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GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING *PRUNUS DOMESTICA* (PLUM) FRUIT EXTRACT ITS CHARACTERIZATION, ANTIMICROBIAL AND ANTIOXIDANT STUDIES

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Keywords:

Zinc oxide nano particles, Phytochemical screening, Antimicrobial studies, Antioxidant activity, SEM studies

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ABSTRACT: Production of nanoparticles using plants is a new area of interest. Plum fruit extract was used for the biosynthesis of zinc oxide nanoparticles. Green synthesis of zinc oxide nanoparticles are considered as a eco-friendly methodusing materials from plants and other natural sources without using anyharmful chemicals. Plum fruit has many medicinal values such as antioxidant activity, cardiovascular activity, anti-inflammatory and antimicrobial effect. Present paper discuss about the green synthesis of zinc oxide nanoparticles using *Prunus domestica* (plum) fruit extract, Its characterization by analytical studies, antimicrobial and antioxidant studies. Plum fruit extract also subjected to phytochemical screening. The active constituents present in Plum were identified as alkaloids, glycosides, phenolics, flavonoids, proteins and carbohydrates. Nano particles were characterized by UV-Visible Spectroscopy, FT-IR and SEM studies. In the UV visible spectrometer absorption peak was observed at 362 nm, which is specific for zinc oxide nanoparticles. Synthesized zinc oxide nanoparticles were subjected to FT-IR analysis to detect the various characteristic functional group associated with the synthesized nanoparticles. The peaks indicate the characteristics functional group present in the zinc oxidenanoparticles. Synthesized zinc oxide nanoparticles were subjected to scanning electron microscopy (SEM) to determine the morphology and particle size of nanoparticles. It can be seen that all the particles were in spherical shape with particle size distribution from 77.5nm to 110 nm. The variation in size may be due to decreased amount of capping agents. The antimicrobial properties of the particles were determined using agar well diffusion and the disc diffusion method using *Bacillus subtills* and *E. coli* bacteria. The green synthesised ZnO NP showed antimicrobial activity against both *Bacillus subtills* and *E. coli* bacteria. The zinc oxide nanoparticles showed more activity against *E. coli* bacteria compared to *Bacillus subtills*. The antioxidant activity of nanoparticles was performed using Hydrogen peroxide scavenging and total phenolic content method, indicates the presence of antioxidant properties.

INTRODUCTION: Nanotechnology is a field of science and technology that deals with the manipulation and control of matter at the nano scale range (i.e., 1-100nm) of individual atoms and molecules. It involves understanding and utilizing the unique properties and behavior of materials and devices at this scale to create new structures, systems, and functionalities.

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Nanoparticles exhibit diverse chemical natures and can be metallic (silver, gold, copper, zinc, etc.), or comprised of metal oxide, silicates, polymers, organics, or carbon. In addition to their diverse chemical nature, nanoparticles can be produced in different morphologies such as spheres, cylinders,

sheets or tubes [1]. This amazing morphological and chemical diversity of nanoparticles results in shape dependent upon on the type of medium from which they are created as well as the number of bioactive compounds present in the medium. Zinc oxide nanoparticle is one of the inorganic compounds of group II–IV semiconductor appears to be white powder and insoluble in water. Zinc oxide (ZnO) NPs have low toxicity and are biodegradable. Zinc oxide nanoparticle is the second most abundant metal oxide after iron and it is inexpensive, safe, and as well as it can be prepared easily. Zinc oxide nanoparticle is naturally known as a strong resistance of microbes. Due to these reasons zinc oxide nanoparticle is extensively used for biological labelling, biological sensing, drug delivery, gene delivery, and nano medicine. Food and drug administration has approved zinc oxide as a safe material. Zinc oxide also can solubilize in an acidic environment therefore, this gives the material an opportunity to be discovered as multifunctional nanocarriers to ease the drug delivering and release processes [2]. Nanoparticles can be generated in various ways (physical, chemical, or biological routes), but generating nanoparticles through physical and chemical pathways results in toxicity issues as well as environmental concerns. The physical pathway (for example, the use of a tube furnace) requires a large amount of space and generates a large amount of heat, raising the environmental temperature around the source material, in addition to being very time consuming. The major drawback to the chemical method of nanoparticle generation is its use of toxic solvents and chemicals, which could cause a great deal of harm to environment. Therefore, the need for a different alternative in nanoparticle generation was felt across the globe, which led to the development of the green nanotechnology (or green nano biotechnology) concept. Green nanotechnology is simple, cost-effective, and environment friendly, and has gained a lot of importance in the recent past [3]. A large number of nanoparticles generated with green nanotechnology have successfully been used in various applications.

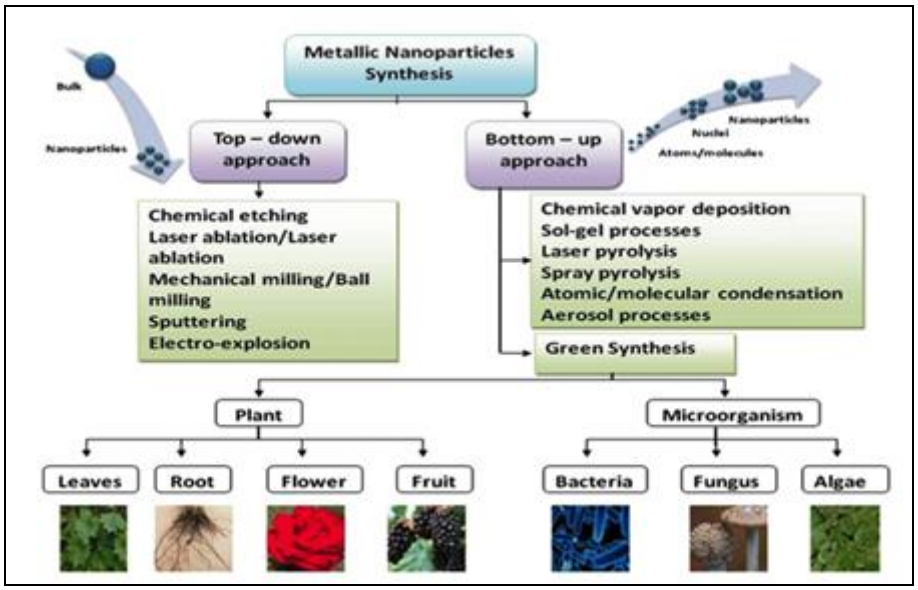

FIG. 1: APPROACHES FOR SYNTHESIS OF NANOPARTICLES

The major advantages of using plant extracts for nanoparticle synthesis is that they are easily available, safe, nontoxic in most cases, have a broad variety of metabolites thatcan aid in the reduction of metal ions, and are quicker in synthesis than microbes [5]. Because of photochemical, plant-assisted reduction is the main mechanism considered for this process. The relatively high levels of the steroids, saponins, carbohydrates, and flavonoids act as reducing agents and phytoconstituents as the capping agents, which provide stability to the nanoparticles. Owing to its nutritional and phytochemical activity, Plum fruit was selected for the green synthesis of ZnO NPs and therefore this study was conducted to characterize *Prunus domestica* fruit extract assisted synthesized ZnO NPs and investigated their antibacterial, antioxidant effect of the synthesized

ZnO NPs. Green synthesis of nano particles were reported for plants and fruit but till date there is no report on the synthesis of ZnO NPs using *Prunus domestica* fruit extract.

MATERIALS & METHODS:

Preparation of the Fruit Extract: The fresh fruits of Plum (*Prunus domestica*) were collected from local market and carefully cleaned with distilled water. They were then chopped into small pieces and removed seeds. The sliced fruits were blended finely using blender with sterile distilled water to obtain fruit broth. The resulting extract was passed through a muslin cloth and then filter by Whatman No. 1 filters paper. The filtered extract was kept at 4◦C until use.

Preperation of Zinc Acetate Dihydrate Solution: Zinc acetate dihydrate(0.5M) solution were prepared by dissolving 4.07g zincacetate dihydrate in 100ml distilled water.

Biosynthesis of ZnO Nanoparticles: The prepared zinc acetate dihydrate solution (100ml) is mixed with the extract of plum fruit (100ml) at the ratio 1:1in a flask. The flask is kept for shaking at 120rpm at room temperature for 2-3 days. The formation of ZnO nanoparticles was identified by the formation of colloidal solution which appear in pale pink colour [6]. Then the solution is filtered using muslin cloth. The filtered particles were washed with distilled water and then dried. They were collected for further studies.

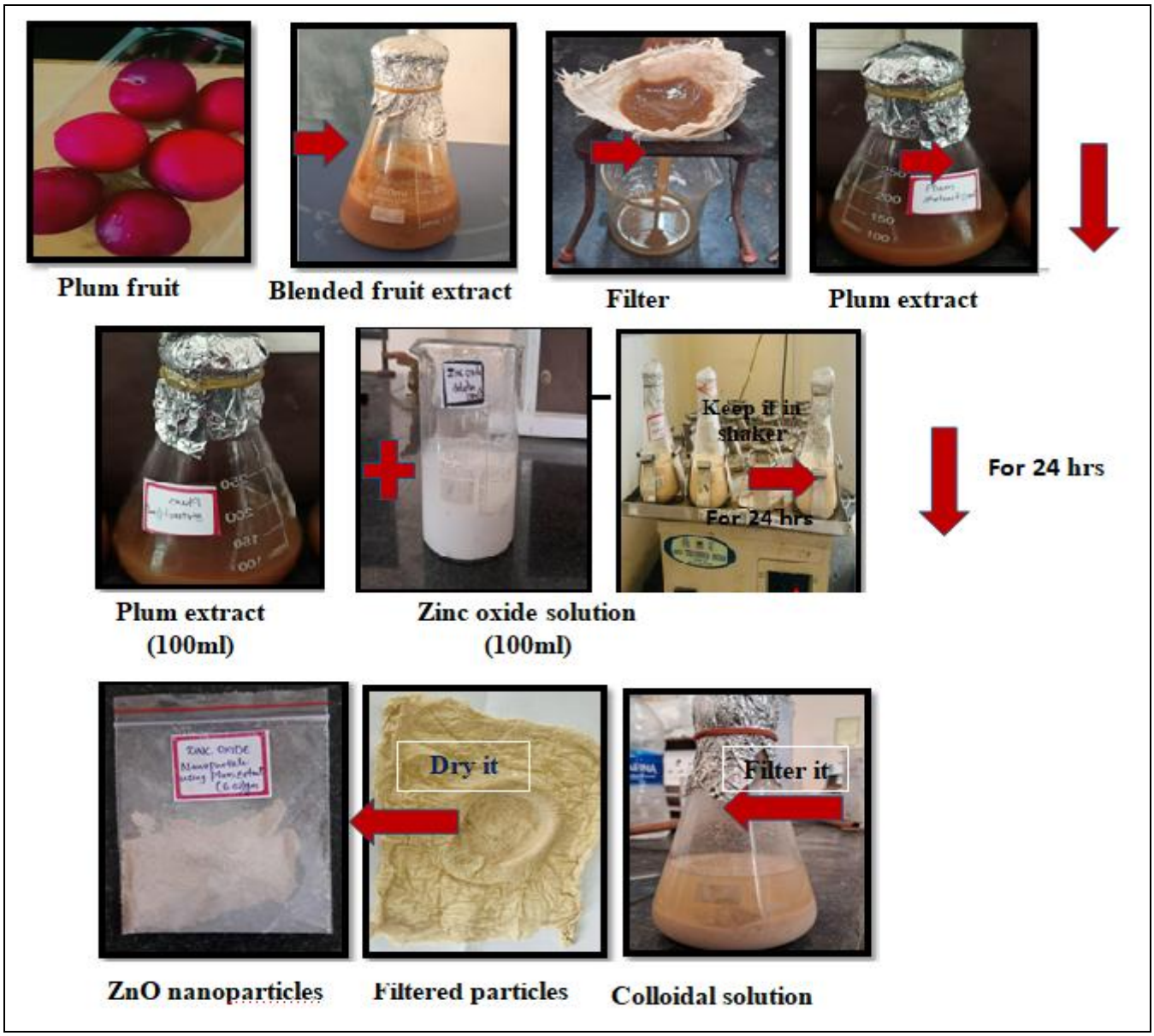

FIG. 2: SCHEMATIC REPRESENTATION OF SYNTHESIS OF ZnO NANOPARTICLES USING PLUM FRUIT EXTRACT

Phytochemical Screening: Phytochemical screening for major constituents is carried out using qualitative methods [7]. Screening tests are performed for flavonoids, glycosides, triterpenoid's, alkaloids, phenols, steroids, proteins.

TABLE 1: PROCEDURE FOR PHYTOCHEMICAL SCREENING

S. no.	Name Of The Test	Procedure	Observation
1.	Test for alkaloids	Sample +Mayers reagent	
a)	Mayer's test	Sample+ Wagner's reagent	Cream ppt/white ppt
b)	Wagner's test	Sample+ Dragandroff reagent	Reddish brown ppt
c)	Dragandroff test	Sample+ Hager's reagent	Orange brown ppt
d)	Hager's test		Yellow colour ppt
2.	Test for Glycosides:	Sample+GAA+$FeCl_3$+ conc.H_2SO_4	Reddish-brown colour
a)	Killer-killani test	Sample+1ml Pyridine+1ml Sodium	appears at junction.
b)	Legal's test	nitroprusside.	Pink or red colour.
c)	Baljeet's test	Sample+ Sodium picrate	Yellow colour
3.	Test for Phenolics:	Sample+5%$FeCl_3$	Bluish Black colour
a)	Ferric chloride test	Sample+ lead acetate	White ppt
b)	Lead acetate test		
4.	Test for Flavonoids:	Powder sample + 5ml ethanol +Mg	Orange, pink , red or purple
a)	Shinoda test	turnings	
5.	Test for proteins:	Sample+ Biuret reagent	Violet or pale pink colour
a)	Biuret test	Sample+ Millon's reagent	White ppt , warm ppt turns to
b)	Millon's test		brick red colour
6.	Test for carbohydrates:	Sample+ Fehling's A and B+ Heat	Red colour ppt
a)	Fehling's test:		

Characterization of ZnO NP: The ZnO NPs initially analyzed by using the Lab India-3660 UV-Visible spectroscopy within the range 200–800 nm. Then FTIR was used to identify the functional groups and various phytochemical constituents involved in the reduction and stabilization of the synthesized nanoparticles [89].

FTIR was carried out using the attenuated total reflectance (ATR) mode with a Jasco FTIR 4100 spectrophotometer (Japan). The results recorded in the range of 4000–400 cm^{-1}. The particle morphology was examined by utilizing a scanning electron microscope (Hitachi, H-7600) that operates under high vacuum and has magnifications ranging from 20x to about 30,000x, as well as spatial resolutions of 50 to 100 nm. Greater magnification of ZnO NPs was attained by decreasing the raster width of the sample, and vice versa, for the fixed sizes of the ZnO NPs.

Antimicrobial Activity: The antimicrobial activity of green synthesized ZnO NPs, *Prunus domestica* fruit extract extract was tested against Gram positive bacteria (*Bacillus Subtilis* (ATCC 6633), Gram negative bacteria (*Escherichia coli* (ATCC 8739), Antimicrobial activity was determined by using the agar well diffusion method and Disc diffusion method [10].

Agar well Diffusion Method: Holes with a diameter of 8 mm were bored in the agar plates. 1ml of three different concentrations of test sample (ZnO NP's) and standard antibiotic solution (Azithromycin) were poured into the wells of respective petri plates under strict aseptic conditions [11].

All the plates are incubated at 37 °C overnight to check the bacterial growth inhibition. Microbial growth is determined by measuring the diameter of the zone of inhibition.

Disc Diffusion Method: Filter paper discs, approximately 6 mm in diameter, were prepared. These discs contained the test compound and a standard antibiotic solution (Azithromycin) at three different concentrations. The prepared discs were placed on the agar surface in their respective petri plates [12].

The petri dishes were then incubated under suitable conditions. The antimicrobial agents present on the discs diffuse into the agar, inhibiting the germination and growth of the test microorganisms. The diameters of the zones of inhibition, where bacterial growth is inhibited, were measured [13, 14].

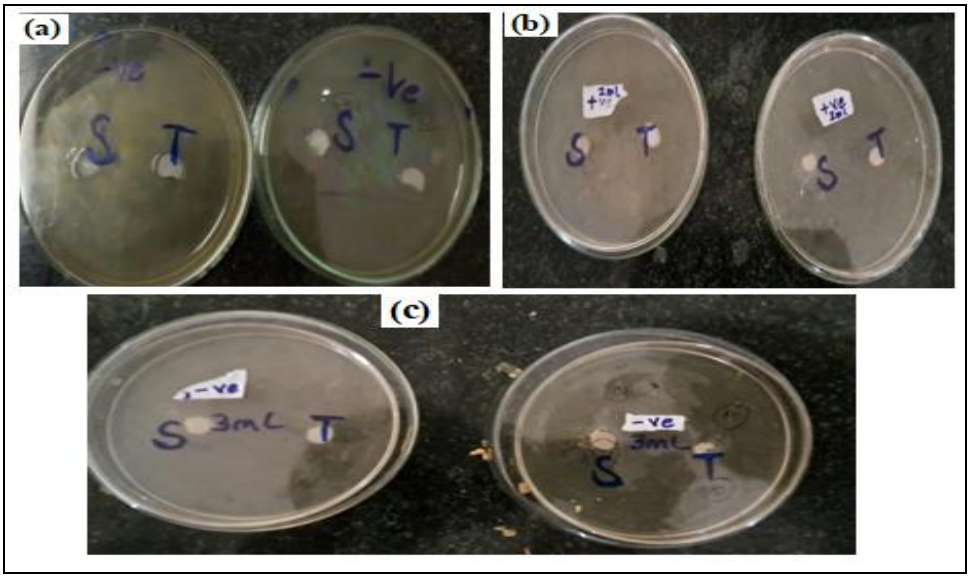

FIG. 3: AGAR WELL DIFFUSED PLATES CONTAINING BOTH TEST SOLUTION (ZnO NPS) AND STANDARD (AZITHROMYCIN

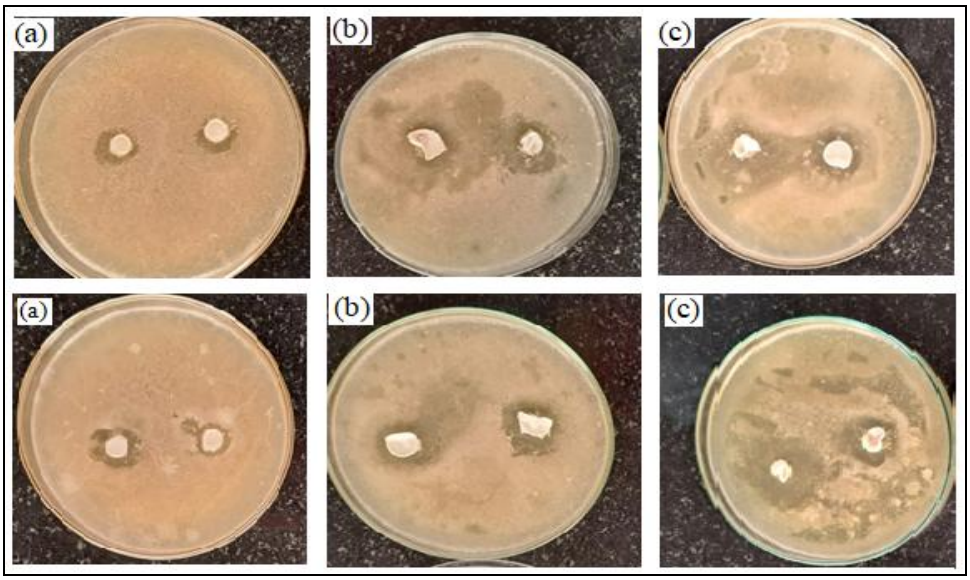

FIG. 4: DISC WELL DIFFUSED PLATES CONTAINING BOTH TEST SOLUTION (ZnO NPS) AND STANDARD (AZITHROMYCIN)

Antioxidant Activity: Antioxidant activity of green synthesized ZnO NPs were determined using Hydrogen peroxide scavenger activity and Phenolic content method [15]. Hydrogen peroxide scavenger activity, In this method, the antioxidant activity of sample solution (ZnO nanoparticles) is compared with standard (Ascorbic acid) the absorbance was recorded using UV-visible spectroscopy at 230nm against the blank (phosphate buffer).

FIG. 5: (A) ASCORBIC ACID (B) SAMPLE AT DIFFERENT CONCENTRATIONS

Total Phenolic Content Method: The total phenolic content was determined using the spectroscopic method and the absorbance was recorded at 760 nm using Gallic acid as standard [16]. The TPC was expressed as Ascorbic acid equivalent (mg AAE) per gram of the dried sample.

RESULTS AND DISCUSSION:

UV-Visible Spectroscopy: The presence of secondary metabolites in plants reduces zinc ions in the solution to zinc oxide [17]. The plant extract not only acts as reducing agents but as stabilizing agents as well. This was confirmed by taking the UV–visible spectrum analysis in the range of 280 nm–800 nm. The spectrum showed a peak at 320 nm, which is specific for ZnO nanoparticles [18]. For ZnO nanoparticles, the absorbance peak is reported between 310 nm and 360nm of wavelength. A UV spectrum of ZnO nanoparticles was recorded by scanning between 250-800nm. From the spectrum, ZnO nanoparticles showed maximum absorbance at 362 nm.

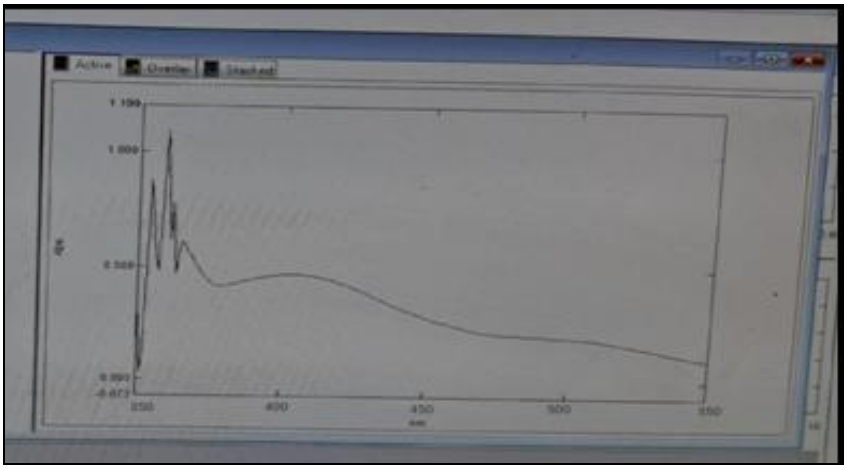

FIG. 6: UV ABSORPTION SPECTROSCOPY OF ZINC OXIDE NANOPARTICLE

Fourier Transform Infrared Spectroscopy (FTIR): The infrared absorption spectrum produced by this characterization technique reveals the chemical bonds in the synthesized nanoparticles [19]. For this, FT-IR Spectrophotometer-Thermo Fisher-Scientific Nicolet iS50 was used for the analysis. Synthesized zinc oxide nanoparticles were subjected to FT-IR analysis to detect the various characteristic functional group associated with the synthesized nanoparticles. In **Fig. 7** the peaks indicate the characteristics functional group present in the synthesized zinc oxide nanoparticles. The absorption peak observed at 576.51 cm^{-1} indicates metal-oxygen (ZnO stretching vibrations).The absorption peak observed from 869.03 cm-1 to 1600 cm-1indicates aldehyde functional groups (C-H stretching vibrations).

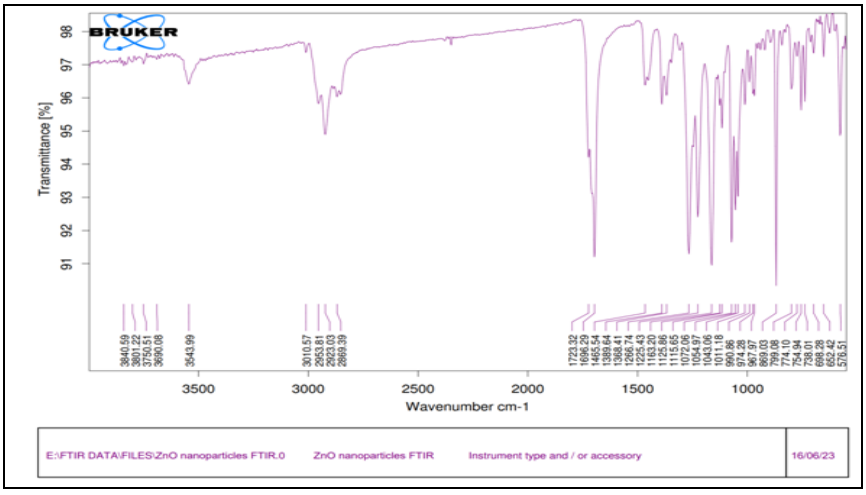

FIG. 7: FTIR SPECTRUM OF ZINC OXIDE NANOPARTICLE

Peak at 1723.3 cm-1indicates ester phospholipids (C=O stretching band). Peak at 3543.99 cm-1indicates stretching vibrations of Phenolic (O-H) groups. The absorption peaks at 869.03 cm^{-1}, 1723.3 cm^{-1}, 3543.99 cm^{-1} may be due to the active constituents present in the plum fruit.

SEM Analysis: SEM images were taken in different magnifications to examine the shape and size of the nanoparticles synthesized, as shown in **Fig. 8.** The surface morphology confirms the formation of nanoparticles in their agglomerated form. Various literature reports the effect of surface morphology and its relationship in the synergistic activity of ZnO [20]. From **Fig. 8** it can be seen that all the particles were in spherical shape with particle size distribution from 77.5nm to 110nm. The variation in size may be due to decreased amount of capping agents.

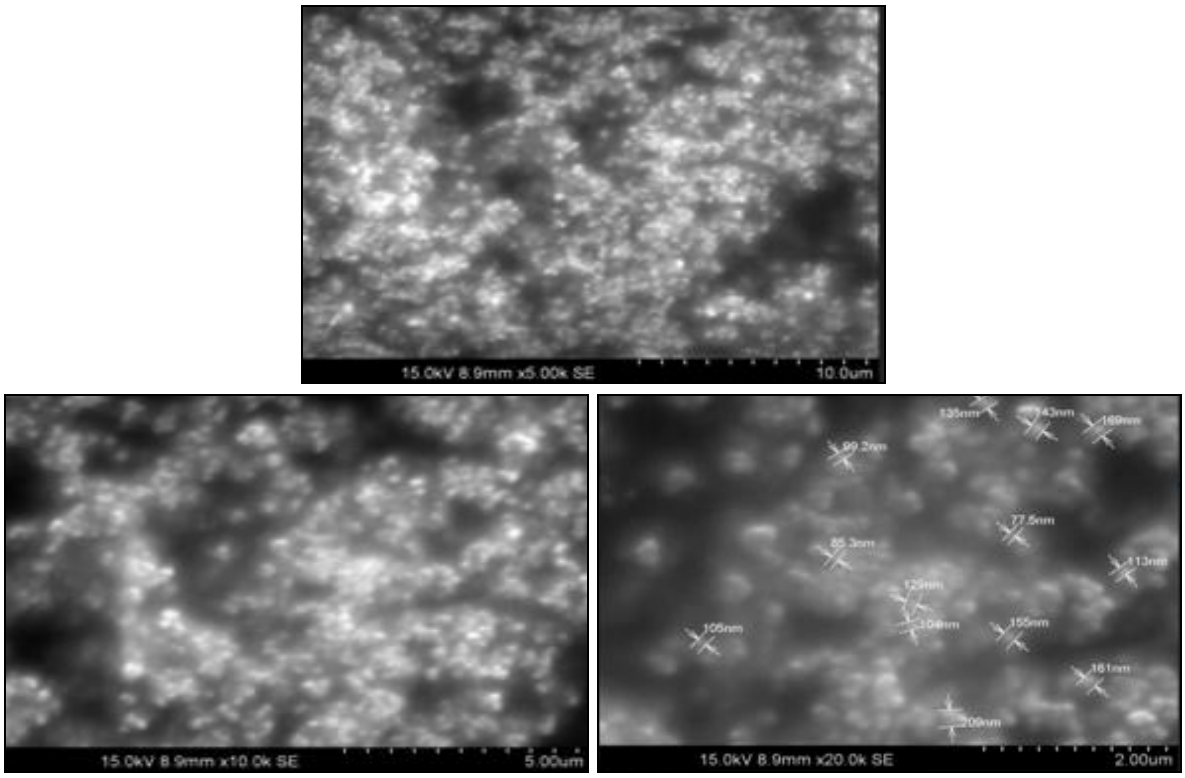

FIG. 8: RESULTS OBTAINED BY THE SEM

Anti Bacterial Activity: Antibacterial activity of Zinc oxide nanoparticles was performed using two methods (i.e., Agar well diffusion method and Disc diffusion method.

Agar well Diffusion Method: In this method, the antibacterial activity of Zinc oxide nanoparticles was performed against two bacterial cultures (i.e., *Bacillus subtills* species and *E. coli* bacteria) using Azithromycin as standard [21]. The green synthesised ZnO nanoparticles showed antimicrobial activity against both *Bacillus subtills* and *E. coli* bacteria. But nanoparticles shows more antibacterial activity against *E. coli* bacteria compared to *Bacillus subtills*.

TABLE 2: ZONE OF INHIBITION AGAINST *BACILLUS SUBTILIS*

S. no.	Conc. (µg/ml)	Sample (ZnO NPs)	Standard (Azithromycin)
1.	1(µg/ml)	1.1 cm	1.3cm
2.	2 (µg/ml)	2cm	2.4cm
3.	3(µg/ml)	3.1cm	3.4cm

TABLE 3: ZONE OF INHIBITION AGAINST *E. COLI*

S. no.	Conc. (µg/ml)	Sample (ZnO NPs)	Standard (Azithromycin)
1.	1(µg/ml)	1.5cm	1.8cm
2.	2 (µg/ml)	2.5cm	2.4cm
3.	3(µg/ml)	3.4cm	3.25 cm

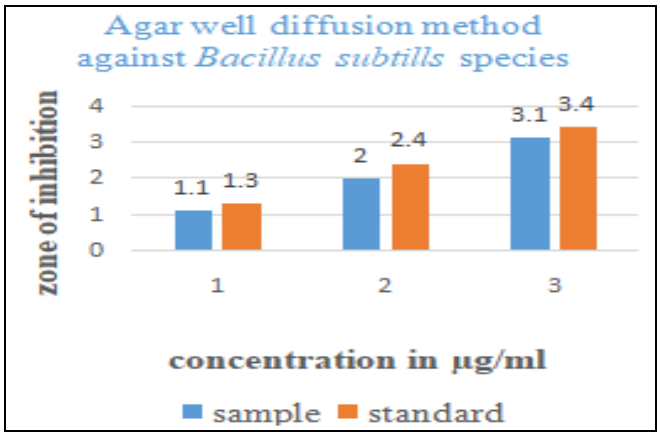

FIG. 9: ZONE OF INHIBITION VS CONC. µg/ml AGAINST *BACILLUS SUBTILLS*

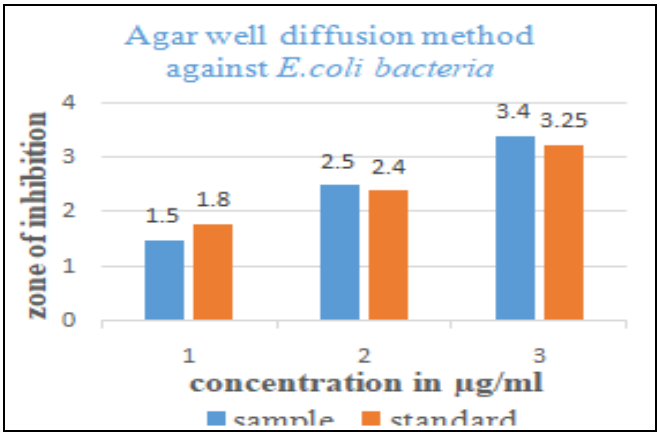

FIG. 10: ZONE OF INHIBITION VS CONC. µg/ml AGAINST *E. COLI*

Disc Diffusion Method: In this method, the antibacterial activity of Zinc oxide nanoparticles was performed against two bacterial cultures (i.e., *Bacillus subtills* species and *E. coli* bacteria) using Azithromycin as standard. The green synthesized ZnO nanoparticles showed antimicrobial activity against both *Bacillus subtills* and *E. coli*. But nanoparticles showed more antibacterial activity against *E. coli* bacteria compared to *Bacillus subtills*.

TABLE 4: ZONE OF INHIBITION AGAINST *BACILLUS SUBTILLS* SPECIES

S. no.	Conc. (µg/ml)	Sample (ZnONPs)	Standard (Azithromycin)
1.	1(µg/ml)	1.3cm	1.8cm
2.	2 (µg/ml)	1.8cm	2.5cm
3.	3(µg/ml)	2.9cm	2.5 cm

TABLE 5: ZONE OF INHIBITION AGAINST *E. COLI* BACTERIA

S. no.	Conc. (µg/ml)	Sample (ZnONPs)	Standard (Azithromycin)
1.	1(µg/ml)	1.5cm	1.3cm
2.	2 (µg/ml)	2.2cm	2cm
3.	3(µg/ml)	2.7cm	2.4cm

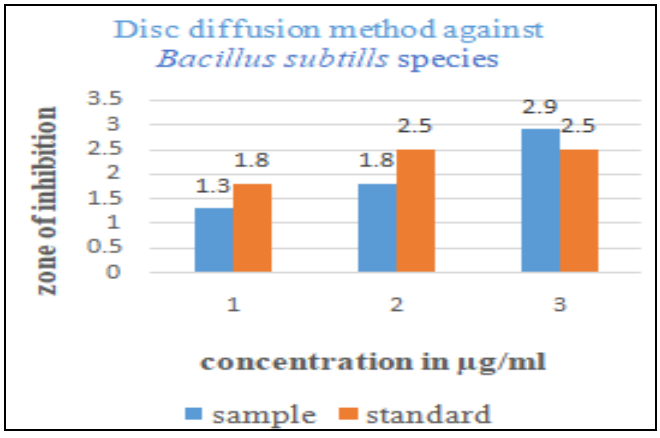

FIG. 11: ZONE OF INHIBITION AGAINST *BACILLUS SUBTILLS* SPECIES

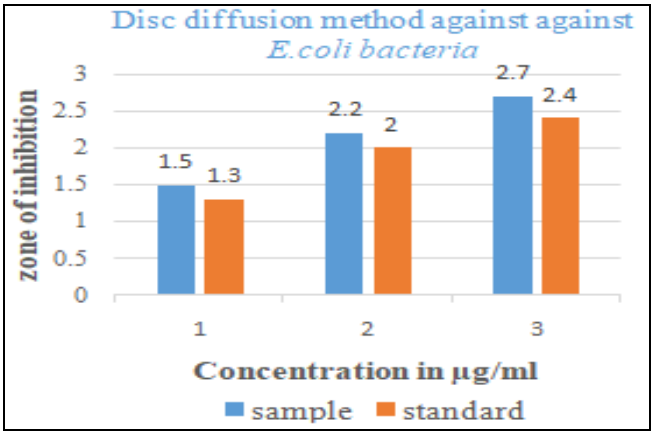

FIG. 12: ZONE OF INHIBITION AGAINST *E. COLI* BACTERIA

Antioxidant Studies: Antioxidant activity of zinc oxide nanoparticles can be determined by using two methods 1. Hydrogen peroxide scavenger activity [22]. In this method, the antioxidant activity of sample solution (ZnO nanoparticles) is compared with standard (Ascorbic acid) the absorbance was recorded using UV-visible spectroscopy at 230nm against the blank (phosphate buffer). ZnO nanoparticles conc. 50, 100, 200, 300, 400 µg/ml has shown % inhibition or scavenging activity is 26.22%,36.24%,46.26, 56.23%, 69.32%. Ascorbic acid at conc. 50, 100, 200, 300, 400µg/ml has shown the %inhibition or

scavenging activity is 18.89%, 27.63%, 41.35%, 52.65%, 63.25%.

Total Phenolic Content Method: The total phenolic content was determined using the spectroscopic method and the absorbance was recorded at 760 nm using Gallic acid as standard. The TPC was expressed as Ascorbic acid equivalent (mg AAE) per gram of the dried sample [23]. The total phenolic content exhibited by Zinc Oxide Nano particles at different concentrations is 8.5, 11. 5, 13.8.

CONCLUSION: Zinc oxide nanoparticles were synthesized using plum fruit, which is an eco-friendly process. The active constituents present in plum fruit were identified using Phytochemical screening. The active constituents are alkaloids, glycosides, phenolics, flavonoids, proteins and carbohydrates. The characterization of ZnO nanoparticles were carried using analytical studies. ZnO nanoparticles showed maximum UV absorbance at 362 nm. FTIR Absorption peak observed at 576.51 cm^{-1} indicates metal-oxygen (ZnO stretching vibrations). All the particles were in spherical shape with particle size distribution from 77.5nm to 209nm. Analysed using SEM. Antibacterial activity of zinc oxide nanoparticles was performed using two methods (i.e., Agar well diffusion method and Disc diffusion method). The green synthesized ZnO nanoparticles showed antimicrobial activity against both *Bacillus subtills* species and *E.coli* bacteria. But nanoparticles show more antibacterial activity against *E. coli* bacteria, compared to *Bacillus subtills* species. Antioxidant activity of Zinc oxide nanoparticles can be determined by using Hydrogen peroxide scavenger activity and Total Phenolic content method, the results indicated presence of antioxidant activity. Overall, the green synthesized Zinc oxide nanoparticles using plum fruit extract shows both antioxidant and antimicrobial properties. The further studies can be carried out in medical field to treat many free radical disease and anti-bacterial disease in animals as well as in human beings.

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CONFLICTS OF INTEREST: Declared None

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