mm for 100 µL and 7 mm for 100 µL for respective strains *Klebsiella aerogene* and *Serratia marcescen*. Significant antioxidant activity was exhibited by NPs through scavenging of 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) free radicals. Thus, the use of aqueous peel extract as a capping agent would improve the antibacterial property of ZnO nanoparticle and can be developed as antibacterial agents against a wide range of micro-organisms to control and prevent the spreading and persistence of bacterial infections. Followed by Edax and Zeta was also included.

INTRODUCTION: Nanotechnology is being used in the production of materials in nanometer-scale [1] range of 1-100 nm [2] which is applied in the field of material science [3]. Nanotechnology can be defined as greatly reduced in chemical and physical materials through the exploitation of matters with specific properties [4].

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The physical approach contains thermal desiccation, synthetic gas degradation, pulsed radiation displacement, metal-organic chemical vapor deposition and atomic shaft epitaxial, while the chemical approaches like solvothermal, sol-gel, sonochemical and electrodeposition development are cost emphatic and volume-extension orientate, they are not eco-friendly in nature [5].

The intensity of nanoparticles is very small as desirable size [6] and has resources of the massive surface area to volume ratio [7, 8]. In the field of nanoscience and nanotechnology ingenious key for design various engineering materials which include ore, alloy oxides, chalcogenides, graphite allotropes in nanoscale level [9] and contaminant management [10]. Green synthesis of nanoparticles has a unique beneficial property like an eco-friendly, low time consuming, inexpensive and most sustainable in which this process has bio-element as reducing agents such as plants, fruits, bacteria, algae and yeast without any participation of chemical hazardous [11].

In recent years, Zinc oxide nanoparticles are accepted as practically favorable and adaptable inert stuff with a wide dimension and also it demonstrates a wurtzite structure [12]. ZnO NPs are semiconductor matter with large strapping space of ~3.37 eV and high obligatory power of 60 meV which displays multiple semiconductor assets and piezoelectric reflex [13]. The collection of blending approach of Zinc oxide has diverse morphology includes nanowires, nanosheets and nanocrystals [14], nanocombs, nanorings, nano-helixes, nanobelts and nanocages [15]. The nano-scaled of ZnO has adopted one of the greatest metal oxides attributable to its premium electrical and optical properties. It's nanosized ZnO substance has a large application in optoelectronics, solar battery, gas sensors, voltage-dependent resistor [16], catalysis, biosensors, and biomedicine, *etc.* [17] The different techniques were used for manufacturing ZnO NPs are Sol-crystallize processing, solid precipitation, organometallic synthesis, aerosol pyrolysis, melting evaporation, microwave practice, automatic milling and mechano - enzymatic synthesis.

Yet, these procedures are generally costly, Energy-accelerated and hazardous [18, 19]. The ZnO is specific in the component for achieving the photocatalytic task, as a substitute for a broadly recycled, similarly overpriced Titanium oxide (TiO_2). While the investigator identified a tantamount photocatalytic system with one and the other of TiO_2 and ZnO. Further, ZnO was superior photocatalyst in lowering the triclopyr which is a herbicide as well as fungicide, insecticide carbetamide, Trashy mill bleaching wastewater, phenol, 2-phenylphenol, blue 19 and acid red 14. These Preference of ZnO photocatalytic properties consist of the wide statistic of effective sites, highly efficient in producing hydrogen peroxide and higher feedback amount [20]. The fresh synthesis technique causes essential profit toward the synthesis of nanomaterials and it explores to reduce infection in dawning [21] Lemon fruit (*Citrus limon*) is a fruit has exhaust worldwide frequently like an edible flavoring or component of the concoction in the fruit juices; *Citrus limon* vest to Rutaceae family and its common name is lemon. Its existence was South East Asia, perhaps in India or Southern China.

It exhibits on the various application like carminative, insectifuge, antiseptic, antibacterial, antivirus, anti-yeast, larvicidal, uricosuric, anti-hepatotoxic and antimutagenic agent [23]. The lemon peel acts as a bio-waste material and in ZnO NPs exist incorporate practicing lemon peel extract as a diminishing agent such as hexamethylenetetramine (HMTA) or cetyl trimethyl ammonium bromide (CTAB) [15]. Limoncello stands as a generic name in Italy which is established for a citrus-based lemon liqueur that was supplied fine frost in the summer season [24]. The juice from the fruit is distinguished by the tremendous quantity of nutrients such as digestive fiber, vitamin C, potassium, citric acid, ascorbic acid, minerals, flavonoids, carotenoids, coumaric acid, limonoids, β- and γ-sitosterol and essential lubricate, given a large diversity of advantageous effect to health, whereas in lemon leaves which are used as a classical remedial worldwide to treat corpulence, diabetes, blood lipid level, cardiovascular pathosis, brain disorder and cancer.

In further, the lemon peel essential lubricate is used in the food manufacturing, aroma, and in ointment [25, 26]. The lemon peel consists of two distinct tissues: flavedo, the peel's superficial stratum, among pigment deviation from green to yellow and a prosperous provenience of essential lubricates, and albedo, a cushioned and cellulosic layer settle below flavedo includes high digestive fiber complacent and bioactive combination with anti-oxidant proprietary [27]. Lemon peel squander constitutes 50-60% of the entire fruit weight and it is the initial consequence and a large-scale source of environmental contamination. Lemon Peel squander is usually recycled for the production of beneficial profit like multi-enzymes or abundant compounds such as single-celled protein, digestive fiber, flavonoids, carotenoids, minerals, citric acid, essential oils and also it contains bioenergy (bio-ethanol) [28].

In the present study, Zinc oxide nanoparticles were synthesized using an aqueous extract of lemon peel and done characterization followed by antibacterial and antioxidant activity using *Serratia marcescence* and *Klebsiella aerogene*.

MATERIALS:

Preparation of Lemon Extract: Fresh lemons were purchased from Katpadi, Vellore District, Tamil Nadu, India. 5 g of lemon peel was taken and made into small pieces. To 250 mL of the conical flask, 100 mL of distilled water was added and boiled in the micro oven for 5 min at 60 ºC. The contents were cooled and filtered using Whatman filter paper. Using the extract, zinc oxide nanoparticles (ZnO NPs) were synthesized.

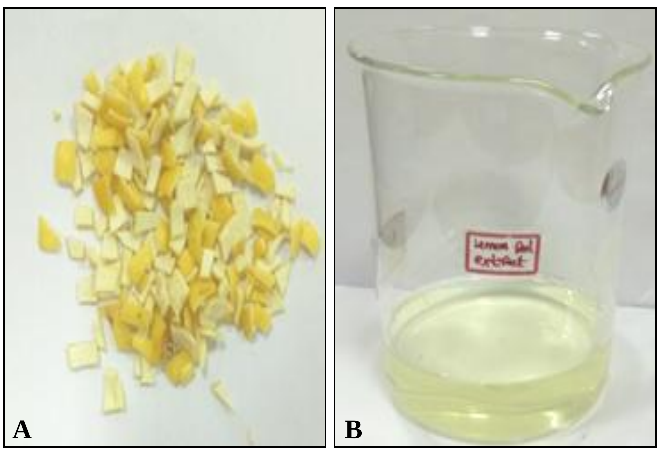


FIG. 1: A) FRESH LEMON PEEL B) LEMON EXTRACT

Methodology:

Synthesis of [ZnO] Nanoparticles: Zinc acetate (M.W: 219.50) and Sodium hydroxide pellet (M.W: 40.00) which is prepared for 1 mM in 50 mL of distilled water. The solution was left undisturbed in the magnetic stirrer at room temperature with 300 rpm for 3 h.

FIG. 2: ZnO + H_2O

Procedure: 50 mL of lemon peel extract was mixed with 50 ml of the prepared solution (ZnO + H_2O), the content was covered with aluminum foil and kept in the shaker at room temperature with 75 rpm for 24 h.

Antibacterial Activity: Muller Hinton agar media was prepared in 250 mL of the conical flask, *Serratia marcescen*, and *Klebsiella aerogene* were two test strains used for the antibacterial activity. Agar media including swab, needle, gel-puncher, Petri dishes were kept for sterilization process at 121 degrees Celsius for 20 min so that no contamination occurred.

After the medium was solidified, the two test strains were swabbed and wells were made to add the samples in various concentrations from the stock (1 mg/mL) of NPs dissolved with distilled water and streptomycin as a positive control was added at the concentration of 100 µL. The plates were incubated at room temperature for 48 h to measure for the zone of inhibition.

Antioxidant Activity: DPPH assay was performed for antioxidant activity. The total volume of 2 mL was made from which 1 mL of 0.1 mM DPPH and 1 mL of a sample prepared from the stock solution (1 mg/mL) taken in a conical flask and covered tightly with aluminum foil to avoid exposure from light. Ascorbic acid was prepared in 1 mg/mL concentration used as the standard. Different concentration was prepared for both lower concentrations like 1 µL to 5 µL and for higher concentration like 10 µL to 20 µL. This was kept undisturbed in a dark place for 30 min and then UV at 517 nm.

RESULTS AND DISCUSSION:

UV-Spectrometer: Ultraviolet spectroscopy is a technique used to quantify the light that is absorbed and scattered by a sample. Nanoparticles have optical properties that are sensitive to size, shape, concentration, agglomeration state and refractive index, which makes UV spectroscopy a valuable tool for identifying, characterizing and study of surface Plasmon resonance [32].

The synthesis of nanoparticles was tracked by using UV-Spectroscopy at the wavelength between 200-800 nm.

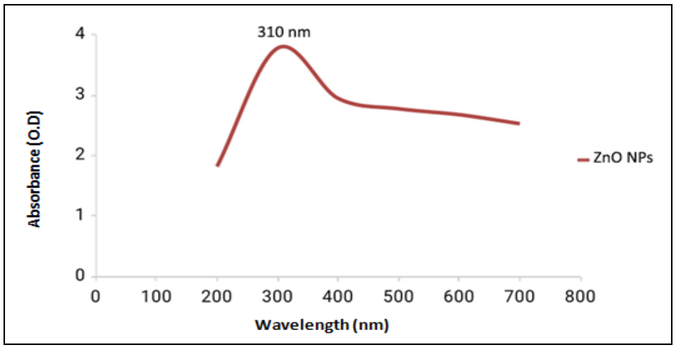


FIG. 3: UV SPECTROMETER

FIG. 4: ZnO NPs

The UV-Vis absorption spectrum of the plain ZnO nanoparticle is shown in the image. The acute absorption in the wavelength of 310 nm formulates the essential bandgap absorption of ZnO crystals due to the electron conversions from the valence band to the initiative band. The synthesis of ZnO NPs was achieved at 6 h with a color change from a light yellow color to a deep yellow color [29].

FT-IR: Fourier Transform Infrared Spectroscopy (FTIR) is an analytical technique used to identify organic, polymeric, and inorganic materials. The FTIR analysis method uses infrared light to scan the samples and observe the chemical groups present.

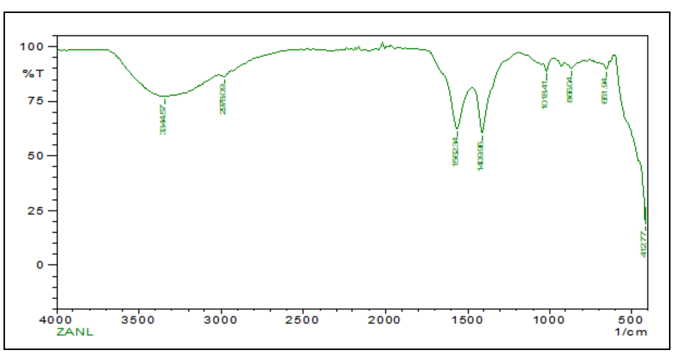


FIG. 5: FT-IR

The functional group of ZnO was determined by FT-IR spectroscopy 400-4000 cm^{-1}. The stretching chains at 502 and 432 cm^{-1} detected in the FTIR spectrum generally stand for the tetrahedral coordination of the Zn and O molecules in the ZnO compound. The peaks at 3344.57 cm^{-1} corresponds to the alcohol/phenol was assigned as stretching vibration of O-H group, 2978.09 cm^{-1} belongs to the group of carboxylic acid, 1562.34 cm^{-1} corresponds to the vibration and bending modes of C=C aromatic group, the peaks at 1409.96 and 1018.41 cm^{-1} were designate to the O-H bond of ethyl alcohol group, the peaks at 866.04 and 651.94 belongs to the bending modes of C-H aromatic group [30].

XRD: The synthesized ZnO nanoparticles were characterized by X-ray Diffraction analysis which results revealed a hexagonal wurtzite structure and the XRD spectrum of ZnO nanoparticles were 2 theta scales.

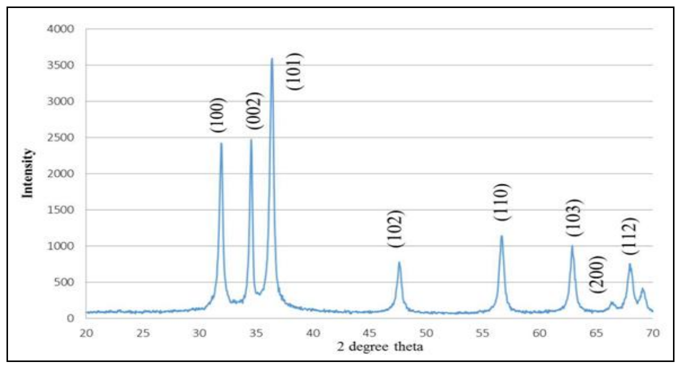


FIG. 6: XRD

XRD has a great possibility for the study of crystal lattice basis and fragment translucent size of the material. The emission peaks listed in the current study event well along with the standard ZnO hexagonal wurtzite system. The emission peaks were detected at 2 theta with the peaks correlate to (1 0 0), (0 0 2), (1 0 1), (1 0 2), (1 1 0), (1 0 3), (2 0 0) and (1 1 2) which is for Zinc oxide nanoparticle [31].

AFM: Atomic force microscopy (AFM) is a form of scanning probe microscopy which is designed to measure local properties, such as height, friction, magnetism, and surface area of nanoparticles. AFM analysis gives us insight into the topography, refinement of nanoparticles. AFM imaging conducted in different magnification ranges 1, 2, 5 and 25 µm. AFM image clearly demonstrates smooth nanoparticle with the capping of photochemical over the surface of nanoparticle [32].

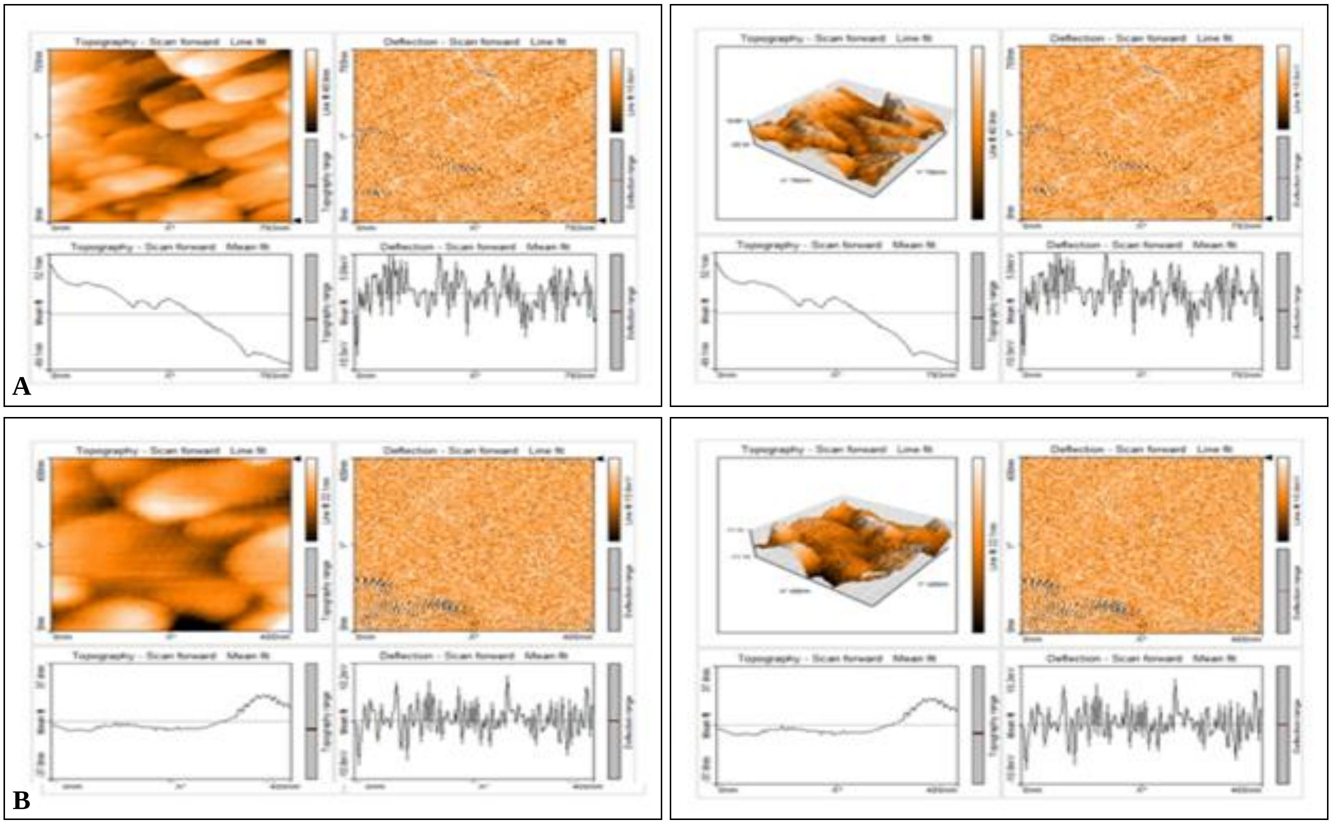


FIG. 7: AFM

SEM: A scanning electron microscope (SEM) is a type of electron microscope designed for directly studying the surfaces of solid objects that appropriate a beam of focused electrons of relatively low energy as an electron probe that is scanned in a regular manner over the specimen.

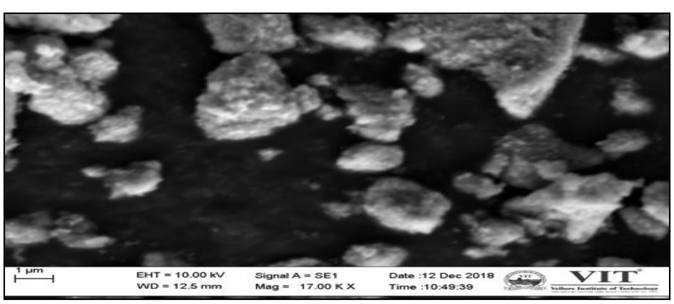


FIG. 8: SEM

SEM image shows the Zinc oxide nanoparticle detected range from 100-190 nm size appears as cubic structure possessed of absolutely a part of single small nanoparticles was detected range from 100-190 nm size. And it appears as cubic structure possessed of absolutely a part of single small nanoparticles [33].

EDAX: Analysis *via* Energy Dispersive X-ray (EDX) spectrometers confirmed the presence of elemental zinc and oxygen signals of the ZnO NPs.

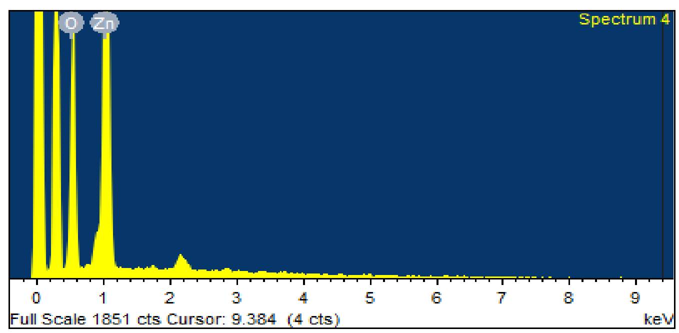


FIG. 9: EDAX

The vertical axis displays the number of x-ray counts while the horizontal axis displays energy in Kev [37]. The ZnO NPs have spherical in nature with the particle size approximately 75 to 80 nm which exhibited the presence of zinc and oxygen [38].

Zeta: Zeta potential analysis is done to determine the stability for the synthesis of zinc oxide nanoparticles. The obtained Mean value was -1.0 mV; the following picture shows the stability pattern.

The ZnO NPs values reveal information regarding the surface charge with the negative. The magnitude of the Zeta potential from -50 mV to +50 mV indicates the particle stability of the colloidal system [39].

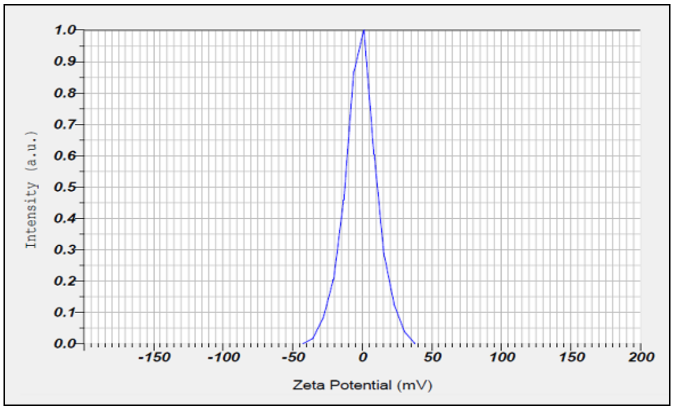
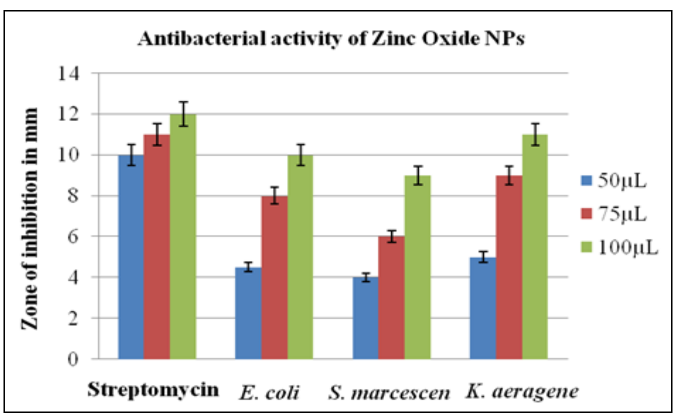


FIG. 10: ZETA

Antibacterial Activity by Well Diffusion: Microbes like bacteria have a miniature opening in the cell membrane. Reactive oxygen species (ROS) are developed from the ZnO NPs actively invades the cell membrane using pores of the cell [35].

The three different types of species namely *E. coli*, *Serratia marcescen* and *Klebsiella aerogene* were selected for the study and antibiotic used was streptomycin, which was the first effective drug developed for the treatment of tuberculosis and also it is the first aminoglycoside antibiotic which was isolated from the actinomycetes bacteria *Streptomycetes griseus* and several related soil microorganism [34]. The following graph describes the bactericidal effect against gram-negative bacteria which cause infection frequently in human beings. As the concentration gets increases the zone of inhibition also improved.



GRAPH 1: SHOWS THE ZONE OF INHIBITION FOR MICROBIAL PATHOGENS INCLUDING STANDARD DRUG STREPTOMYCIN

The above graph shows the maximum zone of inhibition against *Klebsiella aerogene* for 100 µL concentration there was 11.466 ± 3.143 mm formations, which is due to the rapid penetration of synthesized zinc oxide nanoparticle into the gram-negative bacterial cell wall, followed by *Escherichia coli* the zone of inhibition 10.166 ± 2.837 mm for 100 µL, which gets increased apparently when there is raise in concentration of zinc oxide nanoparticles. At the lower concentration of 50 µL for *Serratia marcescen* there was observed a slight fall down in zone of inhibition of 4.166 ± 2.750 mm in diameter when compared to *Klebsiella aerogene* and *Escherichia coli*. Therefore, the present finding clearly revealed that the prepared ZnO NPs using lemon peel extract could be applied as a coating against that enhances the effect of antibacterial activity in paints, cosmetics, food preservatives, flavoring agents, as well as for fabrics.

Antioxidant Activity: The antioxidant potential of synthesized ZnO NPs was estimated as described. The experiment was carried out using DPPH activity estimation. The deep violet color of DPPH turns yellow in the presence of an antioxidant compound. When DPPH is mixed with a hydrogen donor substance, free radicles are reduced and a color change occurs. DPPH free radical scavenging activity was calculated by the following formula DDPH radical scavenging assay was done [36].

% Inhibition = Absorbance of control - Absorbance of sample × 100 / Absorbance of control

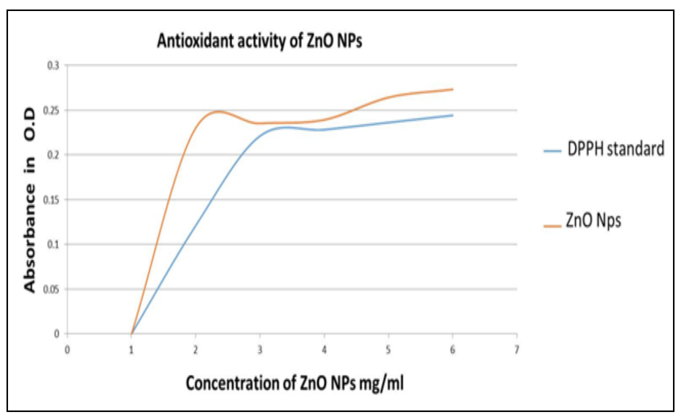


FIG: 11: ANTIOXIDANT ACTIVITY

Lemon has a natural pungent water-soluble antioxidant property. It contains vitamin C which helps to boost the body's immune system and attacks free radicals and toxins in our bodies. At the low concentration of 1 to 5 mg/mL, the absorbance OD at 590 nm the values fall from 0-0.3. The result was obtained in low concentration when compared to the high concentration of ZnO NPs.

CONCLUSION: Green approach for Zinc oxide nanoparticle synthesis was followed in this study. The synthesis method is faster, economical efficient through the source of reducing agent can be obtained from the waste also. Since this is multiple reaction steps conventional energy sources and harmful chemicals. This preparation of Zinc oxide NPs using fresh lemon peel is eco-friendly and can be an effective substitute for the large scale synthesis of ZnO NPs.

XRD studies show that the ZnO NPs synthesized has a good structure as well as characterized for the confirmation. Though, the synthesized ZnO NPs found to exhibit good antioxidant property and antibacterial activity, the future study focuses on drug coating as flavoring agents and its pharmacodynamics.

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