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ORIGINAL ARTICLE

In-vitro Antibacterial and Antioxidant activities of zinc oxide nanoparticles synthesized using *Prunus domestica* L.(Plum) agro-waste (peel) extracts

[1]*Noushin Ajmal, [1]Keerti Saraswat
[1]Department of Basic Sciences and Humanities, Pratap University, Jaipur, Rajasthan
Email: noush.biochem04@gmail.com

ABSTRACT
Biocompatible nanomaterials such as zinc oxide nanoparticles gained attention in the diagnosis and therapeutic as nanomedicine . In present study, the agro-waste(peel) of Prunus domestica L. (Plum) methanolic extract have been utilized for the synthesis of zinc oxide nanoparticles (ZnO NPs). The biosynthesized ZnO NPs were confirmed initially through a visual color change from light yellow to deep yellow, which was further elucidated by FTIR, XRD and SEM analysis. The stability of ZnO NPs was due to capping and stabilizing agent present as polyphenols, carboxyl and amino functional group as depicted by Fourier transform infra red spectroscopy (FTIR) experiment. Scanning electron microscopy (SEM) revealed morphology and size of the ZnO NPs, which was appeared as round shape with 20nm. This was again proved by XRD peak value as 2. Furthermore, biosynthesized ZnO NPs have been evaluated for antibacterial and antioxidant activities. Antibacterial activity as zone of inhibition was found more against gram negative strain, which proved that ZnO NPs damage the cell membrane of gram negative bacteria than gram positive one. Again ZnO NPs showed dose dependent antioxidant activities against four methods (DPPH, H_2O_2 scavenging, NO scavenging and reducing assay). Almost all utilized methods, a moderate antioxidant activity was found at higher concentration of zinc oxide nanoparticles. In conclusion, agro-waste of Prunus domestica L. (Plum) extract is good source of capping and stabilizing agents for the production of metal nanparticles which might be used as potential therapeutic agents as antibacterial and antioxidants.

***Keywords*:** Agro-waste, *Prunus domestica* L, ZnO NPs, antibacterial, antioxidant

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INTRODUCTION

Nanomaterials, characteristically having dimension between 1-100nm are the most widely application based research going on around the globe[1,2]. In recent past, nanomaterails particularly metal nanoparticles studied with great zeal owing to their unique features such as catalytic, optical, magnetic and electrical properties [3,4]. Metal oxides particularly zinc oxide is a important material used in nano range in environmental science, electrochemistry, chemical sensors and biology [5]. These metal oxides are claimed to be safe for human and animals [6]. Recently ZnO nanoparticles gaining demands due to its high thermal stability and flexibility to form varieties nanostructures [7]. ZnO nanoparticles have potential advantage as catalytic reaction process due to its large exposed surface area with relatively high catalytic activity [8].

Zinic oxide nanoparticles exhibit different medical and biomedical applications. Due to its unique feature of intrinsic florescence properties widely used in imaging of human bodies *in-vitro* and *in vivo* [9]. There are several work has been reported regarding the use of various morphology of ZnO nanoparticles as antibacterial [10-12] and antioxidant agents[13,14].

Usually, ZnO nanoparticles are prepared by some important physical and chemical methods such as lithographic, photochemical reduction, irradiation, laser ablation and electrochemical [13]. Unfortunately these methods are very toxic issues to the environment. Therefore, there is growing interest of

researchers working on nanoscience towards the application of green alternatives for the targeted metal nanoparticles [15].

Plums are the taxonomically diverse among stone fruits under the family of Rosaceae. *Prunus domestica* L. are the most grown species worldwide [16]. Fruits of plum is a rich source of antioxidant compounds, such as phenolic acids, anthocyanins, flavanoids and carbohydrates [17,18]. Plumbs contains phenolic compound such as neochlorogenic, chlorogenic acid, crypto chlorogenic acid, as caffeic acid derivative with few amounts of flavanols and flavonols [19].Various literature confirmed the high antioxidant potential of plumb fruits is due to presence of chlorogenic acid [18-20]. Total phenol and phenolic components has been found great variation in plum flesh and peel. There was a significantly higher amount of phenol peel as compared to flesh in plum fruits. [20].

Present research is devoted to synthesize zinc nanoparticle from the extract of Plum fruit peel as agro-waste material to fulfill the requirements of green chemistry approach. Further, the synthesized nanoparticle were characterized by Scanning electron microscopy(SEM), X-ray diffraction study(XRD), and FT-IR for morphological attributes to confirm the structure. Finally, zinc nanoparticle (ZnP) was evaluated for antibacterial and antioxidant adopting standard protocol.

MATERIALS AND METHODS

Chemicals and necessary reagents used in these experiments were procured from sigma Aldrich (USA) with no extra purification employed. I R spectra of plum peel extract and zinc nanoparticles were identified by KBr discs using FT/IR - 4100 JASKO model in the ratio of 1:100. X-ray diffraction (XRD) study was performed on a Rigaku Ultima IV X-ray Diffractometer between angles 2^0 and 80^0, with a scan rate of 2^0/min. Scanning electron microscopy was done using Carl Zeiss EVO LS10 (Oberkochen, Germany).

Preparation of Plum (Prunus domestica L.) peel extract

Zinc nanoparticles were synthesized using Plum peel extract which performed as reducing and capping agent. Plum fruits were purchased from local market. Peel was separated from flesh and washed with water many times till the removal of dust particles. Peel was then spread in full sunlight to remove any moisture residue. It was further cut into pieces and grounded to a small size using mixer grinder. Small size peel powder was then passing through a mesh of fixed size. About, 50g of peel powder was taken in a 250mL glass beaker containing 200 mL methanol. The solution was boiled around 1hr so that it color change from colorless to light yellow. The methanolic extract was cooled to room temperature and filter using whatmann filter paper. The extract was then stored in a refrigerator for the synthesis of zinc oxide nanoparticles (ZnO NPs).

Synthesis of Zinc oxide nanoparticles

To the synthesis of zinc oxide nanoparticle 50 mL of Plum peel extract was boiled for 70-80^{0}C using heating stirrer. To the extract solution 5g of zinc nitrate was added as the boiling progress to 70^{0}C. The mixture continuously boiled until it reduced to deep yellow color paste. Obtained paste was placed in a ceramic crucible and heated in furnace around 400-500^{0}C for 4 hrs. As a result a light yellow colored powder was obtained and mashed in a pestle-mortar to receive a fine powder, which was collected and packed for necessary characterization.

Evaluation of antibacterial activity of Plum (Prunus domestica L.) peel extract mediated Zinc nanoparticles

The antibacterial activity of the synthesized plum (*Prunus domestica* L.) peel -extract-nanoparticles was studied by disc diffusion method as reported previously [21]. The antimicrobial activity was evaluated against some selected pathogenic bacterial strains such as *E.coli* (NCIM 2079), *S.aureus* (NCIM 2079), *P.aeruginosa* (ATCC 10145) and *Bacillus Substilis* (NCIM2439). The sterile discs (6 mm) were kept at core of plate and different concentrations of zinc nanoparticle (12.5, 25, 50 and 100 µg/mL) were added on disc. Gentamycin disc was used as reference standard (50µg/mL) and Plum peel extracts were used as control. The plate was incubated in the upright position at 37 °C for 24 h. After incubation, the antimicrobial evaluation of nanoparticles was estimated as zone of inhibition in mm of disc.

Evaluation of in vitro antioxidant activity of Plum (Prunus domestica L.) peel extract mediated Zinc nanoparticles

Antioxidant activity of the peel extract mediated nanoparticles was determined by DPPH, hydrogen peroxide and nitric oxide radical scavenging and reducing power assays.

DPPH free radical scavenging assay

The free radical scavenging ability of the *Prunus domestica* L. peel extract mediated nanoparticles against 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical was evaluated as per literature [22]. The peel extract mediated zinc nanoparticles at different concentrations (10, 20, 30, 40, 50, 75 and 100 µg/mL)

and standard butylated hydroxytoluene (BHT) was added to test tubes labeled accordingly. To the above test tubes, 1 mL methanolic solution of DPPH (1 mM) was added and thoroughly mixed. All the test tubes were incubated in dark place for 30 min. The absorbance of resultant solution was measured at 517 nm. The DPPH solution without sample or standard was used as blank. The free radical scavenging activity was calculated by following equation and results expressed as % inhibition.

% free radical scavenging = (AC-AS)/AC x 100

Where,

AC- absorbance of blank, i.e, absorbance of methanolic DPPH radical

AS- absorbance of sample + methanolic DPPH radical

Hydrogen peroxide scavenging assay

The H_2O_2 scavenging potential of zinc nanoparticles was studied as described earlier [22]. Various concentrations of zinc nanoparticles and ascorbic acid (reference standard) were taken in test tubes and mixed with 50µL of 5 mM H_2O_2 solution. Blank consisted of H_2O_2 solution devoid of any sample. The mixture was incubated for 20 min at room temperature. The absorbance was measured spectrophotometrically at 610 nm. The percentage of H_2O_2 scavenging was calculated using control (blank) and sample (nanoparticle treated) absorbance.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was estimated by the use of Griess reaction [23]. In the present study, 10 mM solution of $NaNO_2$ prusside buffered in phosphate saline was poured with various concentrations of ZnO NPs and incubated at 30 °C for 2 h. Control was also prepared devoid of any treated samples. On completion of incubtion, 0.5 ml of Griess reagent was transferred to each tube. The absorbance of the resulting chromophore formed during diazotization reaction was measured at 550 nm. The antioxidant ascorbic acid was used as reference standard.

Reducing power assay

The antioxidant activity of zinc nanoparticles can be measured by using reducing power assay. In this assay, antioxidant compounds and related samples convert the oxidation form of iron (Fe^{+3}) in ferric chloride to ferrous (Fe^{+2}). The reducing power was determined by reported method [21]. Different concentrations of zinc nanoparticles were mixed with 2.5 mL of phosphate buffer (200 mM, pH 6.6), and to this 2.5 mL of 1 % potassium ferricyanide was added. The resulting mixture was heated at 50 °C for 20 min and then cooled rapidly. Then, the mixture was precipitated with the addition of 2.5 mL of 10 % TCA solution and centrifuged at 3000 rpm for 8 min. The supernatant was collected and diluted (1:1) with equal volume of Milli-Q water. To this, 1 mL of ferric chloride solution (0.1 %) was added and the absorbance was measured spectrophotometrically at 700 nm. A sample without nanoparticles served as control. BHT was used as standard.

RESULTS AND DISCUSSION

Characterization of biosynthesized zinc oxide nanoparticles

X-ray diffraction (XRD) analysis

Prepared powder nanoparticle was used by x-ray diifractometer to confirm and analyze the ZnO nanoparticle. The x-ray diffraction pattern of the synthesized ZnO nanoparticles from extract of plum peel was demonstrated by major peaks corresponding to 2θ values as 31.5^0, 34.7^0, and 36.5^0 as shown in Fig 1.

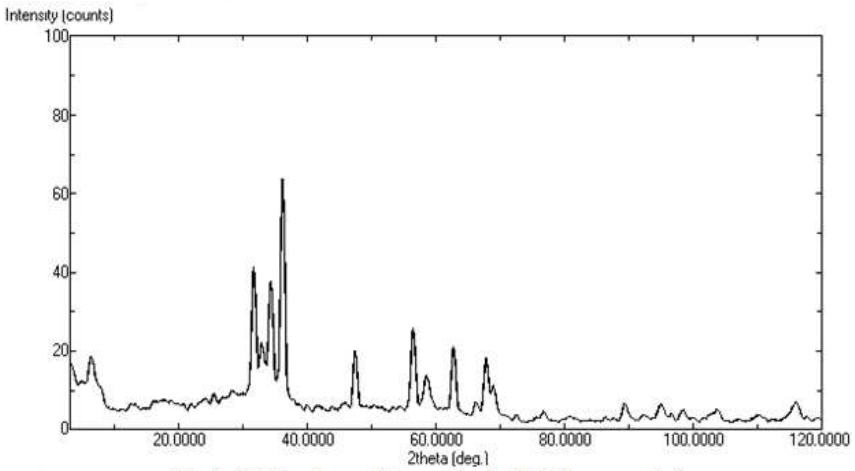

Fig 1. XRD pattern of biosynthesized ZnO nanoparticles

These peaks are in line with the literature report of zinc oxide nanoparticle(JCPDS file no. 5-0566). Further, peaks also seems to be very close to the reported values of zinc oxide nanoparticles with average size of 20nm as calculated by Debye-Scherrer's equation.

FT-IR Analysis

FT-IR spectral analysis is the measurement of IR radiations absorbed by a treated sample ploted against the wavelength. As result of IR vibrational bond in the chemical compounds present in the sample are vibrated which is interpretated as IR-spectrum. In such a way, the biomolecules present in the plants (plum peel in this case) which are solely responsible for the reduction and stabilization processes involves to the conversion of metal to metal Nanoparticles. FT-IR absorbtion spectra of methanolic peel extract of *Prunus domestica* L. before and after the reduction of ZnO NPs are presented in **Fig2 a & b**. The bands of peel extract of *Prunus domestica* L. were identified at 3357, 2928, 1726, 1613, 1418, 1240, 1149, 1061 and 818cm^{-1} (Fig2.a). After successfully synthesized ZnO nanoparticles, FT-IR spectra (Fig2.b) observed strong absorption peak at 3388, 2931 and 1616 is due to alcohol and phenol, C-H stretching of alkane , C=C stretching of alkanes and C-N stretching vibration of aliphatic amines respectively.

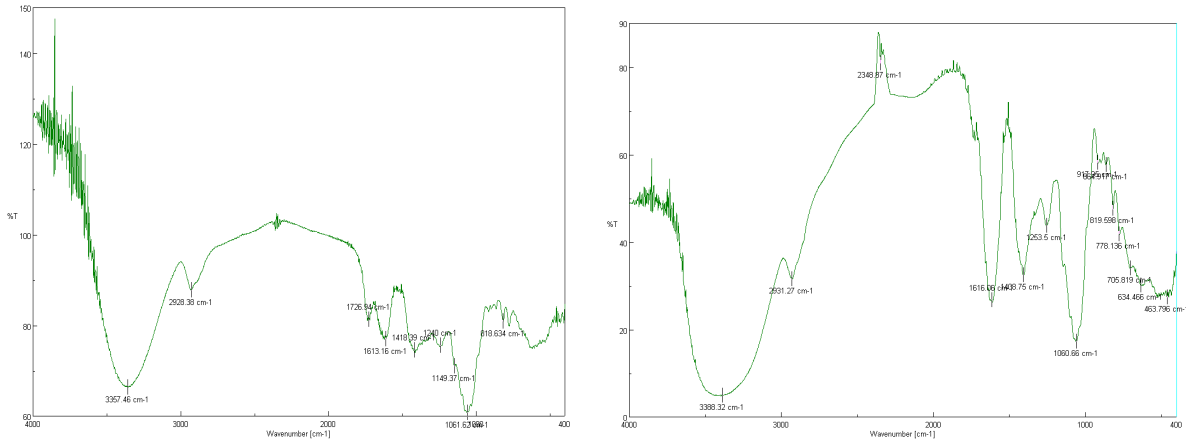

Fig 2. (a) : FTIR analysis of Plum peel extract (**b) :** FTIR analysis of biosynthesized ZnO NPs

Additionally, two new peaks at 634 and 463cm^{-1} were found as characteristic of ZnO NPs which was supported by earlier work [24]. Therefore, from IR spectrum, it was concluded that different biomolecules present in plum peel extract is eventually responsible for bio-reduction and stabilization of ZnO nanoparticles.

Scanning electron microscopy (SEM) analysis

SEM analysis is very important tool to characterize morphology of the metal nanoparticles. In this experiment *Prunus domestica* L.(Plum) peel extract mediated zinc oxide nanoparticles have been synthesized. ZnO NPs are clearly found somewhat round shaped with average size of 20nm (Fig 3).

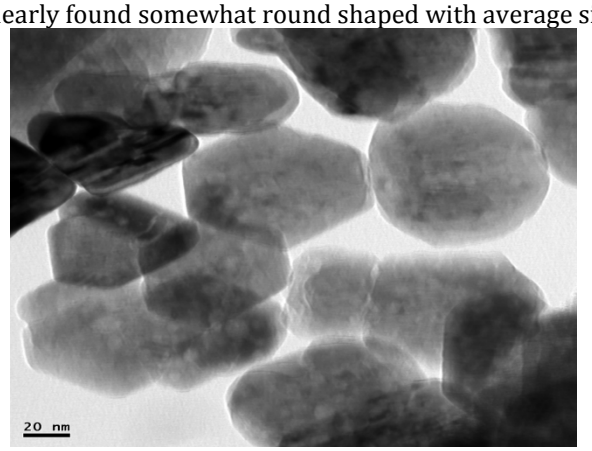

Fig 3. SEM image of biosynthesized ZnO NPs

Biological activities

Evaluation of antibacterial activity of Plum (Prunus domestica L.) peel extract mediated Zinc nanoparticles

The antibacterial activity of synthesized zinc nanoparticles was tested against gram negative and gram positive bacteria by disc diffusion method using Gentamycin as reference standard. Gentamycin at 50

µg/mL concentration was reported to inhibit growth of all tested pathogens. In this study, gentamycin exhibited killing action against all studied pathogen with zone of inhibition highest against S.aureus (27.2 mm) and lowest for P.aeruginosa (17 mm) Fig. 4. Antibacterial activities in terms of zone of inhibition were found in the range of 2-22 mm against all tested strains. Results clearly indicated that antibacterial propensity showed higher against gram negative strain such as E.coli and P.aeruginosa than the gram positive i.e S.aureus and B.substilis. This result is certainly due to the differences in the structure of gram negative and positive bacterial cell wall, which was further supported by some reported work [22].

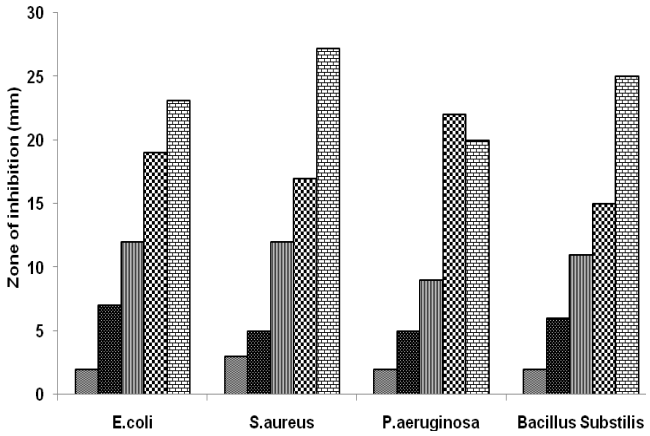

Fig.4 Antibacterial activity of biosynthesized ZnO NPs

Evaluation of in vitro antioxidant activity of Plum (Prunus domestica L.) peel extract mediated Zinc nanoparticles

DPPH free radical scavenging assay

DPPH radical scavenging assay was used for the evaluation of antioxidant potential of the prepared zinc nanoparticles. The red solution containing DPPH turns yellow on addition of ZnNPs indicating the scavenging of free radicals and presence of antioxidant activity. Standard BHT showed concentration dependent inhibition of free radicals with highest scavenging being observed at 100 µg/mL concentration. The % scavenging of iosynthesized zinc nanoparticles was 5-64% i.e it showed moderate free radical scavenging effect which is lesser than standard at all the tested concentrations (Fig 5).

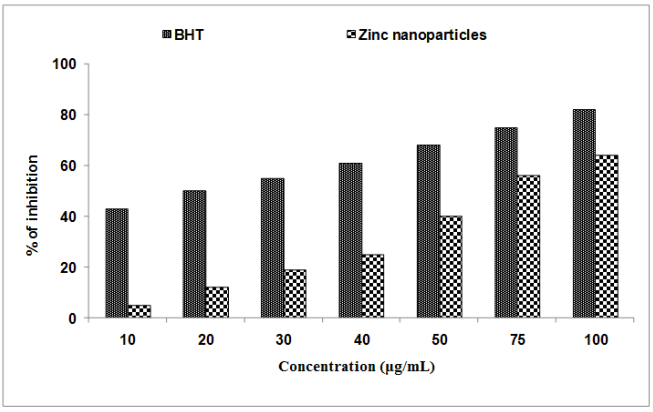

Fig 5. Antioxidant activity of biosynthesized ZnO NPs in DPPH assay

Antioxidant efficacy of biosynthesized ZnO NPs against DPPH may be attributed due to electrostatic interaction between negatively charged bioactive compounds(COOH,OH) present in used extracts of plum peel with positively charged nanoparticles ($ZnO = Zn^{+2}+O^{-2}$) in this experiment[25]. Phytochemicals present in extract is believed to be firmly bound to the ZnO-NPs and thereby increase the antioxidant potential synergistically. Antioxidant activity also depends on the site of attachment of metal nanoparticles with antioxidant agent. A simple and well recognized mechanism is that production of reactive free oxygen species (ROS) as a result of *in- vitro* methods can interact with metal ions to generate

hydroxyl radicals. Further, this antioxidant reacts with a more stable free radicals available, causing free radical scavenging activity.

Hydrogen peroxide scavenging assay

Generation of oxygen free radicals and hydroxyl radicals in living system is due to uninterrupted accumulation of hydrogen peroxide (H_2O_2), which renders cell membrane damage [26].

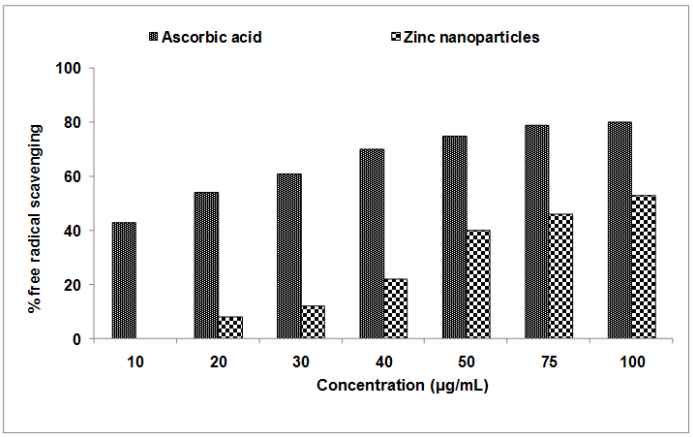

Fig 6. Antioxidant activity of biosynthesized ZnO NPs in H_2O_2 assay

The H2O2 scavenging ability of nanoparticles was measured spectrophotometrically employing ascorbic acid as known standard antioxidant. The H2O2 scavenging of biosynthesized ZnO NPs were found to be less (0-53%) than standard ascorbic acid (>80%). H2O2 scavenging so produced by ZnO NPs might be due to presence of phaytochemicals such as protein, amino acids, flavanoids and polyphenols[27].

Nitric oxide scavenging activity

Nitric oxide is an important bio-regulatory molecule primarily functions in cardiovascular, immune and nervous system [28].

Plum peel mediated zinc oxide nanoparticles showed moderate and lower NO scavenging activity than that of ascorbic acid. The zinc oxide nanoparticles showed a concentration dependent NO scavenging activity and the maximum inhibition observed with 100µg/mL.

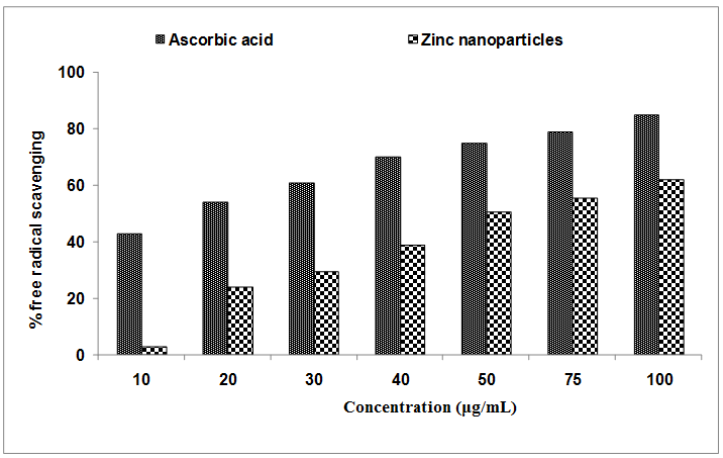

Fig 7. Antioxidant activity of biosynthesized ZnO NPs in NO scavenging assay

Reducing power assay

Reducing power of biosynthesized zinc oxide nanoparticles as shown in Fig 8 below is clearly a dose-dependent response.

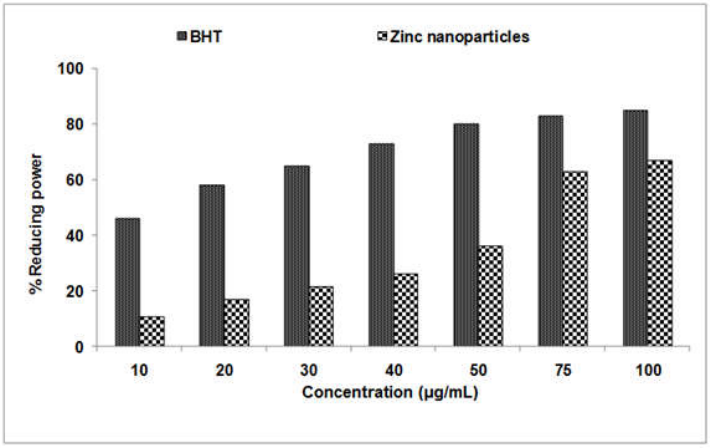

Fig 8. Reducing power of biosynthesized ZnO NPs

ZnO NPs exhibited lesser reducing power than the standard ascorbic acid at all the tested concentrations. The reducing power zinc oxide nanoparticles at 100µg/mL concentration was 67% as compared to 85% of ascorbic acid. Moreover, reducing power of these ZnO NPs was expected to be due to different phytoconstituents in the *Prunus domestica* L. peel extracts.

CONCLUSION

The rapid eco-friendly, simple and efficient protocol were adopted for the biosynthesis of zinc oxide nanoparticles utilizing *Prunus domestica* L.(Plum) agro-waste (peel) has been a revalorized approach. The use of agro-waste extract for the biosynthesis of zinc oxide nanoparticles is merits over harmful, toxic chemicals as reducing and stabilizing agents. Prepared ZnO NPs was well characterized by analytical tool such as XRD, FT-IR and SEM. Somewhat rounded and about 20nm size of zinc oxide nanoparticles was identified, which was further evaluated as antibacterial(using four strains) and antioxidant (by four different methods). ZnO NPs were found more active against gram negative bacteria than gram positive one. Further, antioxidant potential in all studied methods was found moderate as compared to their respective standard used.

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