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Dr. Vishnu Kiran Manam
Senior Scientist / DGM – R&D,
IB Group, Indamara,
Chhattisgarh, India

Corresponding Author:
Dr. Vishnu Kiran Manam
Senior Scientist / DGM – R&D,
IB Group, Indamara,
Chhattisgarh, India

Nano-antioxidants: Investigating chemically synthesized silver nanoparticles

Vishnu Kiran Manam

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Abstract
Silver nanoparticles (AgNPs) have gained significant attention in recent years due to their remarkable biological properties, including antioxidant activity. In this study, silver nanoparticles were chemically synthesized using 1% trisodium citrate as a reducing agent and 0.01 M silver nitrate solution as a precursor. The synthesis process involved vigorous stirring and heating, resulting in a color change from mild colorless to pale brown, indicating the formation of silver nanoparticles. The obtained nanoparticles were centrifuged, purified, and dried for further analysis. Characterization of the silver nanoparticles was performed using UV-visible spectroscopy, X-ray diffraction (XRD), Thermo Gravimetric Analysis, and Transmission Electron Microscopy (TEM). The antioxidant potential of the synthesized AgNPs was evaluated using standard free radical scavenging assays. The results demonstrated that the chemically synthesized silver nanoparticles exhibit significant antioxidant activity, highlighting their potential application in nanomedicine, food preservation, and biomedical industries. This study provides insights into the synthesis, characterization, and functional properties of silver nanoparticles as promising nano-antioxidants.

Keywords: Nanomedicine, silver nanoparticles, chemical reduction synthesis, antioxidant activity

Introduction
Nanotechnology has emerged as a transformative field, offering innovative solutions across various disciplines, including healthcare, food preservation, and environmental applications. Among the diverse nanoparticles synthesized, silver nanoparticles (AgNPs) have garnered immense attention due to their unique physicochemical properties and biological activities, including antimicrobial, antifungal, anti-inflammatory, and antioxidant potential [1], [13], [23], [24], [27]. Silver nanoparticles are particularly promising as nano-antioxidants, given their ability to scavenge free radicals and reduce oxidative stress, which plays a critical role in the prevention of chronic diseases and cellular damage [17].

Chemical synthesis is one of the most widely employed approaches for AgNP production, as it allows precise control over nanoparticle size, shape, and stability. Typically, silver nanoparticles are synthesized through the reduction of silver ions (Ag^+) in the presence of reducing agents such as sodium borohydride, ascorbic acid, or trisodium citrate [21]. In this context, trisodium citrate serves as both a reducing and stabilizing agent, facilitating the formation of stable and uniform silver nanoparticles. A characteristic color change during the reduction process, from pale colorless to brown, is often attributed to the surface plasmon resonance (SPR) of silver nanoparticles, which is a key indicator of successful synthesis [18].

The antioxidant activity of silver nanoparticles is of significant interest due to their potential applications in combating oxidative stress-induced cellular damage. Oxidative stress occurs when there is an imbalance between free radical production and antioxidant defense mechanisms, leading to lipid peroxidation, protein oxidation, and DNA damage [8]. Nanoparticle-based antioxidants, or nano-antioxidants, exhibit enhanced reactivity and bioavailability compared to conventional antioxidants due to their high surface area-to-volume ratio and nanoscale dimensions [2].

The present study focuses on the chemical synthesis of silver nanoparticles using trisodium citrate as a reducing agent and their subsequent characterization using various analytical techniques. The antioxidant potential of the synthesized nanoparticles was evaluated to highlight their efficacy as nano-antioxidants.

This investigation aims to provide a foundation for utilizing silver nanoparticles in biomedical, food, and environmental applications, where antioxidant properties are of paramount importance.

Review of Literature

The increasing interest in silver nanoparticles (AgNPs) is primarily due to their unique physicochemical properties, which result in various biological activities, including antioxidant, antimicrobial, and anti-inflammatory effects. Among these properties, the antioxidant potential of AgNPs has gained significant attention, owing to the growing need for effective nano-antioxidants that can combat oxidative stress-related diseases and enhance therapeutic efficacy. This section reviews the synthesis, characterization, and antioxidant applications of chemically synthesized silver nanoparticles, emphasizing their mechanisms and potential uses.

The synthesis of silver nanoparticles can be accomplished via several methods, with chemical reduction being the most common approach. Silver ions (Ag^+) are typically reduced using chemical-reducing agents like sodium borohydride, glucose, or trisodium citrate. Trisodium citrate is particularly advantageous because it acts as both a reducing and stabilizing agent, ensuring the formation of stable nanoparticles with controlled sizes [21]. The reduction process often involves heating the silver nitrate solution in the presence of trisodium citrate, which facilitates the transformation of silver ions into elemental silver, resulting in nanoparticles that are stabilized by the citrate ions. This method has been shown to produce nanoparticles with excellent stability and well-defined characteristics, including size, morphology, and surface charge [7].

The color change from colorless to brown during the reduction of silver ions is a characteristic feature of AgNPs, attributed to their surface plasmon resonance (SPR) properties [25, 26]. The SPR absorption band is a unique optical signature of silver nanoparticles, and it can be detected using UV-visible spectroscopy, which is a key tool for characterizing the nanoparticles [18]. The particle size and distribution of AgNPs are influenced by factors such as the concentration of the precursor, the type and concentration of the reducing agent, and the reaction time, all of which can be controlled to achieve nanoparticles with desired properties [4].

Silver nanoparticles exhibit significant antioxidant properties due to their high surface area-to-volume ratio and nanoscale dimensions, which enhance their interaction with free radicals and other reactive oxygen species (ROS) [29]. The antioxidant activity of AgNPs is mainly attributed to their ability to scavenge free radicals such as hydroxyl radicals (•OH), superoxide anions (O_2•−), and hydrogen peroxide (H_2O_2), which are associated with oxidative stress and various pathological conditions, including cancer, cardiovascular diseases, and neurodegenerative disorders [17].

Several studies have explored the mechanism of antioxidant action of AgNPs. These nanoparticles are capable of donating electrons to free radicals, thus neutralizing them and preventing oxidative damage. The nanoscale size of AgNPs also allows them to penetrate cellular membranes and interact directly with intracellular ROS, thereby protecting against oxidative stress at the cellular level [15]. For example, silver nanoparticles synthesized using plant extracts, such as *Azadirachta indica* (neem), have demonstrated enhanced antioxidant activity, potentially due to the synergistic effect of both the nanoparticles and the plant-based biomolecules present on their surface [1].

In addition to their direct free radical scavenging ability, AgNPs can also enhance the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). These enzymes play a crucial role in mitigating oxidative stress by breaking down reactive oxygen species (ROS) within biological systems. Studies have shown that AgNPs can boost the expression and activity of these antioxidant enzymes, thereby further enhancing their protective effects against oxidative damage [9, 10, 13, 24].

Due to their potent antioxidant properties, silver nanoparticles have been investigated for a range of biomedical applications, particularly in the field of nanomedicine. AgNPs have shown promise as potential therapeutic agents for treating oxidative stress-related diseases, including cancer and neurodegenerative diseases such as Alzheimer's and Parkinson's [16]. Moreover, their antioxidant properties make them suitable candidates for use in drug delivery systems, where they can protect therapeutic agents from oxidative degradation and enhance their stability.

In addition to their biomedical applications, AgNPs are also being explored in food preservation, water purification, and environmental remediation. The antioxidant properties of AgNPs can help preserve food quality by preventing lipid peroxidation and spoilage caused by oxidative stress. Furthermore, their ability to neutralize free radicals may improve the shelf life of packaged food products [29]. Additionally, AgNPs have shown potential in water treatment by removing toxic pollutants and reactive oxygen species in contaminated water systems [19].

The chemically synthesized silver nanoparticles, particularly those produced using trisodium citrate as a reducing agent, have demonstrated remarkable antioxidant properties, making them valuable candidates for various biomedical and environmental applications. Their ability to scavenge free radicals, enhance endogenous antioxidant systems, and mitigate oxidative stress has positioned them as promising nano-antioxidants. Further research into their synthesis, characterization, and biological effects is essential for advancing their practical applications in nanomedicine, food industries, and environmental science.

Materials & Methods

Chemical Synthesis of Silver Nanoparticles: An aqueous solution of 0.01 M silver nitrate was heated to boiling, and silver nanoparticles were synthesized using 1% trisodium citrate as a reducing agent. During the process, the solution was vigorously stirred while heating. Upon exposure to 1% trisodium citrate, the aqueous silver ions were reduced, leading to a color change from colorless to pale brown, indicating the formation of silver nanoparticles. The resulting nanoparticle solution was stirred on a magnetic stirrer at 90 °C for 20 minutes. The reaction mixture was then centrifuged at 6000 rpm for 15 minutes, and the pellet was collected, rinsed three times with triple-distilled water, and dried in a hot air oven at 80 °C [6, 20, 28]. The synthesized silver nanoparticles were subsequently characterized using UV-visible spectroscopy, X-ray diffraction (XRD), and transmission electron microscopy (TEM).

Characterization of silver nanoparticles

The characterization of silver nanoparticles (AgNPs) synthesized by the chemical reduction method was performed using advanced analytical techniques. UV-VIS spectroscopy (SHIMADZU UV-3150PC) was utilized to monitor the reduction of silver ions within a wavelength range of 200–600 nm, with water as a reference. TEM analysis (JEOL 2000 EX) provided insights into the morphology and structural features of AgNPs, employing a carbon-coated copper grid for sample preparation and operating at 80 keV [14]. X-ray diffraction (XRD) analysis, conducted using a Rich Seifert P3000 with Cu-Kα1 radiation, was applied to determine crystal structure, phase purity, and nanoparticle size using Bragg's law and the Debye–Scherrer equation [3],[5]. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) assessed the thermal stability and purity of lyophilized AgNPs (Hitachi STA7200, TA Instruments Q200).

DPPH free radical scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of the chemically synthesized nanoparticles was evaluated using a spectrophotometric method [12]. For the assay, 0.05 mL of silver nanoparticles at varying concentrations (100–900 µg/mL) was added to a methanolic solution of DPPH (200 µM). A control sample, containing an equal amount of methanol without the test sample, was also prepared. The scavenging activity was determined by measuring the decrease in absorbance of the reaction mixture at 517 nm after 20 minutes of incubation. The reduction in absorbance indicated the quenching of DPPH free radicals. The percentage inhibition was calculated using the appropriate formula [11]. All experiments were performed in triplicates to ensure accuracy and reproducibility.

$$\text{Inhibition (\%)} = \frac{(\text{Control- Test})}{\text{Control}} \text{X } 100$$

ABTS radical cation decolorization assay

The radical cation decolorization assay was conducted using an improved method involving ABTS•$^{+}$, where the oxidant is generated through the peroxydisulfate oxidation of 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{2-}$). The ABTS radical cation (ABTS•$^{+}$) was prepared by reacting a 7 mM ABTS solution with 2.45 mM ammonium persulfate, and the reaction mixture was left to stand in the dark at room temperature for 12–16 hours [22]. For the assay, 0.5 mL of silver nanoparticles at varying concentrations (100–900 µg/mL) was added to 0.3 mL of the ABTS•$^{+}$ solution. Ethanol was used to bring the total volume to 1.0 mL. The absorbance of the reaction mixture was measured at 745 nm using a spectrophotometer, and the percentage inhibition (%) was calculated using the appropriate formula. This experiment was designed to evaluate the antioxidant potential of the chemically synthesized nanoparticles.

$$\text{Inhibition (\%)} = \frac{(\text{Control- test})}{\text{Control}} \text{X } 100$$

Results

Chemical Synthesis of Silver Nanoparticles

The first visual confirmation of the successful synthesis of silver nanoparticles (AgNPs) was observed during the reaction process. When aqueous silver ions were exposed to a 1% solution of trisodium citrate, a reduction reaction occurred, resulting in a distinct color change from colorless to pale brown (Figure 1). This color change is a clear indication of the formation of silver nanoparticles.

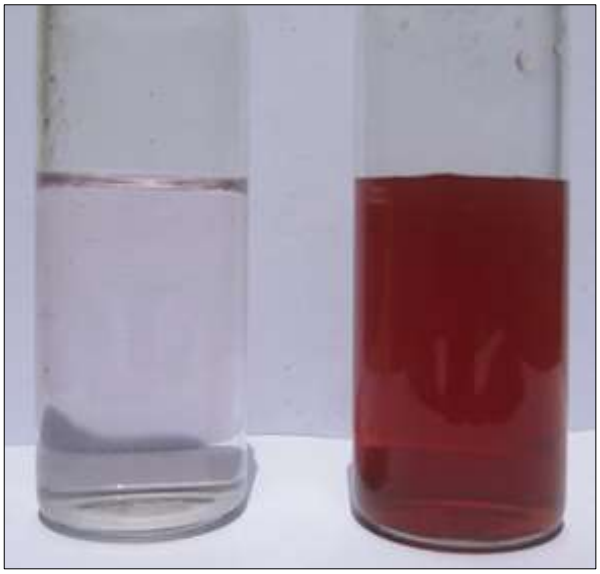

Fig 1: Synthesis of silver nanoparticles by chemical reduction method

Characterization of silver nanoparticles

The chemically synthesized silver nanoparticles (AgNPs) were thoroughly characterized using various analytical techniques. The UV-Vis spectrophotometer analysis revealed a distinct absorption maximum at 420 nm, indicating the formation of polydisperse AgNPs. The peak broadening suggested that the AgNPs had a wide size distribution. X-ray diffraction (XRD) analysis confirmed the crystalline nature of the AgNPs, exhibiting a face-centered cubic (FCC) structure. The XRD pattern showed four distinct diffraction peaks, corresponding to the {111}, {200}, and {220} planes, with a particle size range of 10-50 nm. Thermogravimetric analysis (TGA) was performed to assess the purity and thermal stability of the AgNPs. The results indicated that the AgNPs had a stability of up to 300 °C, with a minor coating of chemical molecules on the surface at around 250°C. Transmission Electron Microscopy (TEM) analysis revealed the diverse morphology of the AgNPs, including spherical, cubic, and hexagonal shapes, with a size range of 5-50 nm. The TEM images also showed the aggregation of the nanoparticles. The comprehensive characterization of the chemically synthesized AgNPs using UV-Vis spectroscopy, XRD, TGA, and TEM provided insights into their physical and chemical properties, including their size, shape, crystallinity, and thermal stability.

DPPH free radical scavenging activity

The chemically synthesized silver nanoparticles (AgNPs) demonstrated a notable antioxidant activity when tested using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. At a concentration of 100 µg/mL, the AgNPs were able to scavenge 60.31% of the DPPH radicals.Moreover, the maximum antioxidant activity observed was 72.34%, which was achieved at a higher concentration of 900 µg/mL (Table 1 and Figure 3).

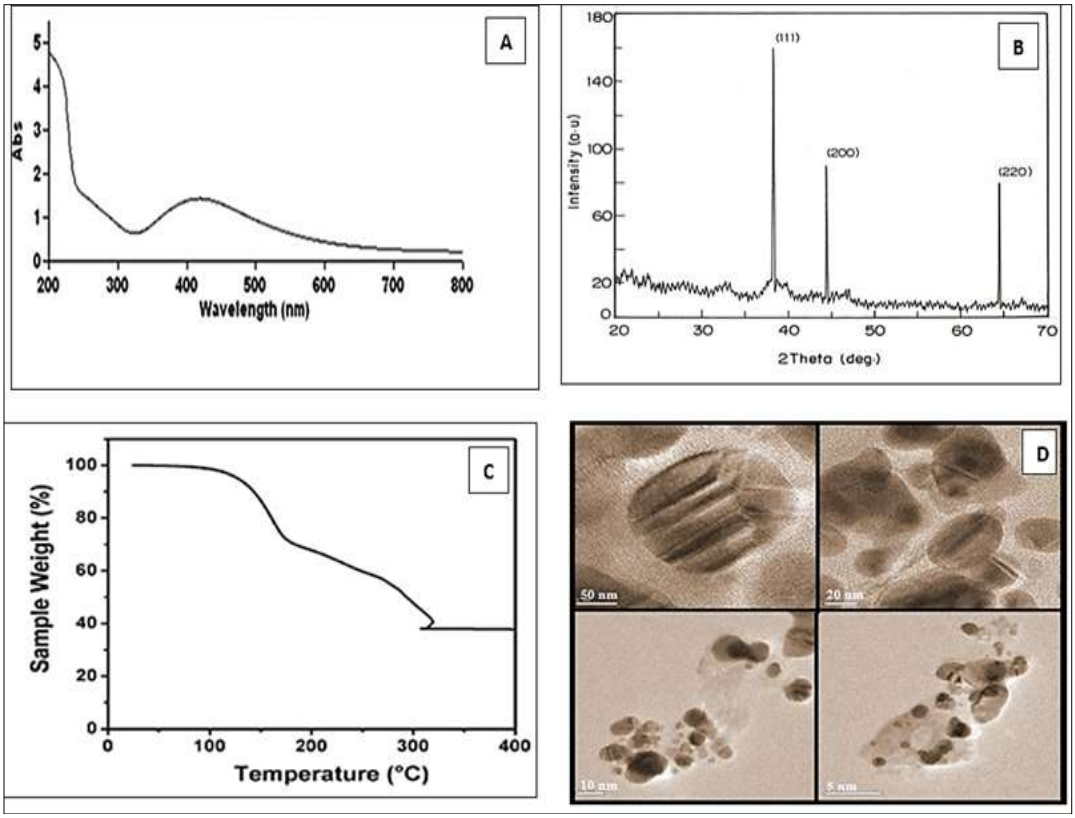

Fig 2: Characterization of silver nanoparticles by chemical reduction method: [A] UV-Vis Spectroscopy analysis, [B] XRD diffraction analysis, [C] Thermogravimetric analysis, [D] Transmission electron microscopic analysis

ABTS radical cation decolorization assay

The chemically synthesized silver nanoparticles (AgNPs) also exhibited a considerable ability to scavenge ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radicals. At a concentration of 100 µg/mL, the AgNPs were able to scavenge 40.39% of the ABTS radicals. Furthermore, the maximum antioxidant activity reached 52.93% when the concentration of AgNPs was increased to 900 µg/mL, (Table 1 and Figure 3).

Table 1: Free radical scavenging activity of DPPH and ABTS of AgNPs by chemical reduction method

S. No	Concentration (µg/mL)	Free radicals scavenging activity (inhibition %)	
		DPPH radical	ABTS radical
1	100	60.31	40.39
2	300	62.57	44.81
3	500	66.82	46.52
4	700	70.53	50.38
5	900	72.34	52.93
F–Value		1.93544	7.5523
P–Value		0	0

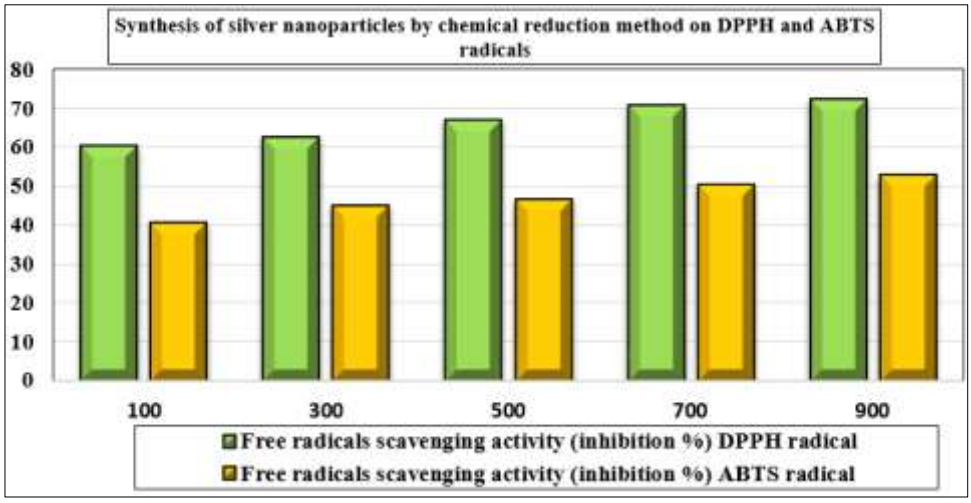

Fig 3: Free radical scavenging activity of DPPH and ABTS of AgNPs by chemical reduction method

IC_{50} values for antioxidant efficacy

The chemically synthesized silver nanoparticles (AgNPs) demonstrated DPPH radical scavenging activity with an IC_{50} (half-maximal inhibitory concentration) value of 68 µg/mL, which was approximately 2-fold higher than the reference antioxidants BHT (32 µg/mL) and lower activity with L-ascorbic acid (72 µg/mL). This suggests that the chemically synthesized AgNPs had a lower DPPH radical scavenging capacity compared to the reference antioxidant L-ascorbic acid and higher activity when compared to BHT.

On the other hand, the ABTS radical scavenging activity of the chemically synthesized AgNPs had an IC_{50} value of 72 µg/mL, which was greater than the reference antioxidants BHT (31.5 µg/mL) and L-ascorbic acid (44.1 µg/mL). This indicates that the chemically synthesized AgNPs had a higher ABTS radical scavenging capacity compared to the reference antioxidants (Table 2 and Figure 4).

Table 2: Free radical scavenging activity IC_{50} of DPPH and ABTS of AgNPs by chemical reduction method against standards BHT and L-ascorbic acid.

Standards & AgNPs by chemical reduction method	Free radicals scavenging IC_{50} value (µg/mL)	
	DPPH radical	ABTS radical
BHT	32	31.5
L-ascorbic acid	72	44.1
Chemical synthesis AgNPs	68	72

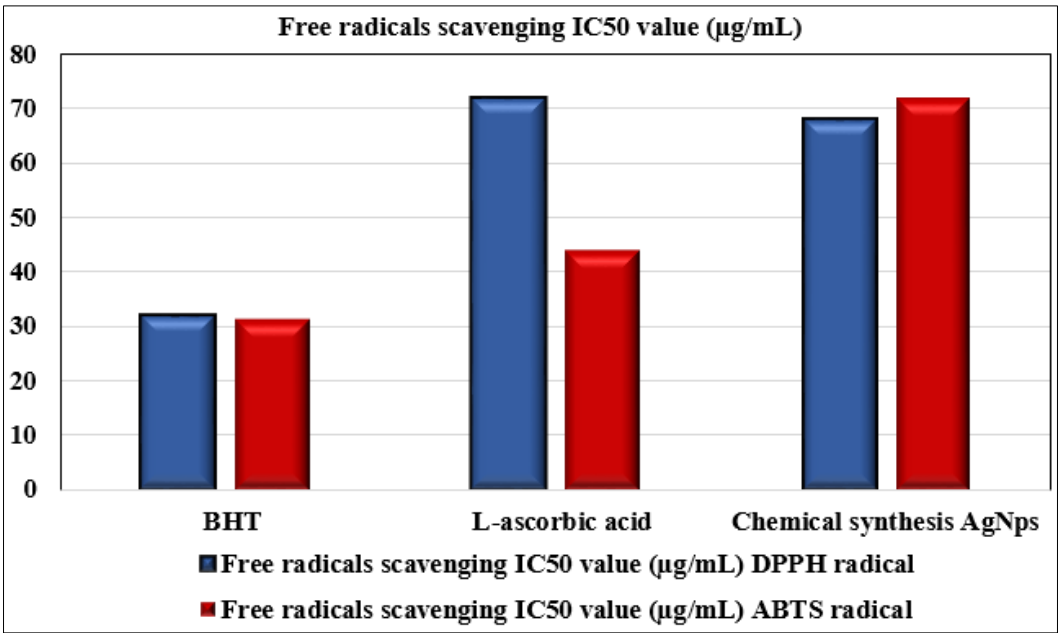

Fig 4: Free radical scavenging activity IC_{50} of DPPH and ABTS of AgNPs by chemical reduction method against standards BHT and L-ascorbic acid.

Discussion

The DPPH and ABTS assays are widely used to evaluate the antioxidant activity of various compounds, including silver nanoparticles (AgNPs). The DPPH assay is based on the reduction of the stable DPPH free radical, which has a maximum absorption at 517 nm and gives a purple color. The ability of antioxidant compounds to pair with the unpaired electron of the DPPH radical makes this assay efficient in screening a large number of samples at minimal concentrations.

The chemically synthesized AgNPs exhibited a concentration-dependent DPPH radical scavenging activity, with a maximum inhibition of 72.34%. The decrease in absorbance at 517 nm was used to quantify the DPPH radical scavenging ability of the AgNPs. Similarly, the chemically synthesized AgNPs also showed a concentration-dependent ABTS radical scavenging activity, with a maximum inhibition of 52.93%. The ABTS assay measures the ability of antioxidant compounds to scavenge the ABTS radical cation, which is a stable and long-lived radical species. The findings suggest that the chemically synthesized AgNPs have the potential to be utilized in pharmaceutical formulations and anti-aging treatments due to their effective radical-scavenging capabilities.

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