Antioxidant Activity of *Abies webbiana* Mediated Zinc Oxide Nanoparticles

T. Vinaya Swetha a, M. Jeevitha b*, S. Rajeshkumar c and Selvaraj Jayaraman d

a Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.
b Department of Periodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.
c Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.
d Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India.

**Authors’ contributions**

This work was carried out in collaboration among all authors. Author TVS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft manuscript. Authors MJ and SR managed the analyses of the study. Author SJ managed the literature searches. All authors read and approved the final manuscript.

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**ABSTRACT**

**Background:** Biological methods for nanoparticle (NP) synthesis using microorganisms, enzymes, and plants or plant extracts have been suggested as possible eco-friendly alternatives to chemical and physical methods.

**Aim:** The aim of the current study is to synthesize Zinc oxide nanoparticles (ZnO NPs) mediated by *Abies webbiana* and to evaluate its antioxidant activity.

**Materials and Methods:** Bio-mediated synthesis of metal oxide nanoparticles using *A. webbiana* is a promising alternative to traditional chemical synthesis. The antioxidant activity of zinc oxide (ZnO) NPs was synthesised using DPPH radical scavenging assay.

**Results:** The ZnO NPs were identified by dark brown color and the surface plasmon resonance was positioned at a peak at 290 nm. Finally, the current study has clearly demonstrated that the ZnO NPs are responsible for significant high antioxidant activities.

**Conclusion:** Therefore, the study reveals an efficient, eco-friendly, and simple method for the green synthesis of ZnO NPs using a green synthetic approach.

*Corresponding author: E-mail: jeevitham.sdc@saveetha.com;*
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1. INTRODUCTION

In this modern period, nanoscience and nanotechnology are growing branches of science that deal with various processes like fabrication and characterization of different nano metals and non-metal of different sizes, shapes and compositions [1]. Nanoparticles are the particles with size ranging from 1 nm to 100 nm providing solutions to environmental and technological challenges and applied in almost all the fields. Nanotechnology is mainly used to produce and process products eco-friendly and to minimize the use of hazardous environments [2].

Green chemistry is known as implementation, development and designing of chemical products and making it eco-friendly and biocompatible to be used in medicine and food industry [3]. It was found to be more suitable than other methods. In this method, the plant extract is used both as a capping and reducing agent for synthesizing the zinc oxide nanoparticles because of the presence of reducing properties in the leaf extract.

The scientific name of Talisapatra is Abies webbiana, it is considered to have antimicrobial and antioxidant properties and hence chosen for this study to synthesize nanoparticles from this [4]. Nanoparticles containing antioxidant and antimicrobial properties are considered as a new trend of medicinal and therapeutic agents and even in the prevention of deterioration of food and pathogenic microorganisms. Green synthesized ZnO NPs exhibited moderate bacterial activity against Gram positive and Gram negative bacteria and free radical scavenging activity [5].

A wide variety of physical and chemical processes for the synthesis of ZnO nanostructures have been developed including laser ablation, hydrothermal, electrochemical depositions [6], sol gel [6,7], chemical vapor deposition [8], thermal decomposition [9], combustion [10,11], ultrasound, [12] microwave-assisted combustion method, [13] two-step mechano chemical–thermal synthesis, [14] anodization, [14,15] co-precipitation, [16] and electrophoretic deposition [16,17] methods. Even though the exact mechanism of shape and size control of these structures have not been established and mentioned in the literature for the preparation of a number of self-assembled structures reported [18]. Chemical synthesis methods lead to the adsorption of toxic chemical species onto the surface that may have adverse effects in medical applications. As a result, researchers in the field of synthesis of nanoparticles and assembly have turned to biosynthesis methods for nanoparticle production, which employ plants, fungi, bacteria and enzymes and represent possible eco-friendly alternatives to chemical and physical methods [19]. To date, green synthesis of ZnO NPs by plants such as Calotropis procera, [20] Aloe barbadensis miller [21], and Poncirus trifoliate [22] have been reported.

2. MATERIALS AND METHODS

2.1 Preparation of Leaf Extract

The A. webbiana leaves were gathered and washed double distilled water to remove dust particles. The leaves were dried and grained with mortar. The leaf extract sample was mingled with 100 mL of double distilled water and kept at 60 degree Celsius in heating mantle. Using whatman No 1 filter paper, the sample was filtered. Then the final extract of the leaf was stored.

2.2 Synthesis of Zinc Oxide Nanoparticles

About 1 mM of 50 mM of zinc sulfate dissolved in 80 mL of double-distilled water. The plant extract of A. webbiana was added with metal solution and was made into 100 mL solution. The color change was observed visually and photographs
were recorded. The solution is kept in a magnetic stirrer/orbital shaker for NP synthesis.

2.3 Characterization of *A. webbiana* Mediated ZnO NPs

The synthesized NPs solution is preliminarily characterized using UV-visible spectroscopy; 3 mL of the solution is taken in cuvette and scanned in double-beam UV-visible spectrophotometer from 300 nm to 700 nm wavelength and the results were recorded at different time periods.

2.4 Preparation of ZnO NPs Powder

The NPs solution is centrifuged using Lark refrigerated centrifuge. The solution of ZnO NPs is centrifuged at 8000 rpm for 10 min, and the pellet was collected and washed with distilled water twice. The final purified pellet is collected and dried at 100–150°C NPs for 24 h. Finally, the NPs powder is collected and stored in an airtight Eppendorf tube.

2.5 Antioxidant Activity

DPPH radical scavenging assay was done to assess the antioxidant property of the extract. The DPPH is considered as a stable lipophilic free radical, having nitrogen at the center exhibiting purple color. The antioxidant donates an electron to the DPPH radical and the change in absorbance occurs at a wavelength of 517 nm and the color changes to pale yellow slowly.

2 mL of the synthesized CuNPs in the concentration range of 10-50 mL (5 different concentrations) were obtained by adding 50% of the methanol solution to equal volume of 0.1mM of DPPH solution at different concentrations and was incubated for 30 minutes in dark at room temperature. The absorbance was measured at 517 nm. Here the Methanol solution mixed with 0.1 mM of DPPH solution was used as control and ascorbic acid was used as a standard. The IC<sub>50</sub> value was calculated.

3. RESULTS AND DISCUSSION

3.1 Visual Identification

Color change was found to be an important factor for the synthesis of ZnO NPs. Here, the ZnO NPs were synthesized from *A. webbiana*; the color change of the mixture was observed at different intervals during the incubation period. The color changed from light brown to dark brown at room temperature suggesting the formation of ZnO NPs. The results depend upon the presence of the color that arises during the reaction. In another study, which involves a color change to yellow, indicating the presence of ZnO NPs where ZnS is converted to ZnO by oxidation [44].

3.2 UV–vis Spectroscopy

The results depend upon the presence of the color that arises during the reaction. The maximum absorption peak was at 290 nm because of excitation of the characteristic surface plasmon resonance band by the synthesized zinc oxide nanoparticles. Since the *A. webbiana* is a rich source of flavonoids and phenolics they are said to play an important role in the reduction process during synthesis of nanoparticles. It was observed that with increase in incubation time, the absorption spectrum steadily increases.

3.3 Antioxidant Property of Zinc Oxide Nanoparticles using *A. webbiana*

The odd electron molecule in the DPPH free radical gave a strong absorption at 517 nm where it turned from light brown to dark brown color. The % inhibition for different concentrations of copper nanoparticles was calculated and is found to be maximum at 50 μl (Fig. 3) concentration. This confirmed the reducing action of DPPH radical indicating its antioxidant property. In another study, it was observed from the spectra that the extract at 517 nm had highest radical scavenging activity at a concentration of 25 μl (60.2%), which is indicative of significant antioxidant activity as potent as DPPH itself [45]. Previous studies has shown that that the extract at 362 nm has the highest antioxidant activity [46]. In another study, the synthesized silver NPs showed comparable free radical scavenging activity at 100 μl [47]. When subjected to DPPH assay to check for its antioxidant activity of grape seed mediated titanium oxide nanoparticles, highest antioxidant activity at a concentration of 50 μl was observed [48].
Fig. 1. Visual observation of colour change

Fig. 2. UV-vis absorption spectra analyses of zinc oxide nanoparticles synthesized using *A. webbiana* recorded as function of time
4. CONCLUSION

This study was done to find a way for a greener approach of synthesizing the ZnO NPs using *A. webbiana* thereby giving pharmacological evidence against the antioxidant activity. The ZnO NPs were identified by dark brown color and the surface plasmon resonance was positioned at a peak at 290 nm. The ZnO NPs synthesized using *A. webbiana* has potent free radical scavenging activity and has a wide array of medical and dental applications.

**CONSENT**

It is not applicable

**ETHICAL APPROVAL**

It is not applicable

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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