Green Synthesis Of Zinc Oxide Nanoparticles Using *Sapindus Mukorossi* Fruit Extact, Characterization And Their Antioxidant Activity

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Received: 02.08.2023; Revised: 01.11.2023; Accepted: 11.11.2023

Abstract: In this paper we presented a biogenic method for the synthesis of zinc oxide nanoparticles (ZnO NPs) using *Sapindus mukorossi* fruit extract. Currently, green synthesis route is most promising route for the synthesis of metallic nanoparticles in which various plant parts are being used. Nanomedicine utilizes biocompatible nanomaterials for diagnostic and therapeutic purposes. The synthesized zinc oxide nanoparticles were initially noticed through visual colour change from dark brown to yellow and further confirmed by surface plasmonic resonance band at 341 nm using UV-Visible spectroscopy. Morphology and size were determined by TEM, XRD analysis revealed the crystalline nature and spherical shape with less than 16 nm average size of synthesized ZnO NPs. The stability of ZnO NPs was due to capping of oxidized polyphenols which was established by Fourier transform infrared spectroscopic study. It could be concluded that *S. mukorossi* can be used efficiently in the production of potential antioxidant ZnO NPs for commercial application. The antioxidant activity of zinc oxide nanoparticles was determined using a DPPH free radical scavenging assay. Results indicated that synthesized zinc oxide nanoparticles of *S. mukorossi* fruit could effectively scavenge the free radicals which shows effective antioxidant activity.

Keywords: zinc oxide nanoparticles • *Sapindus mukorossi* • XRD • TEM • crystalline

Introduction

In the last few decades, synthesis of metallic nanoparticles is one of the emerging fields of nanoscience and nanotechnology. It has wide range of application in the field of catalysis, optics, antimicrobials, biomaterial production effective antimicrobials and antifungal agents in the biomedical field (Chikdu *et al*., 2015). It has vital potential for the utilization as anticancer, anti-platelet, anti-oxidant, anti-bacterial agents. In order to reduce toxic chemicals, the biogenic approaches have been developed which are simple, environment friendly and cost- effective. Metallic nanoparticles mostly prepared from nobel metals such as Ag, Au, Pt, Pu and also metal oxide (i.e. ZnO, CuO, FeO etc) (Xie *et al*., 2011; Bala *et al*., 2015; Purohit *et al*., 2020). Plant based materials such as leaves, bark, fruit, stem, root, flowers, peels and seeds have been utilized for the synthesis of metallic nanoparticles. Biosynthesis using plant extract approaches have many advantages over chemical and physical methods because there is no requirement of high energy and purification equipment for wastes (Gandhi *et al*., 2017). So, the researcher's attention towards the synthesis of metallic nanoparticles using plant extracts has been increased due to their stable formation, controllable size, easy handling and less time consuming. Bioactive constituents act as reducing and capping agent for the synthesis of zinc oxide nanoparticles such as saponins, tannins, flavonoides, alkaloids, terpenoids, steroids, glycosides, polysaccharides, vitamins and proteins etc (Singh *et al*., 2011; Noruzi 2015; Khajuria *et al*., 2021).

By using basic principle of green chemistry an environment friendly approach used for the synthesis of stable nanoparticles by reduction of Zn into ZnO with bioreductants in the fruit extract of *Sapindus mukorossi*. *S. mukorossi* is an important medicinal plant of Himalaya which belongs to *Sapindaceae* family and is popularly known as "reetha'' and regional wise known as several names such as reetha, soapnut, washnut, soapberry, dodan and dodani etc (Kirtikar and Basu, 1991). *S. mukorossi* found in tropical and sub-tropical regions of Asia (Suhagia *et al*., 2011). It has been reported that *S. mukorossi* exhibit many pharmacological activities such as antimicrobial, antifungal, spermicidal, heptaprotective, anti-cancer and used as contraceptive cream (Aneja *et al*., 2010). It contains several phytoconstituents like flavonoids, saponins, sugar, mucilage which are strong reducing agent. It is used for inhibiting tumor cell growth. The fruits are expectorant, emetic, contraceptive and for the treatment of excessive salivation, epilepsy, chlorosis, head lice, migranes, eczema, psoriasis (Upadhayay and Singh, 2011). The powdered seeds are employed in the treatment of dental caries, arthritis, common cold, constipation and nausea, oily skin cleanser and also used for washing hair and a hair tonic. Traditionally, *S. mukorossi* also used as natural surfactant (Anjali *et al*., 2018). Keeping in view the high potential of plant in pharmacological and traditional practices, the fruits of *S. mukorossi* were selected for the synthesis of ZnO NPs. The synthesized zinc oxide nanoparticles were characterized by UV-Vis, XRD, TEM and FTIR. The antioxidant activity of zinc oxide nanoparticles was determined using a DPPH free radical scavenging assay.

Materials And Methods

Materials: The fresh fruit of *S. mucorossi* collected from Advani Forest Region of Pauri (Garhwal), Uttarakhand. $ZnNO_3.6H_2O$ and double distilled water used as solvent and NaOH.

Preparation of fruit extracts: The collected fruits washed with double distilled water to remove filth/dust. Then, these washing fruits kept for drying under shade and grinded into fine powder using mortar-pestle. After that, 10 g of powdered fruits boiled with 200 ml distilled water for 25 min at 65^0c. After filteration using whatman no. 1 filter paper aqueous fruit extract obtained which is used for the synthesis of ZnO nanoparticles.

Synthesis: For the synthesis of ZnO nanoparticles, initially 50 ml of fruit extract was taken and heated on magnetic stirrer at 65^0c for 15 minutes in a 250 ml of Erlenmeyer conical flask. Then 50 ml of 100 mM zinc nitrate hexahydrate solution was added dropwise to it with continue stirring and add few drops of 1 M NaOH solution. Color of the solution changed from dark brown to yellowish indicating the ZnO nanoparticles were synthesized. Then, solution was allowed to cool at room temperature for 24 Hr. After that, mixed solution was centrifuged for 10 minutes at 7500 rpm and washed with deionized distilled water followed with ethanol to remove unwanted materials. Then, obtained material dried in the oven at 50^0c using mortar-pestle to get uniform yellow nanoparticles.

Characterization of synthesized ZnO Nps

Elite double beam UV-Vis spectrophotometer was used to investigate the formation of ZnO nanoparticles. The spectra of the mixtures

($ZnNO_3.6H_2O$ solution and fruits extract) were taken between 300 and 900 nm.

XRD Analysis. X-ray Diffraction (XRD) (PAN Analytical, X-pert pro, Diffractometer) was used for the structural and phase determination of zinc oxide nanoparticles and its spectra was taken in the range of 2ɵ from 0^0 to 80^0. The average particle size was calculated by using Scherrer's equation.

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

TEM Analysis. Transmission electron microscope (JEOL JEM 1400) analysis was used to determine the surface morphology of synthesized zinc oxide nanoparticles.

FTIR Analysis. FTIR spectrometer (Perkin Elmer Model RZX) was used to analyze functional groups of the synthesized zinc oxide nanoparticles in the range 4000-400 cm^{-1} using KBr pellet method.

Result and Discussion

In the present work, aqueous fruits extract of *Sapindus mukorossi* was used to synthesize zinc oxide nanoparticles.

UV-Visible Analysis

It was observed that solution color changes from dark brown to yellow. UV-Vis spectra of the solution was taken in the range 300-900 nm. The color change is due to the surface plasmon resonance phenomenon in zinc oxide nanoparticles (Awwad *et.al*., 2013). Several studies showed the similar results (Vijaykumar *et.al*., 2018; Sati *et.al*., 2020a). The Surface Plasmon Resonance band observed at 341 nm which indicated the formation of zinc oxide nanoparticles. Zinc oxide nanoparticles possesses absorption maximum band in the range of 300-400 nm (Kumar and Badoni, 2017). (Figure 1)

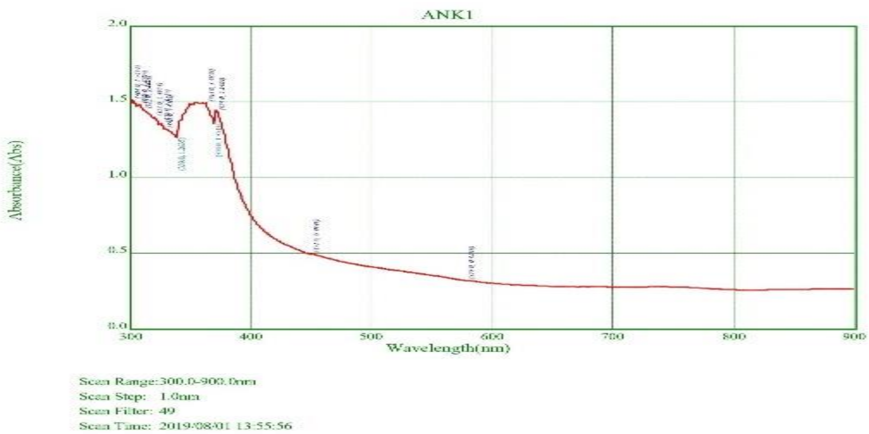

Figure 1. UV-visible spectrum of zinc oxide nanoparticles

XRD Analysis. X-Ray diffraction studies confirms the crystalline nature of zinc oxide nanoparticles. The XRD analysis of synthesized zinc oxide nanoparticles from fruits extract of *S. mukorossi* showed sharp diffraction peaks at 2Ɵ = 32.02^0, 34.58^0, 36.42^0, 47.64^0, 56.76^0, 63.06^0, and 68.14^0 which are indexed to (100), (002), (101), (102), (110), (103) and (201) lattice planes respectively of hexagonal closed packed nano crystals (Fig. 3). Comparing with the standard data, which confirm the nanocrystalline synthesized zinc oxide nanoparticles. The average size of synthesized nanoparticles from fruits extract of *S. mukorossi* is found to be less than 5 nm. Similar results of synthesized zinc oxide nanoparticles using *Viola canescens* leaves extract (Khajuria *et al*., 2019), *Hibiscus subdariffa* leaves extract (Bala *et.al*., 2015), *Parthenium hysterophorus* leaves extract

DOI: https://doi.org/10.51220/jmr.v18i2.4

(Datta *et.al*., 2017) and *Atalantia monophylla* leaves extract (Vijaykumar *et.al*., 2018) were reported.

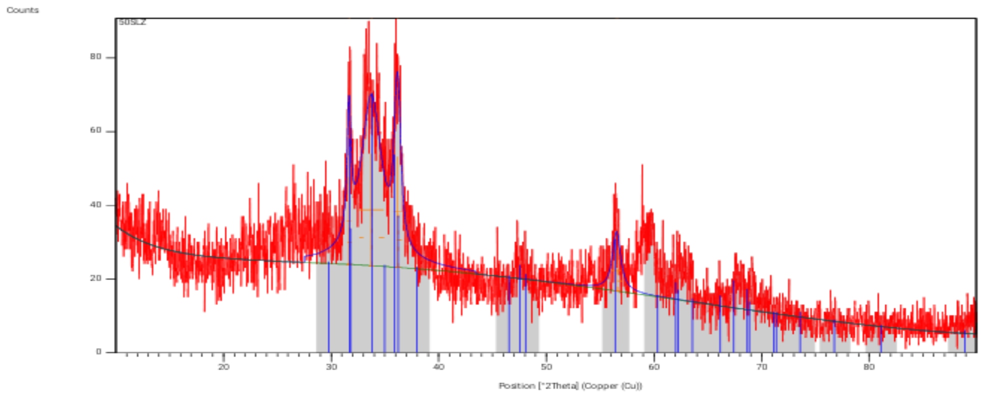

Figure 2: XRD Spectra of zinc oxide nanoparticles

EDX Analysis

Energy dispersive X-ray analysis was used to identify the elemental composition of synthesized zinc oxide nanoparticles. The EDX spectrum was carried out within 1-20 keV X-ray energy range and peaks at 1 keV and 0.5 keV corresponding to Zn and O respectively confirms the formation of zinc oxide nanoparticles (Mohammadi and Ghasemi, 2018; Kandwal *et al*., 2019); (Figure 3).

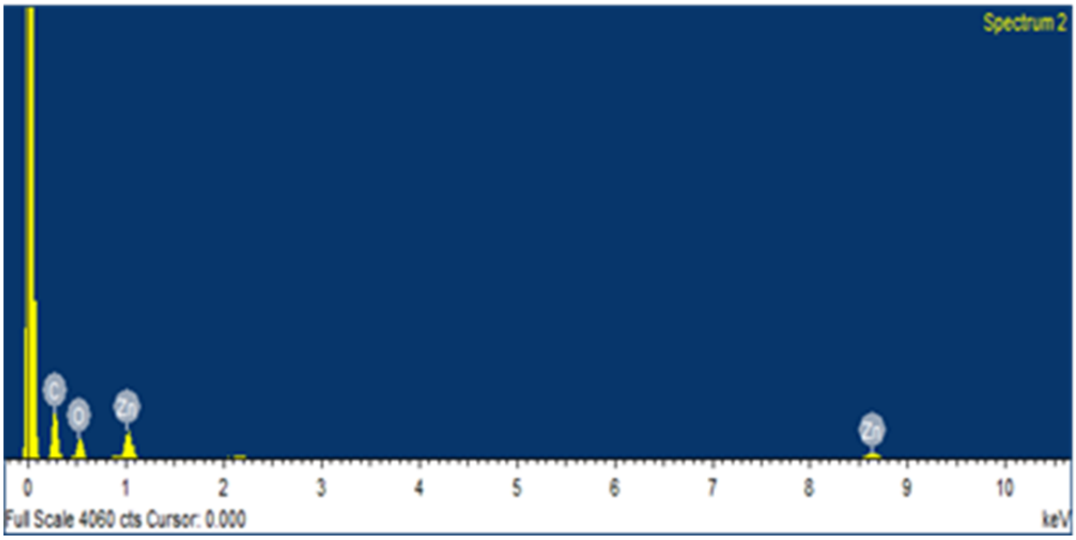

Figure 3. Energy Dispersive Spectra of zinc oxide nanoparticles

TEM analysis

TEM analysis was used to study the surface morphology of zinc oxide nanoparticles (Fig. 4). These results confirmed that zinc oxide nanoparticles were spherical in shape with their average size less than 16 nm. Similar results in the synthesis of zinc oxide nanoparticles using citrus paradisi peel extract (Kumar *et al*., 2014), Parthenium hysterophorus leaves extract (Datta *et.al*., 2017) and *Punica granatum* peel extract (Ghidan *et.al*., 2017; Purohit *et al*., 2021) were reported.

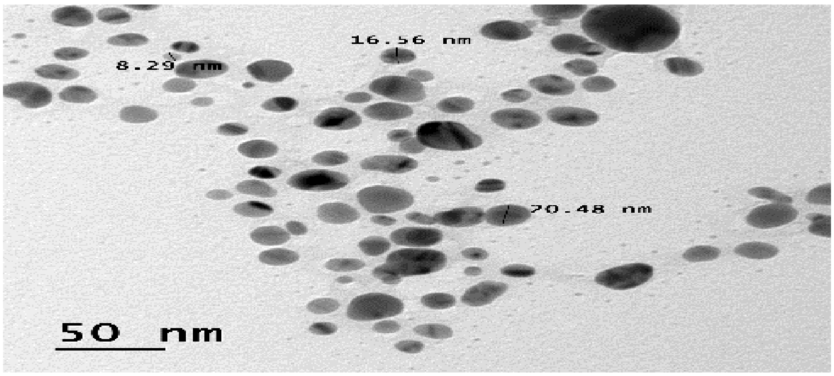

Figure 4. Transmission electron micro-image of zinc oxide nanoparticles

FT-IR analysis: FTIR analysis was done to identify the different functional groups of phytochemicals present on the surface of ZnO nanoparticles. The FT-IR spectra of fruits extract of *S. mukorossi* ZnO nanoparticles in Fig. 5 shows peaks at 3429.1 cm^{-1}, 1588.6 cm^{-1}, 1384.2 cm^{-1}, 1048.5 cm^{-1} and 470.1 cm^{-1}. The absorption peaks at 3429.1 cm^{-1} in the FTIR spectra depicts the O-H stretching vibration of polyphenolic compounds. The peak at 1588.6 cm^{-1} corresponds to carbonyl (C=O) stretching vibration of extensively conjugated compounds (Adam *et.al.*, 2021). The band at 1384.2 cm^{-1} corresponds to C=C stretching of the aromatic compound and at 1048.5 cm^{-1} may depicts C-O stretching of ArOH and at 470.1 cm-1 may be attributed to ZnO nanoparticles which may reveals that *S. mukorossi* fruit extract acts as reducing and capping agents for the synthesis of zinc oxide nanoparticles (Raut and Throat, 2015; Kumar and Badoni, 2017).

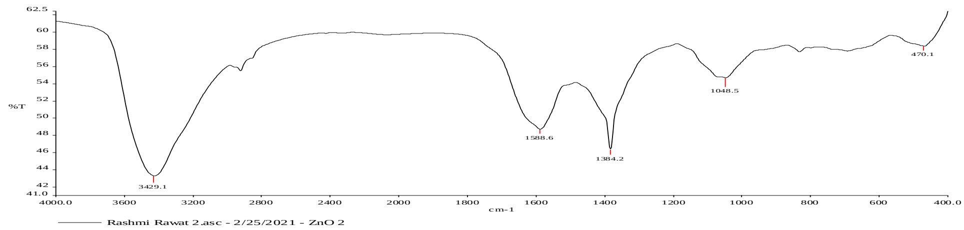

Figure 5. FTIR spectrum of zinc oxide nanoparticles

Antioxidant activity

The antioxidant activity of zinc oxide nanoparticles was determined using a DPPH (1,1-Diphenyl-2-picrylhydrazyl) free radical scavenging assay. Results indicated that synthesized zinc oxide nanoparticles of *S. mukorossi* fruit could effectively scavenge the free radicals which shows effective antioxidant activity (Sowmya *et al*., 2020; Sati *et al*., 2020b).

Fig. 6 is showing the scavenging activity of ascorbic acid as control. Percent inhibition or scavenging at 15 µg/ml (63.46 %), 30 µg/ml (76.79 %), 45 µg/ml (90.9 %), 75 µg/ml (92.62 %) and 100 µg/ml (92.62 %) were obtained. The IC_{50} value of ascorbic acid was 6.99 µg/ml.

Fig. 7 is showing comparable antioxidant activity of fruit extract of *S. mukorossi* mediated ZnO NPs with control ascorbic acid. The scavenging activity of fruit extract of *S.*

mukorossi mediated ZnO NPs increases in a dose dependent manner. Percent inhibition or scavenging at 15 µg/ml (16.82 %), 30 µg/ml (33.21 %), 45 µg/ml (52.94 %), 75 µg/ml (67.05 %) and 100 µg/ml (68.56 %) were obtained. The IC_{50}value of synthesized ZnO NPs of *S. mukorossi* fruit extract was 8.86µg/ml.

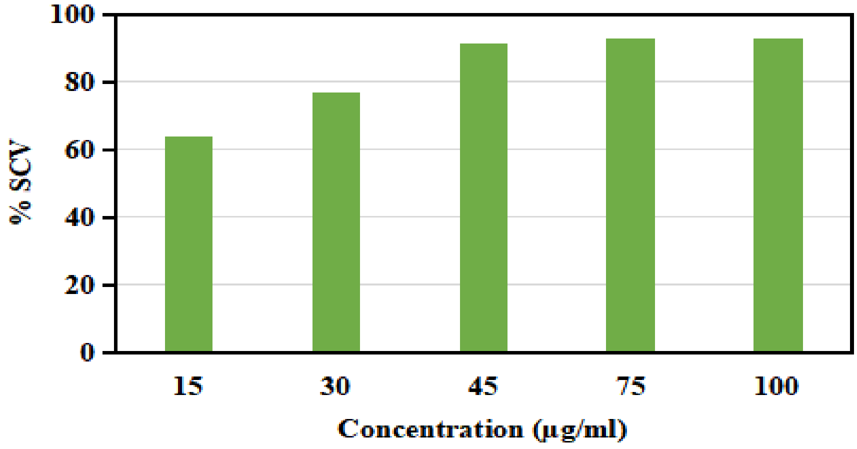

Figure 6. % scavenging of ascorbic acid (standard)

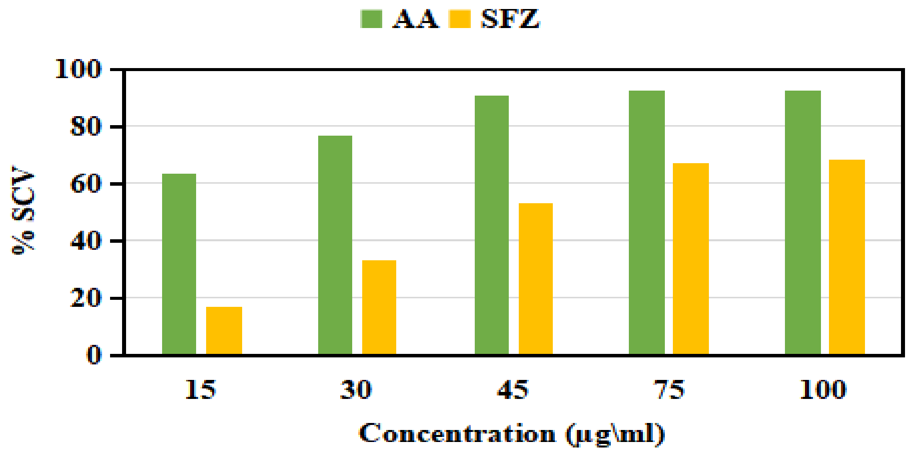

Figure7. % Scavenging of ZnO Nps of *Sapindus mukorossi* fruit extract with ascorbic acid; (AA-Ascorbic acid, SFZ- S. *mukorossi* fruit mediated ZnO NPs)

Conclusion

In this study, we synthesized green zinc oxide nanoparticles using fruits extract of *S. mukorossi*. Characterization techniques (UV-Visible, XRD, FTIR, TEM and EDX analysis) confirmed that the zinc oxide nanoparticles are stable, crystalline in nature, spherical in shape and average crystallite size is less than 16 nm. Synthesized zinc oxide nanoparticles of *S. mukorossi* fruit could effectively scavenge the free radicals which shows effective antioxidant activity. Thus, we can say that, this green route of zinc oxide nanoparticles is simple, cost effective, safe to handle and advantageous to the environment over hazardous chemicals.

Acknowledgements

Authors are thankful to USIC, HNB Garhwal University for providing XRD, Panjab University, Chandigarh for FTIR and CDRI Lucknow for TEM analysis facilities.

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