

Biogenic synthesis of zinc oxide nanoparticles using root extract of *Anacyclus pyrethrum*

Síntesis biogénica de nanopartículas de óxido de zinc utilizando extracto de raíz de *Anacyclus pyrethrum*

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ABSTRACT

Introduction: Nanoparticle synthesis can be carried out via various physical, chemical, and biogenic methods. Nanoparticles are easily synthesized with the help of bacteria and plant extracts. Utilizing root extract in the biogenic synthesis of nanoparticles is a non-toxic and environmentally beneficial method.

Objective: The present study is aimed to synthesize zinc oxide (ZnO) nanoparticles using an eco-friendly biological approach. The study also aims to explore and compare the antioxidant potential of the synthesized ZnO nanoparticles with that of the *Anacyclus pyrethrum* root extract.

Methods: This method makes use of aqueous extract from *Anacyclus pyrethrum* roots. The phytochemical analysis of the root extract has been analyzed using qualitative methods and the antioxidant potential has been determined using DPPH analysis. The ZnO nanoparticles have been characterized using spectrophotometric analysis.

Results: The aqueous extract was found to contain significant phytochemicals that act as a significant reducing agent that aid in stabilization of the synthesized nanoparticles. The synthesized ZnO nanoparticles exhibited absorbance at 362 nm. The root extract was found to possess significant antioxidant potential owing to the presence of polyhydroxy phytochemicals

Conclusions: The antioxidant potential of the biosynthesized ZnO nanoparticles was significantly higher than that of the root extract. Thus, the root extract of *A. pyrethrum* as well the biogenic ZnO nanoparticles can be explored for pharmaceutical benefits.

Keywords: nanotechnology; zinc oxide; root extract; biosynthesis; antioxidant.

RESUMEN

Introducción: La síntesis de nanopartículas puede llevarse a cabo a través de varios métodos físicos, químicos y biogénicos. Las nanopartículas se sintetizan fácilmente con la ayuda de bacterias y extractos de plantas. La utilización de extracto de raíz en la síntesis biogénica de nanopartículas es un método no tóxico y beneficioso para el medio ambiente.

Objetivo: El presente estudio tiene como objetivo sintetizar nanopartículas de óxido de zinc (ZnO) utilizando un enfoque biológico ecológico. El estudio también tiene como objetivo explorar y comparar el potencial antioxidante de las nanopartículas de ZnO sintetizadas con el del extracto de raíz de *Anacyclus pyrethrum*.

Métodos: Este método utiliza extracto acuoso de raíces de *Anacyclus pyrethrum*. El análisis fitoquímico del extracto de raíz se ha analizado mediante métodos cualitativos y el potencial antioxidante se ha determinado mediante análisis

DPPH. Las nanopartículas de ZnO se han caracterizado mediante análisis espectrofotométrico.

Resultados: Se encontró que el extracto acuoso contenía fitoquímicos significativos que actúan como un agente reductor significativo que ayuda en la estabilización de las nanopartículas sintetizadas. Las nanopartículas de ZnO sintetizadas exhibieron absorbancia a 362 nm. Se descubrió que el extracto de raíz posee un potencial antioxidante significativo debido a la presencia de fitoquímicos polihidroxiados

Conclusiones: El potencial antioxidante de las nanopartículas de ZnO biosintetizadas fue significativamente mayor que el del extracto de raíz. Por lo tanto, el extracto de raíz de *A. pyrethrum* y las nanopartículas biogénicas de ZnO pueden explorarse en busca de beneficios farmacéuticos.

Palabras clave: nanotecnología; óxido de zinc; extracto de raíz; biosíntesis; antioxidante.

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Introduction

The application of materials with dimensions on the nanoscale is the primary focus of nanotechnology.⁽¹⁾ Nanoparticles (NPs) are materials with at least one dimension in the nanoscale and have incredibly fine sized particles, ranging in size from 1-100 nm.⁽²⁾ NPs have unique features and a very high surface area to volume ratio due to the nanoscale dimension. Due to their fine size and substantial surface area, NPs have special chemical and physical characteristics.⁽³⁾ Depending on their size, NPs exhibit optical characteristics that impart diverse colors through the visible region absorption. The physicochemical features that

result from the fine size of NPs give rise to various imaging, mechanical, electrical, and optical capabilities that are highly sought after for applications in the ecological, commercial, and medical fields.⁽⁴⁾ Because they outperform their bulk equivalents in terms of tunable physicochemical properties including electrical and thermal conductivity, melting point, catalytic activity, and light absorption, NPs and nanomaterials have gained much importance.⁽⁵⁾ Increased drug compliance, improved treatment efficacy and lower side effects can be achieved when NPs are used in the optimal dosage range in pharmaceuticals.⁽⁶⁾

Due to their tunable biological, physical, and chemical competencies with improved execution compared to their bulk component parts, nanomaterials have assumed primacy in technological progress.⁽⁷⁾ An increasing number of companies are exploring NPS in electronics as a viable option for sensors and flexible displays. Zinc oxide NPs (ZnO NPs) have been investigated in recent studies for their high excitation binding energy and vast bandwidth.⁽⁷⁾ ZnO NPs are utilized in a variety of biomedical applications, such as bio sensing, bioimaging, and drug delivery.⁽⁸⁾ They are also employed in environmental applications, such as water purification and photocatalysis.⁽⁹⁾ In addition to being used as an additive in the production of concrete and ceramics, ZnO NPs are also used in the production of rubber and cigarette filters, calamine lotion, creams and ointments used to treat skin conditions, food products like breakfast cereals, and as a coating agent in various paints.⁽¹⁰⁾

The synthesis of NPs using bacteria, fungus, algae, plants, etc. is referred to as "green synthesis" as this method is affordable, safe, green, and environmentally friendly.⁽¹¹⁾ There are numerous reports available in the literature on the green synthesis of ZnO NPs using various fruits or plants such as Aloe Vera,⁽¹²⁾ green tea,⁽¹²⁾ and fruits.⁽¹³⁾ However, a review of the literature revealed that less study has been done on the green synthesis of ZnO NPs using roots of any plant extract that can benefit from numerous phytochemicals found in these plants. Keeping these points in mind, this work is aimed to explore the green synthesis of ZnO NPs using the root extract of *Anacyclus pyrethrum* (aka Akarkara), a perennial

medicinal herb that is rich in phytochemicals.⁽¹⁴⁾ The antioxidant potential of the root extract and the ZnO NPs has also been explored and compared for the potential medicinal benefits.

Methods

Materials: *A. pyrethrum* roots were collected from Kasna, Uttar Pradesh, India. The plant was identified at the University School of Agricultural Sciences (RBU, Chandigarh) under voucher with Ref. No. RBU/USAS/HOD/22/198. All the chemicals used in the study were purchased from Sigma Aldrich Chemicals, New Delhi.

Preparation of the precursor solution: 0.1097 g of zinc acetate dihydrate was dissolved in 100 mL of deionized water to obtain the precursor solution.

Preparation of root extract: *A. pyrethrum* roots were washed with tap water followed by distilled water to remove any contaminants. The cleaned roots were sun dried and powdered. 25 g of root powder was boiled in 100 ml of deionized water on a hot plate at 80 °C. The extract was cooled before filtering it through Whatman filter paper and kept in the refrigerator.

Synthesis of NPs: Precursor solution and root extract were mixed in a volume ratio of 1:9, 2:8, 3:7, 4:6, and 5:5 and stirred for 1 hour using a magnetic stirrer after addition of 1 mL of 0.1 M NaOH leading to precipitation with a color change. The maximum precipitation and color intensity was achieved using a 2:8 root extract to metal salt solution ratio. The solution was filtered and the precipitates were calcined at 100 °C to obtain ZnO NPs. The effect of temperature variation on the synthesis of ZnO was also studied.

Phytochemical analysis of extract: The extract was analyzed for the presence of various phytochemicals using preliminary qualitative approach discussed by Nagano and Batalini.⁽¹⁵⁾

Characterization technique: The formation of NPs was confirmed by UV-vis. analysis using Agilent Cary 60 UV-Vis double beam spectrophotometer. XRD analysis was performed by using Panalytical X.Pert Pro X-ray Diffractometer. The functional group analysis was performed using Perkin Elmer Spectrum, RX-I Fourier transform infrared spectrophotometer.

Antioxidant potential: A modified DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical test was used to analyze the antioxidant potential of the root extract and the synthesized ZnO NPs.⁽¹⁶⁾ 1.5 ml of freshly made control (DPPH reagent solution in methanol) was mixed with 1.5 ml of the sample solution (10-150 ppm) and left untouched for 30 minutes. The absorbance of the resultant solution was measured at 517 nm and compared to that of Vitamin C as standard. % antioxidant activity was calculated using absorbance of sample (A_s) and absorbance of control (A_c) through equation.

$$\% \text{ antioxidant activity} = \left(1 - \frac{A_s}{A_c} \right) \times 100$$

A graph of % antioxidant activity vs. concentration of the sample was plotted and linear regression was used to calculate the IC₅₀ value.⁽¹⁷⁾

Results and discussion

Phytochemical analysis: The phytochemical analysis of *A. pyrethrum* root extract revealed the presence of phenol, tannins, terpenoids, diterpenoids, alkaloids, amino acid, carbohydrates, glycosides, steroids, and flavonoids in the extract.⁽¹⁸⁾ The qualitative phytochemical results have been summarized in table.

Table - Phytochemical screening of root extract

Reagent Name	Observation	Inference
Ferric chloride test	Red coloration	Phenol present
Lead acetate test	White precipitates	
Mayers' test	White creamy and yellow ppt.	Alkaloids present
Wagner test	Brown precipitates	
Ninhydrin test	Purple coloration	Amino acid present
Molisch's test	Deep violet coloration	Carbohydrates present
Fehling test	Brick red ppt.	
Conc. sulphuric acid test	Reddish colour precipitates	Glycosides present
Salkowashi test	Bluish green ring formation	Terpenoids present
Copper acetate test	Green coloration	Diterpenoids present
Lead acetate test	Red precipitates	Tannins present
Salkowashi test	Red coloration	Steroids present
Lead acetate test	Reddish brown ppt.	Flavonoids present

Biogenic synthesis of ZnO NPs: *A. pyrethrum* root extract was used to synthesize ZnO NPs. Figure 1 depicts the UV spectra of ZnO NPs synthesized from *A. pyrethrum* aqueous root extract. The presence of ZnO NPs was confirmed by an absorption peak at 362 nm. The temperature gradient was also used to synthesize ZnO samples at various reaction temperatures. It was found that ZnO NPs could not be synthesized at ambient temperature. Literature reports that the size of biosynthesized NPs can be adjusted by varying the temperature of the solution.⁽¹⁹⁾ It has been reported that increasing the temperature induced an increase in the size of ZnO NPs, indicating that the reduction rate of metal ions increased with temperature, because high temperatures result in excessively fast reaction kinetics.⁽²⁰⁾

Characterization of ZnO NPs: Characterization of ZnO nanoparticles using XRD to support the proof that the synthesized nanoparticles are pure ZnO nanoparticles.⁽³⁾ The XRD diffractogram of ZnO nanoparticles is shown in figure 2 that shows the XRD diffraction pattern produced by ZnO nanoparticles synthesized using root extract. The resulting diffractogram shows fairly sharp peaks which proves that crystalline ZnO nanoparticles have been formed.⁽²¹⁾ This is indicated by the 2θ values at 35.54, 37.61, 39.11, 47.23, 56.11, 58.21, and 68.31 with Miller's indices (100), (002), (101), (102), (110), (103) and (112), respectively.⁽²²⁾ The diffractogram data was used to provide information on the grain size distribution of ZnO nanoparticles. The size distribution of the ZnO nanoparticles that were successfully synthesized varied in size from 10-30 nm. These results support the analysis by UV spectrophotometer which showed that ZnO nanoparticles were formed using bioreductors from the root extract.

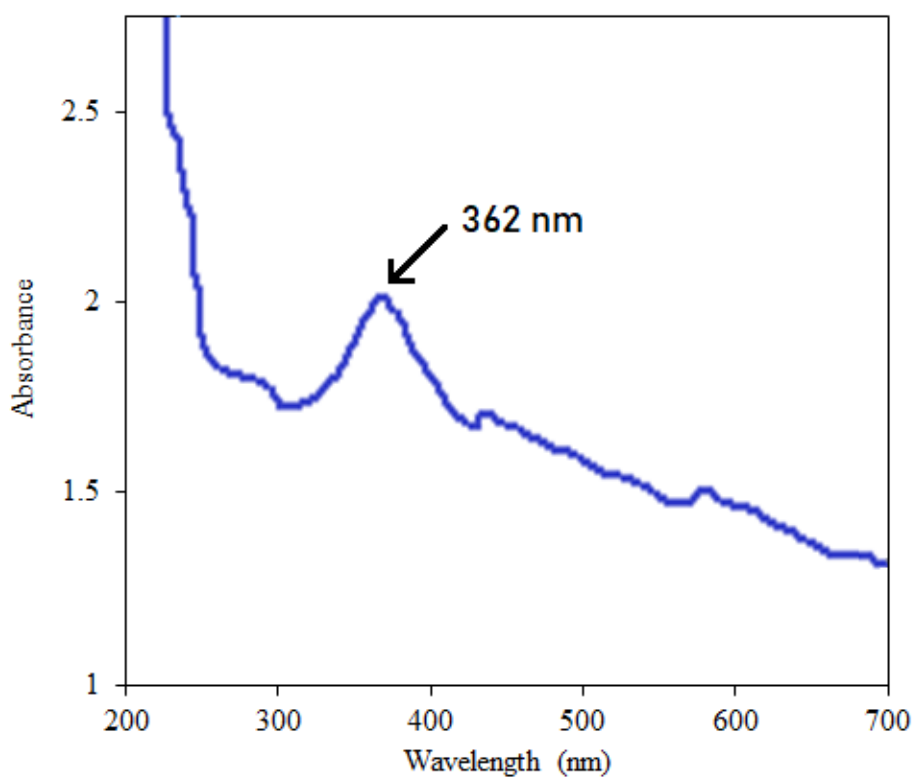


Fig. 1 - UV Spectra of ZnO NPs.

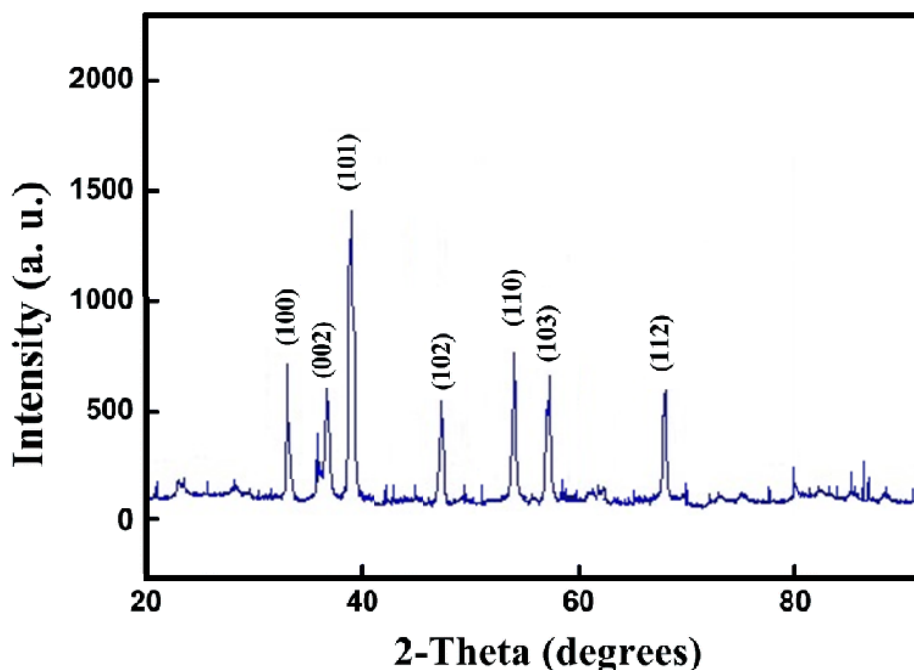


Fig. 2 - XRD diffractogram of ZnO NPs.

The FTIR spectrum showed the presence of different functional groups in the root extract as well as the synthesized nanoparticles. The absorption band of the root extract at (figure 3a) 3422 cm^{-1} is characteristic of the O-H stretching vibrations originating from the group of compounds contained in flavonoids, tannins, terpenoids, saponins and polyphenols.⁽²³⁾ The general characteristic of phenolic compounds is indicated by the presence of absorption band at 1626 cm^{-1} while bands in the region of $1043\text{--}1068\text{ cm}^{-1}$ indicates the presence of CO group.⁽³⁾ The absorption band of wave number 2921 cm^{-1} indicates the presence of C-H stretching vibrations.⁽³⁾ Figure 3b shows a shift in the absorption bands indicating that there is an interaction between functional groups and nanoparticles. The shift in the spectrum of the root extract after the formation of silver nanoparticles in the O-H, C=O and C-O groups indicates that these groups play a role in the silver metal reduction reaction.⁽²²⁾ The absorption band of nanoparticles (figure 3b) at wavenumber 3441 cm^{-1} indicates the presence of O-H stretching vibrations. The

band at 3134 cm^{-1} indicates the presence of a methylene group. The band at 1614 cm^{-1} indicates the presence of phenolic compounds. The band at 1387 cm^{-1} indicates the presence of C-O stretching vibrations. The band at 1021 cm^{-1} indicates the presence of a bending C-O group.⁽²¹⁾

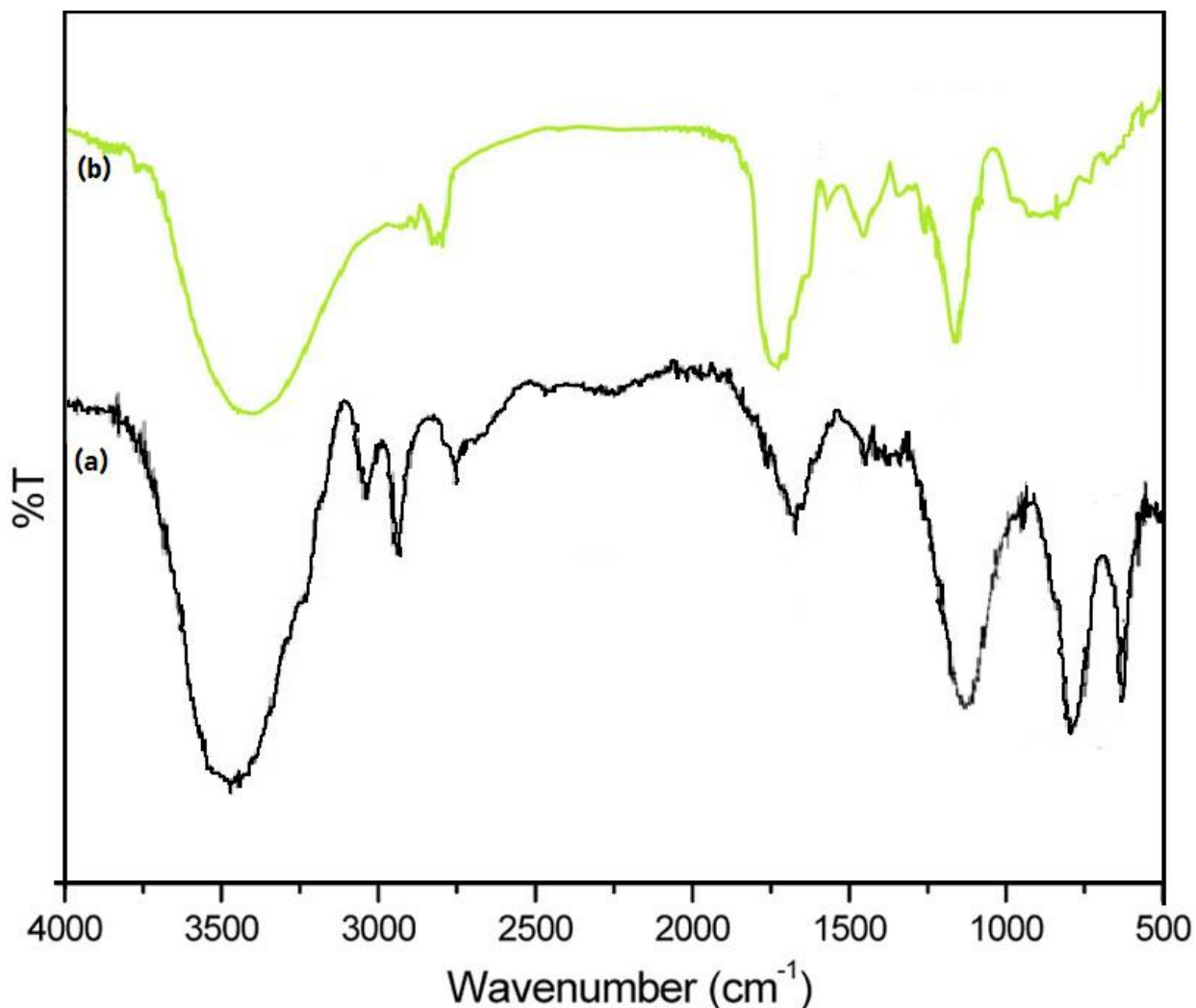


Fig. 3 - FTIR Spectra of (a) Root extract (b) ZnO NPs.

Antioxidant potential of root extract and ZnO nanoparticles: Antioxidants are substances that are able to slow down or prevent the oxidation process caused by free radicals.⁽¹⁶⁾ The measurement results of the antioxidant activity of the root extract and ZnO nanoparticles with varying concentrations can be seen in figure 4.

Based on the antioxidant activity of root extract and ZnO nanoparticles, it shows that the greater the concentration (ppm) used, the greater the antioxidant activity.⁽³⁾ The antioxidant activity of the root extract is lesser than the antioxidant activity of ZnO nanoparticles. The antioxidant activity of the comparison compound, namely vitamin C has been used by many researchers.⁽²⁴⁾ Vitamin C used as a comparison or positive control had antioxidant activity much greater than root extract and ZnO nanoparticles at a concentration of 5 ppm. The IC₅₀ value is inversely proportional to the antioxidant activity of the root extract and ZnO nanoparticles. The greater the antioxidant activity, the smaller the IC₅₀ value.⁽²⁵⁾

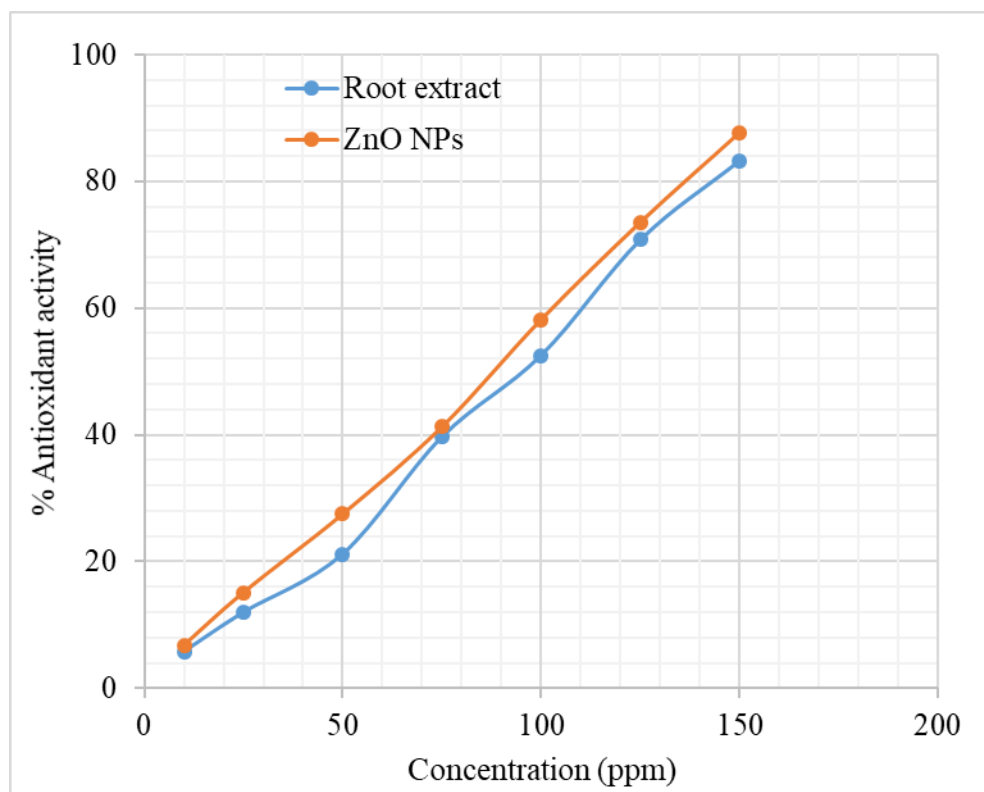


Fig. 4 - Antioxidant potential of root extract and ZnO NPs.

IC₅₀ is a value that indicates the inhibitory ability for 50% of free radicals by a sample concentration (ppm).⁽²⁶⁾ IC₅₀ value was obtained from several steps, namely calculating the concentration value and the inhibition value for each

percentage of DPPH free radical inhibitor activity from the root extract, ZnO nanoparticles and vitamin C.⁽²⁷⁾ Based on the IC50 values for root extract (39.78 µg/mL) and ZnO (45.72 µg/mL), ZnO nanoparticles have a significant antioxidant power as compared to the root extract with a lower IC50 value whose antioxidant power is moderate which is influenced by the number of antioxidant compounds contained in the extract such as flavonoids, saponins, steroids and alkaloids ⁽²⁹⁾. These functional groups get binded to the ZnO nanoparticles and add to their antioxidant activity.⁽²⁹⁾

In this work, *A. pyrethrum* root extract was used as reducing agent as well as capping agent for the synthesis of metal oxide NPs. The synthesis of ZnO NPs was confirmed by the absorption peak at 360 nm and a color change of the solution. The phytochemical analysis of *A. pyrethrum* root extract confirmed the presence of many phytochemicals such as terpenoids, flavonoids, amino acid, alkaloids, phenol, carbohydrates and tannin etc. These phytochemicals facilitate the formation of nanoparticles. Based on the results of the study, it can be concluded that the root extract of *A. pyrethrum* is able to function as a bioreductant in the process of synthesizing ZnO nanoparticles. The synthesis of the slightly pale ZnO nanoparticles having an absorption band at 362 nm with a particle size ranging between 10-30 nm. The root extract and ZnO nanoparticles were found to have significant antioxidant potential and hence can be explored for potential applications in pharmaceutical sector.

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Conflict of interests

The authors report no conflict of interest.

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