

Biosynthesis of Silver Nanoparticles Using *Tridax procumbens* Linn. Leaf Extract and Evaluation of Antioxidant Activity

Gomathi R¹ and Rohini D*

Department of Biochemistry, School of Life Sciences,
Vels Institute of Science, Technology & Advanced Studies

*e-mail: drrohinidurairaj@gmail.com

Abstract

Nanoparticles are ultrafine units with dimensions measured in nanometres (nm; 1 nm =10⁻⁹ meters). Nanoparticles exist in the natural world. In general, nanoparticle-based technologies centre on opportunities for improving the efficiency, sustainability, and speed of already existing processes. The nanoparticles can not only circulate widely throughout the body but also enter cells or be designed to bind to specific cells. An eco-friendly approach for green synthesis of nanoparticles using natural plant and leaf extracts is gaining notable importance.

Objectives: The present study aimed to synthesize silver nanoparticles (AgNPs) using *Tridax procumbens* leaf extract (TPL) and evaluate their antioxidant activity.

Methods: UV-vis spectroscopic, Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Energy dispersive X-ray (EDX) and X-ray diffraction (XRD) techniques respectively. The antioxidant property was evaluated by DPPH, NO, Fe²⁺ chelate, OH and H₂O₂ assays.

Results: The leaf extracts exhibited significant results for the biosynthesis of AgNPs by using silver nitrate as a reducing agent, the synthesis of TPL-AgNPs was ascertained by colour change from yellow to green. The UV-Visible spectroscopy revealed the absorption was observed at 439.20nm. The synthesized TPL-AgNPs have been appropriately characterized using UV-vis spectroscopy, FTIR, SEM, TEM, XRD and EDX analysis. The average size of obtained TPL-AgNPs particle was found to be 76.24 ± 13.19 nm with polydispersed and spherical in shape. Antioxidant activity was tested against DPPH, NO, Fe²⁺ chelate, OH and H₂O₂ assays and the best scavenging assay were observed in DPPH with IC₅₀ value of 52.66 µg/mL. TPL-AgNPs exhibited 92% inhibition at 100 µg/mL concentration.

Conclusion:

Eco-friendly green synthesis offers a sustainable approach for the production of biologically active silver nanoparticles and TPL extract with significant antioxidant potential.

Keywords: *Tridax procumbens*, silver nanoparticles, characterization, anti-oxidant activity.

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Introduction

Nanoparticles are defined as particles that are between 1 and 100 nm in size. Because the surface area compared to volume ratio of these materials has grown, they exhibit new, distinct, and superior physical and chemical characteristics to their massiveness structure [1,2]. One viable method for utilizing underutilized plants in dryland research is the application of nanotechnology. Nanotechnology is an inspiring design that uses biological components and provides a broad, creative platform for the study and conversion of biological systems [3]. Recently, there have been significant

improvements in nanotechnology, such as the use of numerous physicochemical agents and prospective techniques that involve nano-sized metal particles to create novel drugs. These developments make nanotechnology useful for usage in pharmaceutical and natural products [4]. Nanoparticles are essential in electronics, chemistry, medicine, and catalysis. Three distinct techniques chemical, physical, and biological are used to create nanoparticles. Because it is quick, effective, safe, affordable, and environmentally benign, the green synthesis of silver nanoparticles from plants is one of the most intriguing fields [5]. Depending on their

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fundamental shapes, nanoparticles might be composed of metals or nonmetals. Gold, silver, copper, semiconducting, and magnetic (cobalt, nickel) minerals make up the majority of metallic nanoparticles. In contrast, materials based on carbon make up the majority of non-metallic nanoparticles. Because metallic nanoparticles have unique electrical, optical, and catalytic capabilities, they have been the subject of extensive investigation [6]. Among other nanoparticles, silver nanoparticles (AgNPs) have drawn a lot of fascination because of their unique properties [7]. The applications for silver nanoparticles are varied and include drug delivery, biosensing, the creation of nanodevices, and medicine [8]. Due to their natural capping agents and non-toxic mixtures, plants provide an excellent platform for the synthesis of nanoparticles. Silver nanoparticles are widely used in many different disciplines, especially in the clinical and pharmaceutical industries [9]. *Tridax procumbens*, also known as “coat buttons” is a perennial plant from the Asteraceae family, native to Central and South America [10]. *Tridax procumbens* is a very promising species that has many bioactive compounds including alkaloids, flavonoids, tannins, terpenoids, which shows but not limited to: immunomodulatory, anti-oxidant, anti-hepatotoxic, analgesic, antidiabetic, anti-inflammatory, antifungal, neuroprotective and antimicrobial activities [11,12,13,14]. Since oxidative stress contributes to neurodegenerative disorders such as Parkinson’s disease, antioxidant-rich nanoparticles may offer therapeutic effects. Parkinson’s disease is the second most common neurodegenerative disease worldwide with incidence and prevalence on the rise along with changing population demographics [15,16].

Materials and Methods

Biosynthesis of silver nanoparticles (AgNPs)

Fresh leaves of *Tridax procumbens* were collected from Chengalpattu district, Tamil Nadu, India. It was verified by the University of Madras' Botany Department in Guindy. The remaining reagents and chemicals utilized in the study were of analytical grade. For the hydroethanolic leaf extract, the fresh leaves were cleaned with distilled water, shade-dried for 20 to 30 days, and ground into fine powder. About 10 g of leaf powder were subjected to cold extraction following the maceration method using hydroethanolic solution [Ethanol and water (70:30)] for 24 h with intermittent shaking. The extract was further filtered through Whatman No. 1 filter paper

and the filtrate was stored at 4°C for further analysis [17]. In the biosynthesis of AgNPs, 5 mL of *Tridax procumbens* leaf extract was mixed with 45 mL of freshly made 1mM silver nitrate aqueous solution (AgNO₃) in a 250 mL Erlenmeyer flask and continuously stirred for 5 hours at room temperature in the dark with a rotation speed of 200 rpm . A control setup without the leaf extract was also maintained in parallel. After incubation, the leaf extract solution colour changed from light yellow to green, while the silver nitrate solution without the leaf extract remained colour unchanged [18]. The mixture was then incubated for 1 h and the AgNPs were isolated through centrifugation at 15,000 rpm for 1 h. The isolated AgNPs were further washed with deionized water, dried and stored in a refrigerator for further characterization studies

Characterization of techniques

To determine the absorbance value of the nanoparticles in the range of 200–1000 nm, a UV-Vis spectrophotometric study was performed [19]. FTIR analysis was performed using Spectrophotometer system, which was used to detect the characteristic peaks in ranging from 400-4000 cm⁻¹ and their functional groups [20]. The peak values of the UV and FTIR were recorded. SEM-EDX, or scanning electron microscopy combined with energy-dispersive X-rays, was employed to examine the composition and structure of TPL-AgNPs. Using imageJ software, a histogram of the TPL-AgNPs' size distribution was produced [21]. Transmission electron microscopy (TEM) technique was used to visualize the morphology of the TPL-AgNPs [22]. The instrument was operated at an accelerating voltage of 200 kV with ultra-high-resolution of 0.2 nm and magnification of 2,000,000 X. TEM's grid size is 3 mm diameter which was prepared placing a 5µl of the TPL-AgNPS solutions on carbon-coated copper grids and drying under mercury lamp and then analysed. The crystalline structure of the TPL-AgNPs was investigated by X-ray diffraction (XRD), using Cu-K α as the radiation source ($\lambda=1.54$), the XRD investigation was performed at a voltage and current of 40 KV and 30 mA, respectively. Temperature-dependent strengths were recorded at ambient temperature in steps of 0.05, throughout a range of 25 to 80°C, with the diffractometer attached to a computer for data collection and characterization displays. The scanning of biosynthesized silver nanoparticles exercised in 2 θ region range from 30° - 80° and the data is analysed [23].

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Anti-oxidant activity

DPPH radical scavenging activity

The DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay is commonly employed to evaluate the antioxidant potential of the relevant samples and ascorbic acid (as a standard).

The scavenging activity was determined by assembling the ability of the samples to donate hydrogen atoms or electrons, and the IC₅₀ values were calculated. The DPPH radical scavenging assay was studied through discoloration technique. To test various concentrated solutions (20–100 µg/mL), 2 mL of 0.1 mM DPPH (standard solution) was added to 0.5 mL of synthesised AgNPs and *Tridax procumbens* leaf extract diluted in methanol. Additionally, ascorbic acid standard solutions were also included for comparison. After mixing the solutions were allowed to incubate for 30 minutes in the dark at room temperature. UV-Vis spectrophotometer was used to determine the absorbance against the standard at 517 nm to evaluate the radical scavenging activity [24,25,26].

Nitric oxide scavenging activity

Nitric oxide (NO) operates as a signal that radically changed our perception of free radicals from hazardous byproducts of oxidative metabolism to important cellular regulators and it is a crucial bioregulatory molecule involved in various physiological processes, including immune response, neurological function and cardiovascular health. Nitric oxide (NO) radicals were generated from sodium nitroprusside. A mixture of 2 mL of 10 mM sodium nitroprusside, 0.5 mL of 0.2 M phosphate-buffered saline (pH 7.4) and various concentrations (20–100 µg/mL) of AgNPs and *Tridax procumbens* leaf extract was incubated at 25°C for 150 minutes. Following incubation, After the incubation period, 0.5 ml of Griess reagent was added (1% sulphanilamide, 2% H₃PO₄ and 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance was measured at 540 nm and the percentage of inhibition was calculated using the equation above [27]. Additionally, the antioxidant capacity was quantified by determining the IC₅₀ value, which represents the concentration required to inhibit 50% of radical activity, calculated by comparing with a standard ascorbic acid linear plot.

Fe²⁺ chelating activity

The metal chelating activity of the extract was assayed by Dinis *et al.*, (1994) method [28]. To 0.5

ml of extract, 1.6 ml of deionized water and 0.05 ml of FeCl₂ (2mM) was added. After 30s, 0.1 ml ferrozine (5mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 minutes at room temperature, the absorbance of the Fe²⁺- Ferrozine complex was measured at 562 nm. The chelating activity of the extract was quantified by determining the IC₅₀ value, which represents the concentration required to inhibit 50% of radical activity, calculated by comparing with a standard ascorbic acid linear plot.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity characterizes a compound's ability to neutralize highly reactive hydroxyl radicals thereby preventing cell damage and also it protects biomolecules including DNA, proteins, and lipids from oxidative damage and is a crucial indicator of antioxidant ability [29]. The hydrogen peroxide was added to the reaction mixture containing 60µl of 1.0mM FeCl₃, 90µl of 1mM 1,10- phenanthroline, 2.4 ml of 0.2M phosphate buffer (Ph 7.8), 150µl of 0.17M H₂O₂, and 1.5 ml of extract at various concentrations (20–100µg/mL) of AgNPs and *Tridax procumbens* leaf. After incubation at room temperature for 5 min, the absorbance of the mixture at 560 nm was measured with a spectrophotometer. The percentage of inhibition was then calculated by comparing with a standard ascorbic acid linear plot.

H₂O₂ radical scavenging activity

One kind of reactive oxygen species (ROS) that can build up in living cells is hydrogen peroxide, which can then produce additional dangerous ROS including hydroxyl and peroxide radicals. These species have the potential to seriously impair cell membranes and a substance's capacity to neutralize hydrogen peroxide and stop it from turning into dangerous hydroxyl radicals [30]. 1ml of hydrogen peroxide solution was added into various concentrations (20–100µg/mL) of AgNPs and *Tridax procumbens* leaf extract and incubated for 5 minutes at room temperature. After 5 mins, 2 ml of potassium dichromate: acetic acid reagent was added and the tubes were incubated for 10 minutes at room temperature. The absorbance value of the reaction mixture was recorded at 700 nm. The percentage of inhibition was calculated by comparing with a standard ascorbic acid linear plot [31].

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Result and Discussion:

Material characterization

A study on photosynthesis of Ag nanoparticles by the aqueous leaf extract was carried out in this work. During the visual observation, silver nitrate

incubated with leaf extract showed a colour change from light yellow to green after 5 hrs whereas no colour change could be observed in silver nitrate without leaves extract. The appearance of green colour in extract treated flask is clear indication for the formation of Ag nanoparticles [32] (**figure:1**).

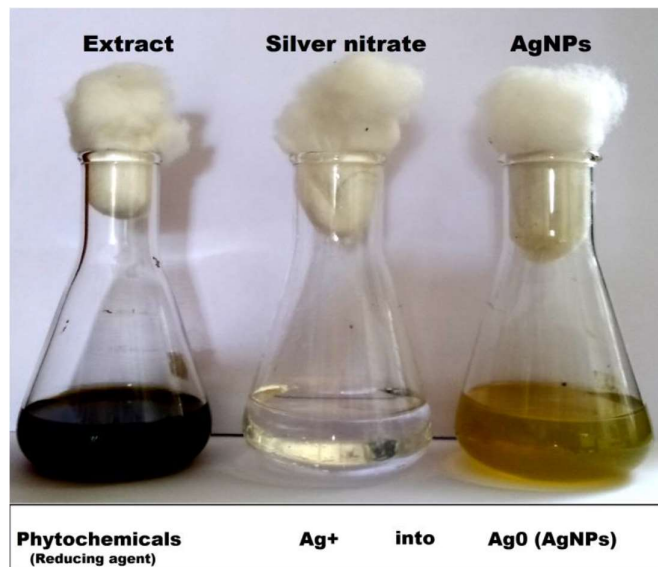


Figure: 1 Colour changes before (*Tridax Procumbens* Leaves extract) and after (AgNPs) the process of reduction of Ag⁺ to Ag nanoparticles and control (AgNO₃)

AgNO₃: 1 mM AgNO₃ (White colour)

AgNPs: 1 mM AgNO₃ in the presence of extract after 5 hours of incubation (Green colour)

Silver nanoparticles (AgNPs) were synthesized through an eco-friendly approach using silver nitrate (AgNO₃) solution and *Tridax procumbens* leaf extracts (as both capping and reducing agent) was characterized by various techniques. The formation of the reduced silver nanoparticles in colloidal solution was monitored by using a UV-vis spectrophotometer. In the UV-vis spectra of the reaction mixture of silver nitrate solution with extract the peak (**figure:2**) was observed at 439.20nm indicating the presence of silver nanoparticles which is synthesized by the extract [33].

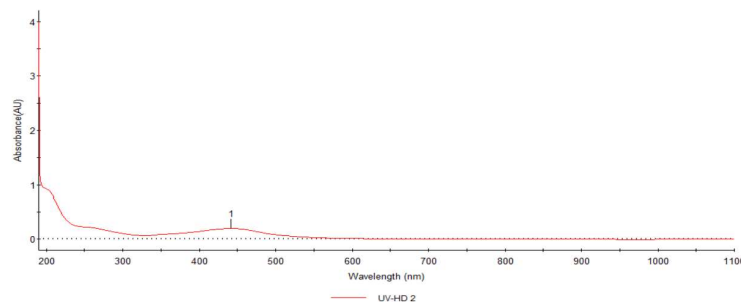


Figure: 2 UV-Visible Spectroscopic analysis of AgNPs

The FTIR spectra provide information about the organic molecules on the surface of nanoparticles. The FTIR spectrum used to identify the various functional groups present in a AgNPs represent in **Figure 3**. The peaks in

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the region 3436.60cm^{-1} were assigned to O-H stretch, H-bonded, indicating the presence of hydroxyl groups such as alcohol and phenol compounds. 2075.26cm^{-1} were assigned to $\text{C}\equiv\text{C}$ stretch, indicating the presence of Alkyne groups. 1634.22cm^{-1} were assigned to N-H bend, indicating the presence of 1° amines. 667.74cm^{-1} is assigned to N-H wag, indicating the presence of 1° and 2° amines [34]. FTIR spectra showed the presence of alcohol, phenol, hydroxyl, alkyne and amine groups in the extract responsible for predicting their role in nanoparticle synthesis and the reduction of silver ions as well as the capping agents for the stability of biogenic nanoparticle solution [35,36].

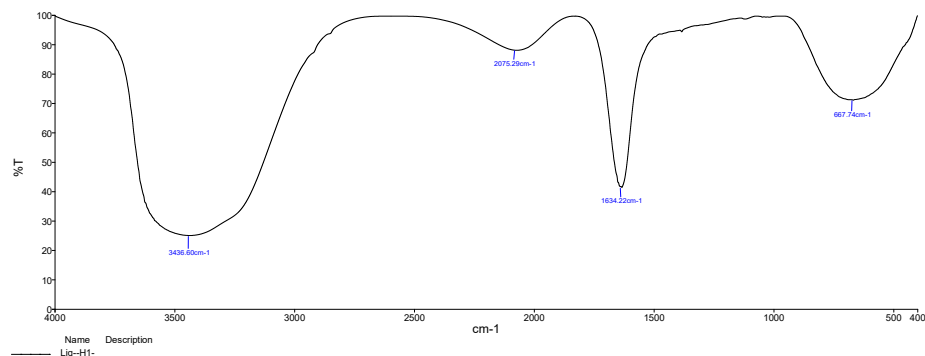


Figure: 3 FTIR Spectroscopic analysis of AgNPs

X-ray Diffraction (XRD) analysis was performed to characterize the synthesized AgNPs and to evaluate their purity and crystalline structure. The XRD pattern of the AgNPs displayed five distinct diffraction peaks at 38.85 , 45.61 , 53.92 , 63.76 and 77.85 2θ values corresponding to the planes (111), (200), (211), (220) and (311) representing Bragg's reflections of metallic silver [43]. This results clearly indicates the formation of crystalline cubic face-centred structure, as identified in the standard powder diffraction card of JCPDS, silver file No. 04-0783 (Fig. 2(a)). The average crystallite size was determined using Debye-Scherrer formula, $D = 0.9 \lambda / \beta \cos \theta$, where ' λ ' is the wavelength of X-ray (0.1541 nm), ' β ' is the FWHM (Full Width at Half Maximum), ' θ ' is the diffraction angle and ' D ' is the particle diameter size. The synthesized AgNPs were in the range of 18 – 61 nm [40,41,44,45].

SEM analysis revealed high-density, polydispersed spherical nanoparticles with sizes ranging from ~ 44 – 99 nm, confirming their nanoscale nature (<100 nm) **Figure:4**. ImageJ-based histogram analysis showed an average particle size of 76.24 ± 13.19 nm, with most particles distributed between 60 – 100 nm and minimal aggregation, indicating good dispersion and crystalline characteristics [37,38,39]. TEM analysis, offering higher spatial resolution than SEM, revealed polydispersed, spherical nanoparticles. The synthesized AgNPs ranged in size from 18 to 61 nm, confirming their nanoscale dimensions **Figure:5** [40,41].

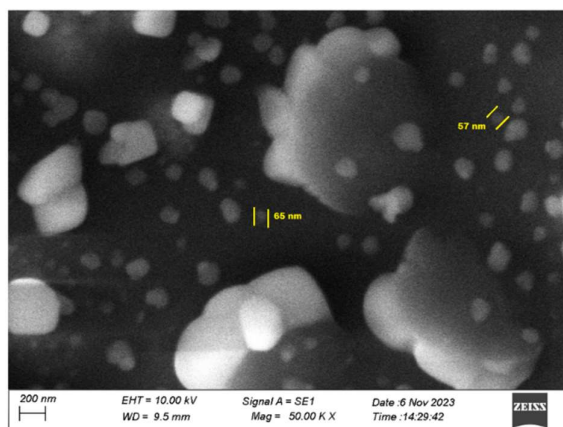


Figure: 4 Scanning Electron Microscopy analysis of NPs

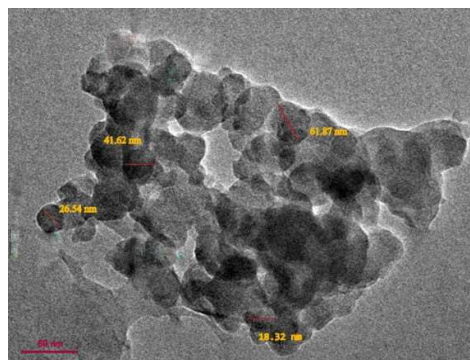


Figure: 5 Transmission Electron Microscopy (TEM) analysis of AgNPs

The assessment of the elemental composition on the surface of the biosynthesized nanoparticle was done using EDX spectroscopy. The EDX elemental analysis of the synthesized silver nanoparticles showed highest proportion by weight of silver (60.36%) followed by oxygen (30.01%) and sodium (9.63%) **Figure 6**. EDX peaks corresponding to element silver show the presence of silver as the ingredient element and the formation and purity of silver nanoparticles synthesized from extract [42].

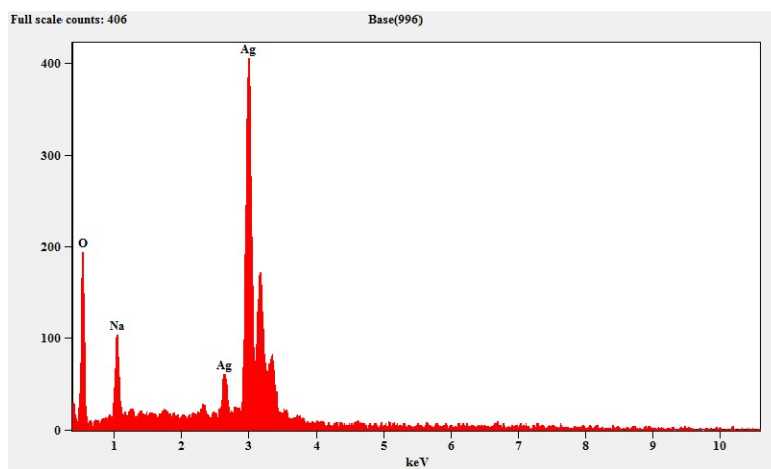


Figure: 6 EDX spectrum of AgNPs

In-Vitro Anti-oxidant activity

DPPH: Hydro-ethanolic leaf extract (TPL) and TPL-AgNPs were evaluated for their ability to scavenge DPPH radicals and compared with standard ascorbic acid. The percentage of inhibition for the TPL extract, TPL-AgNPs, and standard ascorbic acid at 20 $\mu\text{g/ml}$ was 17.70 ± 1.37 , 19.39 ± 1.29 , and 22.59 ± 1.21 , whereas the percentages for 100 $\mu\text{g/ml}$ were 83.23 ± 1.25 , 87.19 ± 1.17 , and 94.91 ± 1.09 , respectively **Figure:7a**. As the concentration of the tested substances increased, a progressive rise in the percentage of inhibition was noted [46]. TPL extract, TPL-AgNPs, and conventional ascorbic acid were shown to have respective IC_{50} values of 56.74, 52.66, and 48.96 $\mu\text{g/ml}$. TPL-AgNPs demonstrated the highest antioxidant activity among the studied samples.

Nitric oxide scavenging activity: The percentage of inhibition for the TPL extract, TPL-AgNPs, and standard ascorbic acid at a concentration of 20 $\mu\text{g/ml}$ was 16.49 ± 1.54 , 18.71 ± 1.37 , and 21.33 ± 1.23 , whereas the percentages for 100 $\mu\text{g/ml}$ were 84.94 ± 1.41 , 86.78 ± 1.42 , and 95.54 ± 1.31 in respectively **Figure:7b**. TPL extract, TPL-AgNPs,

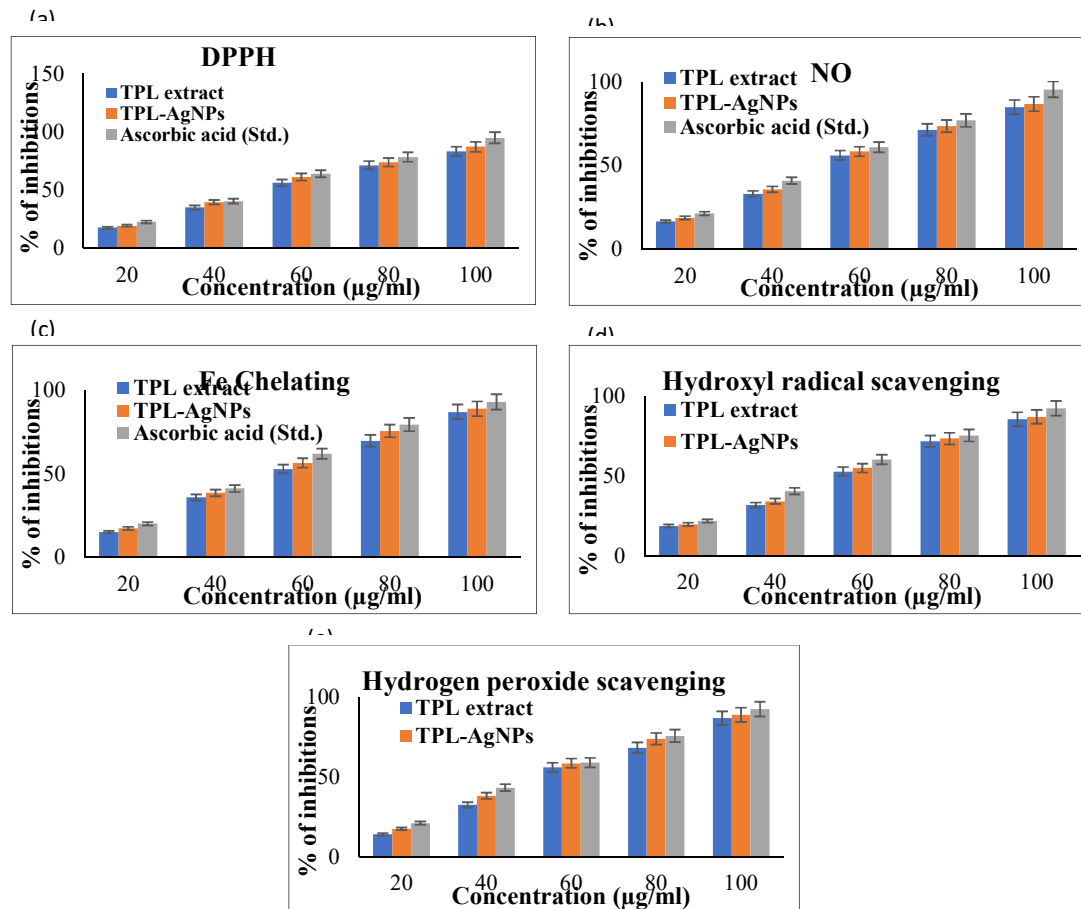
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and standard ascorbic acid were shown to have respective IC₅₀ values of 57.25, 54.65, and 50.07 µg/ml. The antioxidant activity of TPL-AgNPs is superior to that of the other examined samples [47,48].

Iron chelating activity: TPL extract and TPL-AgNPs' ability to chelate iron was evaluated and contrasted with that of regular ascorbic acid. The percentage of inhibition at 20 µg/ml was 15.07±1.35, 17.26±1.22, and 20.09±1.31, but at 100 µg/ml, it was 86.97±1.18, 88.85±1.36, and 92.93±1.25 in respectively **Figure:7c**. For every tested concentration, the percentage of antioxidant activity increased in a dose-dependent manner. TPL extract, TPL-AgNPs, and conventional ascorbic acid were shown to have respective IC₅₀ values of 57.68, 54.13, and 50.14 µg/ml. TPL-AgNPs had the highest activity of all the evaluated samples [49].

Hydroxyl radical scavenging activity: The hydroxyl radical scavenging capabilities of TPL-extract and TPL-AgNPs were evaluated and contrasted with those of regular ascorbic acid. At 20 µg/ml, the percentage of inhibition was 18.77±1.32, 19.77±1.28, and 21.89±1.25, but at 100 µg/ml, it was 85.69±1.27, 87.15±1.33, and 92.51±1.25 in respectively **Figure:7d**. For all tested concentrations, the percentage of antioxidant activity increased in a dose-dependent manner. The TPL-extract, TPL-AgNPs, and conventional ascorbic acid were shown to have respective IC₅₀ values of 57.39, 55.47, and 50.67 µg/ml. TPL-AgNPs had higher hydroxyl radical scavenging activity than TPL-extract [50].

Hydrogen peroxide scavenging activity: The ability of TPL-extract and TPL-AgNPs to scavenge hydrogen peroxide was evaluated and contrasted with that of regular ascorbic acid. The percentage of inhibition at 20 µg/ml was 14.19±1.28, 17.58±1.35, and 21.18±1.24, whereas at 100 µg/ml, it was 86.86±1.37, 88.98±1.26, and 92.58±1.29 in respectively **Figure:7e**. For every tested concentration, the percentage of antioxidant activity increased in a dose-dependent manner. TPL-extract, TPL-AgNPs, and conventional ascorbic acid were shown to have respective IC₅₀ values of 58.17, 53.82, and 50.37 µg/ml. TPL-AgNPs had the highest activity of all the evaluated samples [51].



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Figure 7. *In-Vitro* antioxidant studies of TPL extract, TPL-AgNPs and std. ascorbic in (a) DPPH radical scavenging activity, (b) Nitric oxide scavenging activity, (c) Iron chelating activity, (d) Hydroxyl radical scavenging activity and (e) Hydrogen peroxide scavenging activity.

Conclusion

The biosynthesized AgNPs through the reduction of silver nitrate using *Tridax procumbens* leaf extract exhibited a significant antioxidant radical scavenging activity, with the effect increasing in a dose-dependent manner. The AgNPs displayed superior antioxidant activity, approaching that of the standard ascorbic acid. Hence, this green eco-friendly synthesis method provides a promising alternative to traditional physical or chemical approaches for the production of nanoparticles, in particular *Tridax procumbens* leaf extract. The leaf-mediated synthesis process inherently have bioactive phytoconstituents which are biocompatible and offer a range of potential applications such as anti-inflammatory, antiarthritic and anti-oxidant properties. These findings suggest that green-synthesized TPL-AgNPs possess promising antioxidant properties and may have potential applications in biomedical and pharmaceutical fields.

Declaration of competing interest

The authors claim that no known conflicting financial interests or personal relationships appeared to have an impact on the work that was published in this paper.

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