

EVALUATION OF ANTIOXIDANT POTENTIAL OF ZINC OXIDE NANOPARTICLES SYNTHESIZED USING THE MUCILAGE FROM PLANT SEEDS

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Abstract

In this study, zinc oxide nanoparticles (ZnO@NPs) were fabricated using plant seed mucilage, investigating their diverse properties and applications. ZnO@NPs exhibited noteworthy antioxidant potential with DPPH scavenging activity ranging from 32.7 to 60.2 $\mu\text{g AAE } \mu\text{g/mL}$, FRAP activity from 43.4 to 71.2 $\mu\text{g AAE } \mu\text{g/mL}$, total phenolic content (TPC) from 41.6 to 70.2 $\mu\text{g GAE/mL}$, and total flavonoid content (TFC) from 43.8 to 79.4 $\mu\text{g QCE/mL}$. these findings highlight the versatile properties of the green synthesized ZnO@NPs, showcasing their potential in environmental remediation, and antimicrobial formulations, and as promising candidates for further exploration in the biomedical fields and for the purification of industrial waste water.

1 Introduction

In the industrial sector, organic dyes are an important class of pollutants, which are used in the clothing, paper industry, in suede, in nutrition and for printing and medical applications. About 60% of the dyes used in textile industry for pigmentation are discarded and released to hydrosphere after the textile process. The MB is a cationic azo dye. These are the by-products of these industries that are the most significant contributors to the ecological pollution. Colouring agent in silk, paper, hemp, wood and chemical treatment in printing and dying industries, MB causes vomiting, anxiety, respiratory problems and sweating. MO dye is an important colorant in textile and leather dyeing. MO can be carcinogenic and mutating and can prove to be a serious environmental hazard. The

agricultural industry is a source of dye to water and soil through the extensive use of organic phosphorus insecticides that produce P-nitrophenols (PNPs) in both water and soil which are harmful to aquatic organisms as well as unsuitable for drinking. Additionally, PNPs are highly toxic and carcinogenic and are one of the worst pollutants, according to the United States Environmental Protection Agency (EPA), as it has a limit of 1-20 mg/L for commercial sewage [1-7]. The tannery waste water discharge into the wastewater stream and soil can create a lot of environmental problems because the waste water is carcinogenic and highly toxic. In order to provide a sustainable environment for next generations, efficient approach for treating the organic dyes in effluents is need of the hour. Compared to the traditional water treatment

methods such as biological processes, flocculation, chemical treatment etc. photooxidation has been found to be an effective, economic and environmentally friendly method to degrade dyes and wastewater [8-11].

A recently new development in this field, is semiconductor photocatalysis, where the pollutants are destroyed by the nanomaterials at room temperature and pressure. This highlights the need to research and developing photocatalytic materials with enhanced efficiency for oxidative degradation. Recently, there has been a lot of research into using transistors, like TiO_2 , ZnO , WO_3 , and ZrO_2 as photocatalysts to degrade different types of Pigments. Among these, ZnO and TiO_2 are becoming popular in solar cells, photocatalytic systems and light-emitting devices. On a larger scale, though, ZnO is more cost-effective than TiO_2 , making it favourable on the commercial scale [12-15].

The unique properties of ZnO@NPs such as photocatalytic activity, non-toxicity, large band gap, photosensitivity, high binding energy and excellent chemical stability at room temperature make them attractive for numerous applications in various areas, including catalysis, antioxidant activity, environment protection, electronics, cosmetics, communications, biology, and pharmaceuticals. In addition, its antimicrobial, antifungal, anti-diabetic, and insecticidal properties suggest a wide range of medical applications such as bio-sensing, drug transport and nanomedicines, which makes the use of different parts of plant extracts a promising technique for the synthesis of ZnO@NPs . The antimicrobial potential of green-synthesized ZnO@NPs prepared using water-based floral extract of different plants [16-20].

The aim of this work is to study the antioxidant activity by different methods like DPPH, TPC, TFC and FRAP activity. Consequently, a simple and inexpensive approach to synthesize ZnO@NPs , its remarkable catalytic and optical properties, and the reducing and stabilizing effect of plant seed mucilage pave the way for a potential application in the health and industrial fields.

2 Materials and methods

2.1 Materials

The *Salvia spinosa* seeds were collected from the local market palace of Sargodha. Zinc Acetate (Merck, Germany), DPPH (Sigma- Aldrich®), n-Hexane (EMSURE ACS, Malaysia) and Ethanol (Sigma- Aldrich®) were purchased from the local market and were of analytical grade, identified and authenticated by a botanist of the Department of Botany, University of Sargodha, Sargodha, Pakistan. Deionized water (DH_2O) was used for solution preparations and extractions.

2.2 Extraction of mucilage

The mucilage from the seeds was extracted according to the procedure reported previously.

2.3 Synthesis of nanoparticles

The synthesis of ZnO@NPs was followed by the protocol already reported procedure with slight modification where 0.68 g/ 100 mL zinc acetate aqueous solution was drop wise added in 50 mL of mucilage with continuous and vehement stirring for 30 min [21, 22]. The solution was then heated to 70°C till a pale yellow-colored precipitate was formed. This pale yellow precipitate was centrifuged 20 min at 6000 rpm, washed with DH_2O two to three times and then calcined at 450°C . The dried ZnO@NPs were scraped and packed and labelled as ZnO@NPs and stored for further analysis.

2.4 Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was executed in accordance with methodology illustrated by peers with some amendment [23, 24]. In brief, different concentrations of ZnO@NPs solution, in the range of 100 to 500 $\mu\text{g}/\text{mL}$ combined with 2.5 mL of sodium phosphate buffer (PBS) (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide and incubated for 20 min at 50°C . Then 10% trichloroacetic acid solution (w/v) was added and it was centrifuged for 10 min at 3000 rpm. 2.5 mL of deionized water and 0.1% ferric chloride solution were added to the supernatant. Then absorbance was determined at 700 nm by a spectrophotometer (Shimadzu UV-1800). The antioxidant potential of FRAP was calculated. 40

2.5 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The methodology of researchers [25, 26] was followed and different concentrations of ZnO@NPs (100-500 µg/ mL) were mixed with 2 mL of methanol and 1 mL of DPPH free radical solution in methanol (0.1 mM). The solutions

$$(Absorbance\ of\ control\ 517 - Absorbance\ of\ sample\ 517) / Absorbance\ of\ control\ 517 \times 100 \quad (1)$$

2.6 Total polyphenol content (TPC)

The Folin-Ciocalteu method was used to estimate the total polyphenol content [27]. First, 2 mL of DH₂O and 250 µL of 1N Folin Ciocalteu's phenol were mixed with 100, 200, 300, 400 and 500 µL of ZnO@NPs and placed in the dark for 8 min at room temperature. Then 750 µL of 20% Na₂CO₃ solution and 950 µL of DH₂O were added. The

$$X = \frac{y}{0.0019}, R^2 = 0.9659 \quad (2)$$

2.7 Total flavonoid content (TFC)

To determine the concentration of the flavonoids, the colorimetric method using aluminium chloride (AlCl₃) was used following the protocol used by peers [28]. In brief, 0.75 mL of methanol was mixed with each of the concentrations of ZnO@NPs (1 mg/mL) ranging from 100 µL to 500 µL and the final volume was made up to 2 mL using DH₂O. Then, 300 µL of 5% sodium nitrate and 300 µL of 10% AlCl₃ were added to each sample and then left to settle for 10 min. Further,

$$X = \frac{y}{0.0022}, R^2 = 0.9883 \quad (3)$$

3 Results and discussion**3.1 FRAP test**

The ferric reducing antioxidant assay (FRAP) was used to measure total antioxidant activity. It is widely used because of its function as a 'secondary' antioxidant, able to neutralise the damaging effects of free radicals and break their chain reactions. The antioxidant activity of ascorbic acid is believed to be due to its high hydroxyl content and the presence of free hydroxyl groups to neutralize the harmful free radicals [29]. The purpose of this study is to remove the potassium

were then thoroughly mixed and then incubated for 30 min in dark environment at ambient temperature. To determine the absorbance, a spectrophotometer (Shimadzu UV-1800), set at 517 nm was used. FRAP's antioxidant potential was calculated using the following equation (1).

solutions were left in the dark environment for 30 min and absorbance was measured with a Shimadzu UV-1800 spectrophotometer at 765 nm. The obtained results were expressed as g GAE/mL of ZnO@NPs, using GA as a reference. The estimation of the level of scavenging was carried out with the following equation (2).

2 mL of 1 mol/ L NaOH was added to the solution and the total volume was made up to 5 mL with DH₂O. The test solution was left at room temperature for 40 min and then the absorbance was recorded at 510 nm in the spectrophotometer (Shimadzu UV-1800). The results were obtained by using quercetin as the reference standard and expressed as mg of quercetin equivalents per g (mg quercetin/g) of ZnO@NPs. The level of scavenging was calculated using the equation (3).

ferrocyanide (K₃Fe(CN)₆) that was deposited in the wood using a solution of trichloroacetic acid (TCA). Upon the addition of FeCl₃, a complex is formed which show a chromatic range from green to blue. The reducing power of greenly synthesized ZnO@NPs was assessed by measuring their antioxidant activity by converting Fe⁺³ to Fe⁺². Radical scavenging activity was evaluated against the ascorbic acid standard curve and the values obtained ranged from 43.4 µg (AAE µg/mL) to 71.2 µg (AAE µg/ mL) within the concentration range (100-500 µL) as shown in the standard curve

in Figure (1). The mechanism of antioxidant activity of the synthesized nanoparticles is enhanced by simple electron transfer, sequential proton loss and electron transfer, proton transfer or transfer of hydrogen atoms that stabilize the radicals.

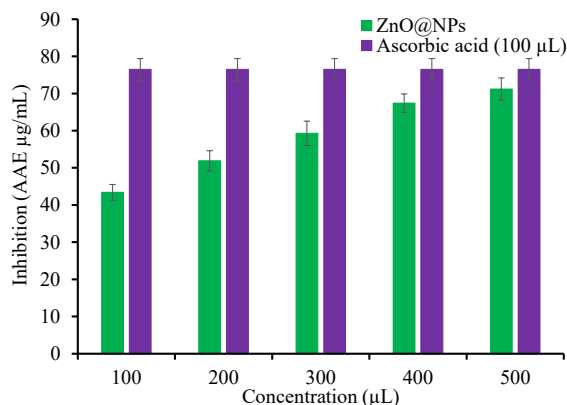


Fig. 1. FRAP test of ZnO@NPs

3.2 DPPH test

The elimination of DPPH (free radicals) by ZnO@NPs was evaluated using the DPPH assay over a range of doses (100-500 µL). The efficacy of ZnO@NPs were tested and compared with the conventional antioxidant ascorbic acid for their ability to reduce the DPPH radical. With the increase in concentration of ZnO@NPs, its ability to scavenge the DPPH radical was observed to increase. The DPPH assay is useful to measure the antioxidant activity of a chemical compound. This indicates that the bioactive compounds present in the extract can have an impact on this process. Scavenging activity of ZnO@NPs (100 to 500µL) against DPPH was determined and shown in Figure (2). The percentage of DPPH radical suppression obtained by the various concentrations of ZnO@NPs ranged from 32.7 to 60.2 µg as AAE µg/mL.

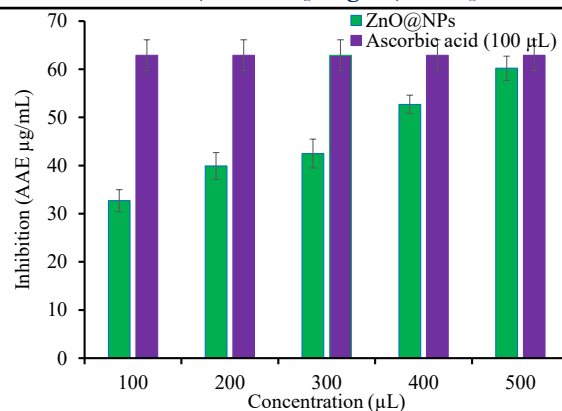


Fig. 2. DPPH test of ZnO@NPs

3.3 TFC test

The total phenolic content (TPC) and total flavonoid content (TFC) have also been assessed, using a standard curve of gallic acid (GA) and quercetin (QC) absorbance against concentration are shown in (Figures (3 and 4)). The study showed that total phenolic content (TPC) and total flavonoid content (TFC) was higher with increasing concentration and recorded as 34.78, 41.6 TO 70.2 µg (GAE µg/mL) and (43.8 to 79.4 µg (QCE µg/mL), respectively. The overall results obtained validate a possible antioxidant action that could be explained by the bioactive components that are able to reduce the metal ions and generate nanoparticles [30, 31].

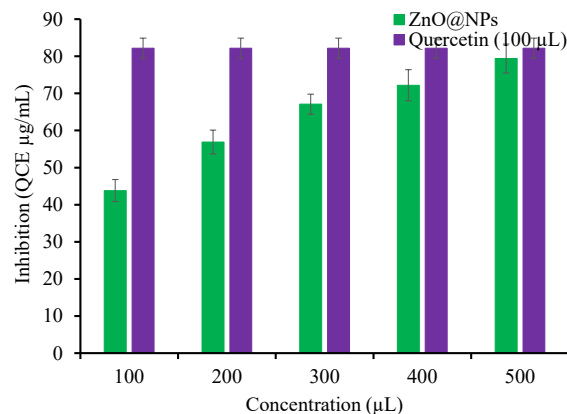


Fig. 3. TFC test of ZnO@NPs

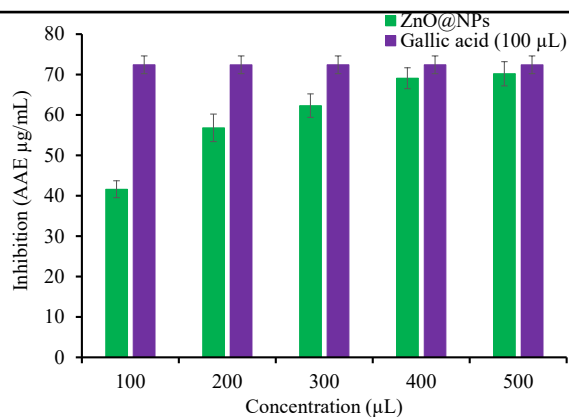


Fig. 4. TPC test of ZnO@NPs

5. Conclusion

In the present work, the green synthesis of ZnO@NPs were carried out by using mucilage from *S. spinosa* seeds which acted as stabilizing and reducing agents. The antioxidant activity of the nanoparticles was significant, with the ability to scavenge DPPH radicals and reduce ferric ions. In particular, such nanoparticles can be used as a low-toxic and harmless substance to clean industrial and other waste waters. Industrial effluents are responsible for introducing pollutants in the environment particularly in water bodies, among which dyes with high COD and BOD should be removed from the effluents. Methylene blue, methyl orange and 4-nitrophenol are commonly used dyes in paper, textile and leather industries for dyeing various products, but pose serious concerns for the public health and environment. The dyes contaminated water can be efficiently purified by these ZnO@NPs and hence can be used in water treatment, which also includes industrial waste water/effluents.

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