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Research Article

Determination of antioxidant potential of biochemically synthesized silver nanoparticles using *Aloe vera* gel extract

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Abstract

Biosynthesis of nanoparticles by using plant extracts is presently under development. The study has been focused on the biosynthesis of silver nanoparticles (AgNPs) using aqueous extract of *Aloe vera* gel as well as to determine their antioxidant potential. UV-Vis spectrophotometric analysis showed surface plasmonic resonance (SPR) band at 440 nm, which is specific for AgNPs. The Transmission Electron Microscopy (TEM) revealed that the synthesized AgNPs were spherical in shape with an average particle size of 66.6 nm. Fourier Transform Infrared Spectroscopic (FTIR) analysis of the aqueous extract before and after the synthesis of AgNPs revealed the presence of different functional groups related to phenolic and polyphenolic compounds such as tannins and flavonoids, and other metabolites like proteins, which may be responsible for the synthesis and stabilization of AgNPs. The antioxidant potential of the synthesized AgNPs was determined by using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, metal chelating and reducing power assay. Antioxidant assessment showed enhanced dose dependent antioxidant potential of the synthesized AgNPs as compared to the crude extract, which can gain attention of the pharmaceutical industry for preparation of antioxidants of natural origin as the synthetic ones are suspected to be carcinogenic. Present study also supports the advantages of green method for the nanoparticles synthesis.

Keywords: green synthesis, AgNPs, bioreduction, *Aloe vera* gel, characterization, antioxidant potential.

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Introduction

Antioxidant can be defined as the substance that prevents or inhibits the accumulation of free radicals in the human body. Number of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated in human body as byproducts of various types of metabolic activities, which are referred as free radicals, forming a large group of

reactive organic species. Free radicals can be either neutral or ionic entities containing one or more unpaired electrons, produced continuously in human body and are also capable of independent existence. ROS and RNS include species such as hydroxyl radicals (OH[•]), superoxide radical anion (O₂^{•-}), hydrogen peroxide (H₂O₂), singlet oxygen species (¹O₂) and nitric oxide (NO) respectively (1).

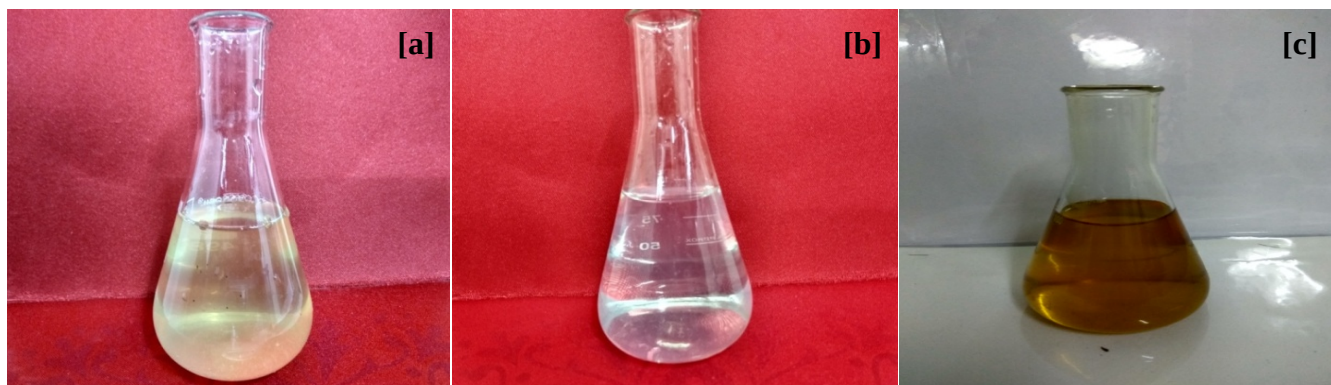


Fig. 2: (a) Aqueous extract of *Aloe vera* gel, (b) 1mM AgNO₃ solution and (c) AgNPs synthesized using aqueous extract of *Aloe vera* gel and 1 mM AgNO₃ solution.

Elevated levels of ROS generate oxidative stress leading to various types detrimental effects, which includes enzyme activation/deactivation, lipid peroxidation of cellular membranes, DNA breakage, altered lipid-protein interaction and eventually promoting mutations that initiate tumor progression (2,3). If the balance between free radical generation and their eradication is maintained, the harmful effects caused by them in the body can be minimized.

Many of the human disorders like arthritis, atherosclerosis, ischemia, central nervous system injury, reperfusion injury of many tissues, diabetes, gastritis and cancer is caused due to oxidative damage by free radicals and reactive oxygen species (4,5,6,7). To protect cellular molecules against the oxygen radical-induced damage the human body possesses multiple endogenous defense systems. These endogenous defense mechanisms include both enzymatic and non-enzymatic systems. The enzymatic defense system includes antioxidative enzymes like catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) (8). Against oxidative damage, these enzymes are our frontline defenders. Whereas the non-enzymatic defense system includes antioxidants like glutathione, vitamin E and C etc. which also protect the body from the oxidative stress induced damages. However, these innate defense systems are insufficient against severe oxidative stress, and overproduction of oxidative radicals may cause tissue damages.

A number of synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), α -tocopherol, tert-butylhydroquinone (TBHQ) and trolox are extensively used in non-food industries, but their use in the food industries is prohibited because they are alleged to be extremely carcinogenic (9). As a result, the development and utilization of more efficient antioxidants of natural derivation are preferred (10).

To treat various diseases and conditions, plants have been used as a source of traditional medicine for many years. Various types of

medicinal plants serve as outstanding source of phytochemicals with potent antioxidant properties (11).

In past few decades thorough investigation has been carried out for novel types of antioxidants from several plant materials (12,13). Myriad of phenolic and polyphenolic compounds present in plants are found have effective antioxidant properties (14). These properties of medicinal plants when merged with nanoparticles can be beneficial for the medical science. Nanoparticles have different physical, chemical and biological properties from their bulk counterparts. They have high surface to volume ratio (15). Hence, higher antioxidant potential of nanoparticles as compared to the extract is anticipated due to the preferential adsorption of the antioxidant material onto the surface of the nanoparticles from the extract. Out of all the metallic nanoparticles silver nanoparticles grabs more attention due to its unique physical, chemical and biological properties.



Fig. 1: The *Aloe vera* plant

Aloe vera is a unique plant which is a rich source of many chemical compounds and plays a significant role in the international market. From centuries the *Aloe vera* plant has been known and used for its health, beauty, medicinal and skin care properties. *Aloe vera* (Fig. 1) is perennial succulent belonging to the Lily (Liliaceae family) (16) and is granted with vast array of healing benefits. About 75 nutrients and 200 biologically active

compounds including sugar, anthraquinones, saponins, vitamins, enzymes, minerals, lignin, salicylic acid and amino acids has been now reported as constituents of *Aloe vera*. Previous studies suggests that the organic extracts of *Aloe vera* gel possess potent *in vivo* and *in vitro* antioxidant potential (17,18). Though the information on the physiological properties and antioxidant potential of the *Aloe vera* gel are known, antioxidant properties of the AgNPs synthesized using it are not thoroughly worked. Present study has been carried out to assess the *in vitro* antioxidant potential of silver nanoparticles synthesized using aqueous extract of *Aloe vera* gel.

Materials and Methods

Collection of plant sample: The *Aloe vera* plant was collected from Centre of Excellence on MAPs (Medicinal and Aromatic Plants) and NTFP (Non Timber Forest Products), Indira Gandhi Krishi Vishwavidyalaya, Raipur, India (21.2382° N, 81.7048° E) and was identified by Dr. P.K. Joshi, Principal Scientist and Team Leader, Centre of Excellence on MAPs and NTFP, Indira Gandhi Krishi Vishwavidyalaya, Raipur, India.

Synthesis of silver nanoparticles: Silver nanoparticles in the present study were synthesized by standard published procedures with slight modifications.

- The fully expanded leaves of *Aloe vera* was selected from three years old plant, washed with distilled water and was subjected to surface sterilization with 70% ethyl alcohol followed by 0.1% HgCl₂.
- The parenchymatous covering of the leaves was peeled and the gel was drained out. Slurry was formed with the help of grinder.
- Aqueous extract of the gel was prepared by using methodology as described by Niko with slight modifications (19). 10 g of the gel formed was then mixed with 100 ml of distilled water and kept in water bath at 60°C for 30 minutes.
- Using Whatman filter paper no. 1 the extract was then filtered and stored at 4°C for further use (Fig. 2a).
- One mM solution of silver nitrate (Molychem, M.W 169.87, 99.9%, Thane, India) was formed by dissolving 17 mg AgNO₃ in 100 ml of distilled water (Fig. 2b).
- AGAgNPs (AgNPs synthesized using *Aloe vera* gel extract) were synthesized by addition of 10 ml aqueous AGE (*Aloe vera* gel extract) to 40 ml aqueous 1 mM silver nitrate solution by constant stirring at 75°C for 40 minutes at neutral pH (Fig. 2c).
- The synthesized AGAgNPs were purified by centrifugation at 17000 rpm for 20 minutes.

Purified AGAgNPs were used to determine *in vitro* antioxidant potential.

Characterization of the synthesized AgNPs

The bioreduction of silver ions by aqueous extract of *Aloe vera* gel was monitored by measuring the UV-visible spectrum of the reaction medium. UV-visible spectral analysis was done by using UV-visible spectrophotometer (Systronics, Double beam spectrophotometer, 2203) and the absorption maximum was scanned at the wavelength of 200-700 nm. The studies on size and morphology of AGAgNPs were performed by transmission electron microscopy. Transmission electron microscope (TEM) samples were prepared by placing a drop of dispersed AGAgNPs solution onto carboncoated copper grid. The micrographs were obtained on TECNAI G2 Spirit (FEI, Netherland) equipped with Gatan digital camera operated at an accelerating voltage at 80 kV. Fourier Transform Infrared Spectroscopic (FTIR) analysis of the aqueous extract before and after the synthesis of AgNPs was done to determine the presence of different functional groups responsible for the synthesis and stabilization of AgNPs.

Total phenolic and flavonoid content measure

Total phenolic content measure: Determination of total soluble phenolic compounds in the *Aloe vera* aqueous extract and the synthesized AGAgNPs was done by using Folin-Ciocalteu reagent following the methodology of Slinkard and Singleton (20). Gallic acid was used as standard. Briefly, 1 ml of extract solution was diluted with 1 ml distilled water. 2 ml of Sodium carbonate (2%) followed by Folin-Ciocalteu reagent (0.5ml) was added, then the reaction mixture was allowed to stand for 2 hr at 25°C with intermittent shaking. The absorbance was measured at 760 nm. Total phenolic content in the aqueous extracts of AGE and AGAgNPs was measured by using calibration curve of gallic acid (R²=0.99) and content was expressed as gallic acid equivalents (microgram of GAE).

Total flavonoid content measure: Determination of total flavonoid content in the aqueous AGE and AGAgNPs were determined by aluminum colorimetric method as described by Bibi *et al.*, (21). The reaction mixture was prepared by adding 1 ml of the sample to 10% aluminum chloride, 1 M potassium acetate and distilled water. The reaction mixture was then incubated at room temperature for 30 mins and the absorbance was measured at 415 nm. Flavonoid content in the aqueous extracts of AGE and AGAgNPs was measured by using calibration curve of quercetin (R²=0.99) and content was expressed as quercetin equivalents (microgram of QE).

Determination of antioxidant activity

DPPH free radical scavenging assay: DPPH free radical scavenging activity of AGE and AGAgNPs

was measured by using the method described by Blois (22). 0.1 mM DPPH solution was prepared in methanol. 1 ml of the solution was added to 3 ml of aqueous extract of AG and AGAgNPs at different concentrations (0.25, 0.50, 0.75 and 1.00 mg/ml). Mixture was shaken vigorously and allowed to stand at room temperature for 30 min, and then absorbance was measured at 517 nm against blank sample, using UV-Vis spectrophotometer (Systronic). Standard antioxidant BHT was used as positive control and DPPH methanol reagent without sample was used as control. Higher the free radical scavenging activity, lower is the absorbance of the reaction mixture. Radical scavenging was expressed as the inhibition percentage and was calculated using the following formula (23):

$$\% \text{ of inhibition} = \frac{O.D_{\text{control}} - O.D_{\text{test}}}{O.D_{\text{control}}}$$

Where,

$O.D_{\text{control}}$ = absorbance of the DPPH methanol reagent (without sample),

$O.D_{\text{test}}$ = absorbance of the test solution i.e AGE/AGAgNPs/Standard.

Metal chelating activity

Ferrozine quantitatively chelates with Fe^{2+} forming a red colored complex. In the presence of other chelating agents, the reaction gets limited which leads to the decrease in the color intensity of the ferrozine- Fe^{2+} complexes. The chelating activity of the aqueous extract of AG and AGAgNPs on Fe^{2+} was measured by the method of Decker and Welch (24). Aliquot of 1 ml of different concentrations (0.25, 0.50, 0.75 and 1.00 mg/ml) of the aqueous extract of AG and AGAgNPs were added to 2.5 ml of distilled water. FeCl_2 (2 mM, 0.1 ml) and ferrozine (5 mM, 0.2 ml) were added to the reaction mixture and was left for 10 minutes at room temperature. The absorbance was then measured at 562 nm. Higher chelating power is indicated by lowered absorbance. The chelating activity on Fe^{2+} of the test extracts were compared with that of EDTA. Chelating activity was calculated by using the following formula:

$$\% \text{ of Chelating activity} = \frac{O.D_{\text{control}} - O.D_{\text{test}}}{O.D_{\text{control}}}$$

Where,

$O.D_{\text{control}}$ = absorbance value of the solution without sample (control);

$O.D_{\text{test}}$ = absorbance of the test solution i.e AGE/AGAgNPs/Standard.

Reducing power assay

The reducing power of the aqueous extracts of AGE and AGAgNPs was measured by using the methodology of Oraiza (25). 0.5 ml of each concentration (0.25, 0.50, 0.75 and 1.00 mg/ml) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The reaction mixture was then incubated for 20 min at 50°C. In it, Trichloroacetic acid (10%, 2.5 ml) was

added. The mixture was centrifuged at 6000 rpm for 10 min at 4°C. The supernatant (2.5 ml) was collected and 2.5 ml of distilled water and 0.5 ml ferric chloride (0.1%) was added to it. Antioxidants present in the test samples would results in reduction of Fe^{3+} / Ferricyanide complex to the ferrous form (Fe^{2+}). The Fe^{2+} can, thus be monitored by measuring the formation of the Perl's Prussian blue color at 700 nm (26). The reducing power of the aqueous extract was compared with that of L-ascorbic acid as standard antioxidant. Increased absorbance values indicate a higher reducing power.

Statistical analysis

The statistical analysis was carried out by using R. Mean and standard deviation of all the experiments was intended from triplicates ($n=3$) and represented in the table (Mean \pm S.D) and figures (error bar). Variations within the experimental groups was determined by ANOVA.

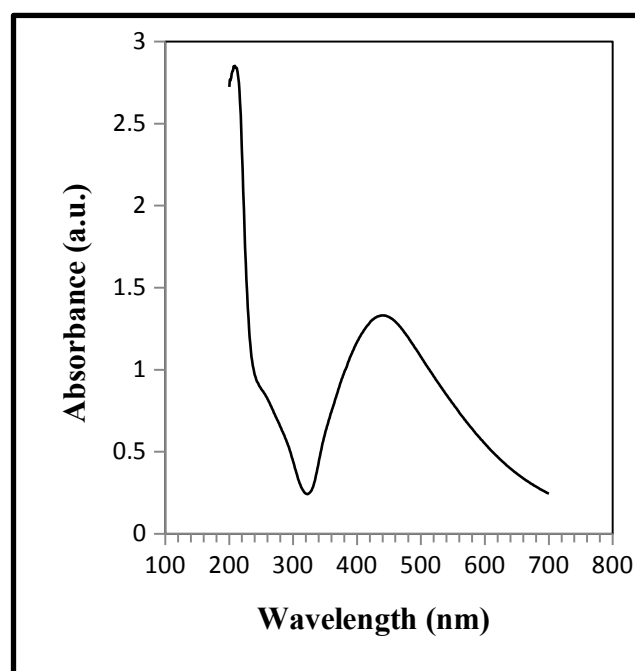


Fig. 3: UV-Vis Spectrum of AGAgNPs.

Results and Discussion

Characterization of the synthesized AGAgNPs:

In present study the green synthesis of AgNPs using aqueous extract of *Aloe vera* gel has been elucidated. When the aqueous AGE was mixed with 1 mM AgNO_3 solution and incubated at 75°C for 40 minutes at neutral pH, color changed to yellowish brown (Fig. 2c), which indicates the formation of AgNPs, this is preliminary identification of AgNPs formation. Color change in metal nanoparticles is due to the excitation of surface plasmon vibrations (27). Reaction medium containing AgNPs was subjected to UV-Vis spectral analysis where it showed sharp absorbance at 440 nm which is specific for AgNPs (Fig. 3). Two control reactions (Fig. 2a and 2b) were kept i.e. aqueous solution of AgNO_3 which was without any

Table 1: Total phenolic and flavonoid content determination in AGAgNPs and AGE. Values are presented as mean \pm SD from triplicate investigation.

Extract	TPC μg of GAE/mg	TFC μg of QE/mg
AGE	201.85 \pm 1.44	46.04 \pm 0.57
AGAgNPs	146.85 \pm 1.44	21.37 \pm 1.15

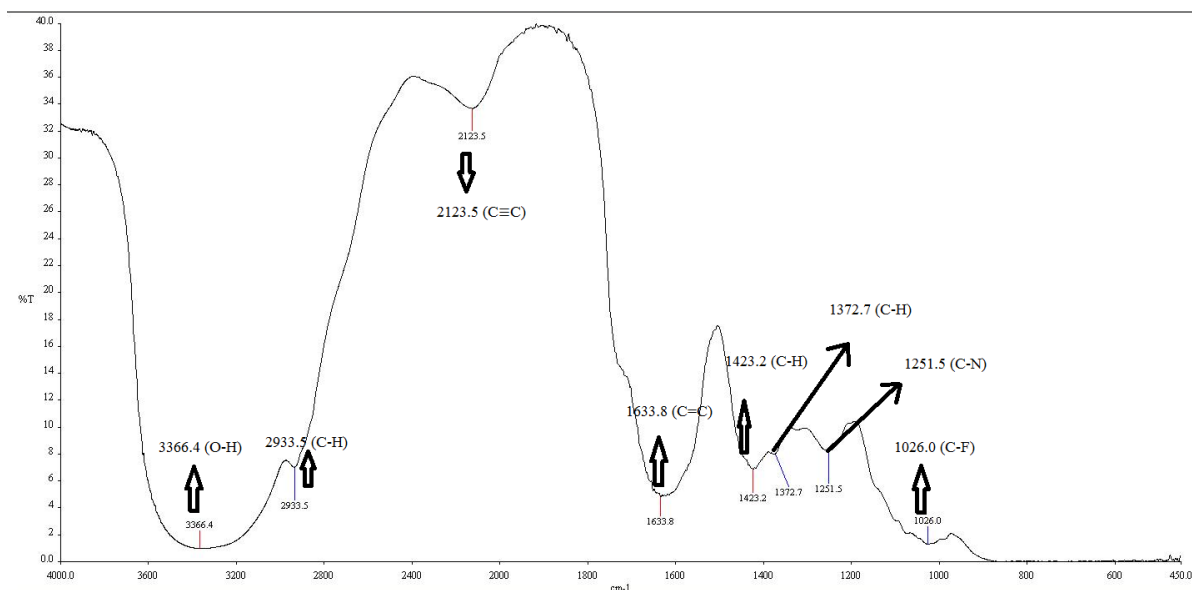


Fig. 5: FTIR Spectrum of AGE

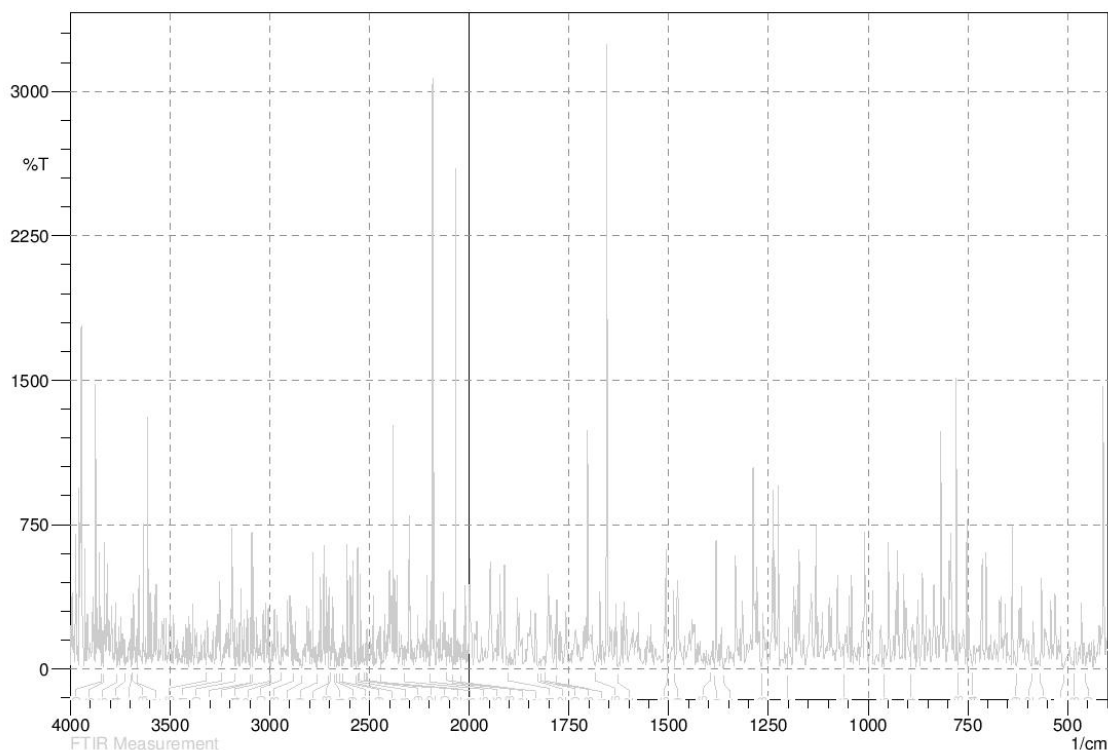


Fig. 6: FTIR Spectrum of AGAgNPs

colour and aqueous AGE that exhibit pale yellow colour. Reaction mixture containing silver nitrate solution and aqueous AGE showed yellowish brown colouration, which indicates the formation of AgNPs. Parallel control experiments didn't show any absorption at 440 nm. TEM analysis provided details about the morphology and size of the synthesized AGAgNPs. The AGAgNPs formed was

found to have an average size of 66.6 nm and spherical in shape and capped by plant constituents that prevented their aggregation. Silver nanoparticles of similar size were prepared by flower extract of *Rhododendron dauricum* (28). Natural capping offers supplementary advantage of the stability in the synthesis through green chemistry route as shown in Fig. 4. This stability is

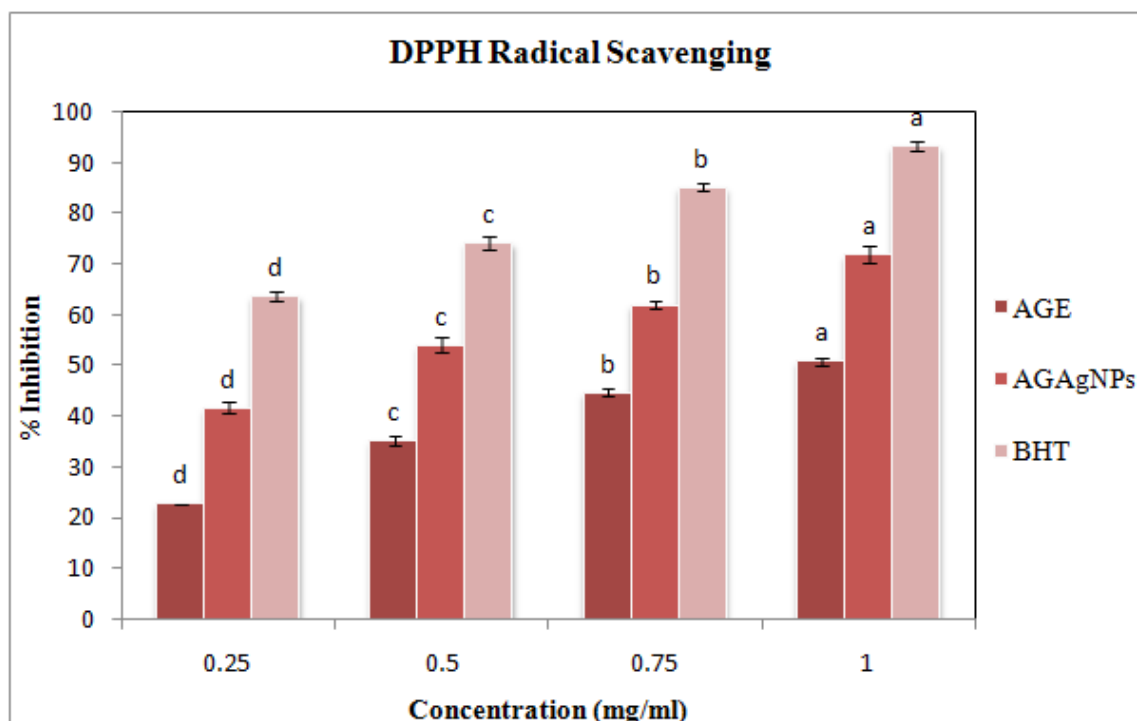


Fig. 7: DPPH free radical scavenging activity of AGE, AGAgNPs and BHT (Standard). Each bar represents Mean \pm S.D of three separate observations. The results were statistically significant at $p < 0.05$.

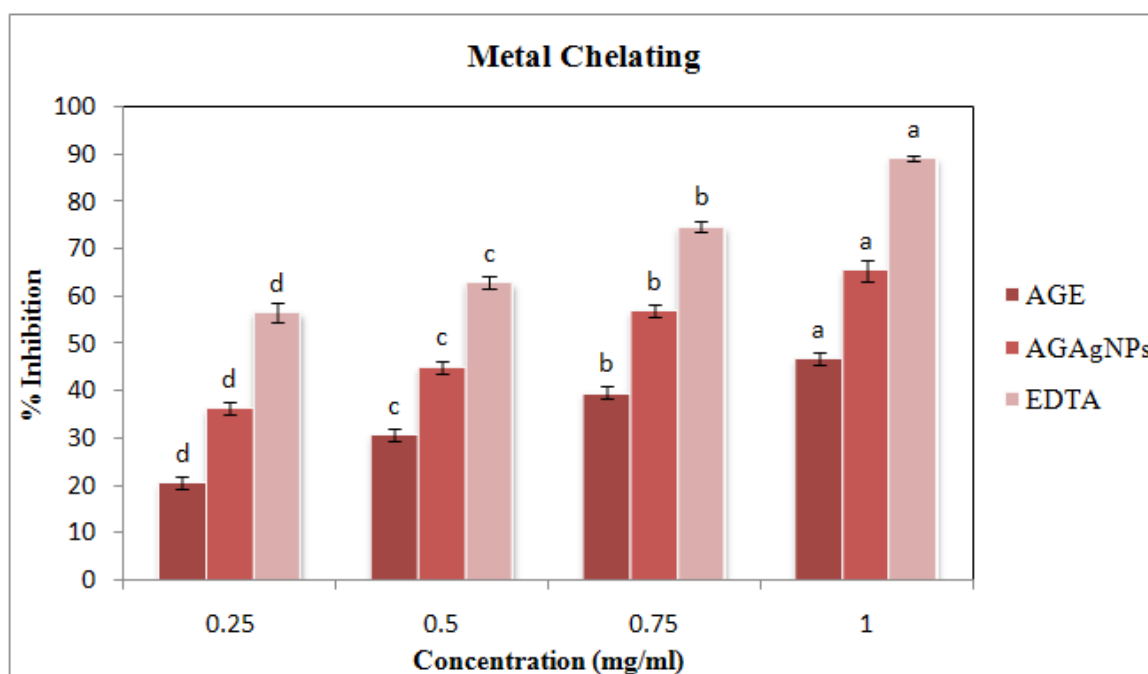


Fig. 8: Metal chelating activity of AGE, AGAgNPs and EDTA (Standard). Each bar represents Mean \pm S.D of three separate observations. The results were statistically significant at $p < 0.05$.

attributed to the phytoconstituents present in the extract, these results are in consensus with the reports by (29). The FTIR absorption spectra of the *Aloe vera* gel aqueous extract before and after the formation of AgNPs is shown in Fig. 5 and 6 respectively. Absorbance bands in Fig. 5 are observed in the region of 450-4000 cm^{-1} are 3366, 2933, 2123, 1633, 1423, 1372, 1251 and 1026 cm^{-1} . These absorbance bands of the FTIR spectrum are identified to be allied with the stretching vibrations for O-H (hydrogen bonded alcohols and

phenols), C-H (Alkanes), $\text{C}\equiv\text{C}$ (Alkynes), $\text{C}=\text{C}$ (Alkenes), C-H (Alkanes), C-H (Alkanes), C-N (Amines, Amides) and C-F (Aliphatic fluoro compounds) respectively which proves the presence of phenolic and polyphenolic compounds such as flavonoids and tannins, and other metabolites like proteins (30) and disappearance of these band after bioreduction (Fig. 6) gives confirmation for the participation of these phytoconstituents in the formation and stabilization of AgNPs.

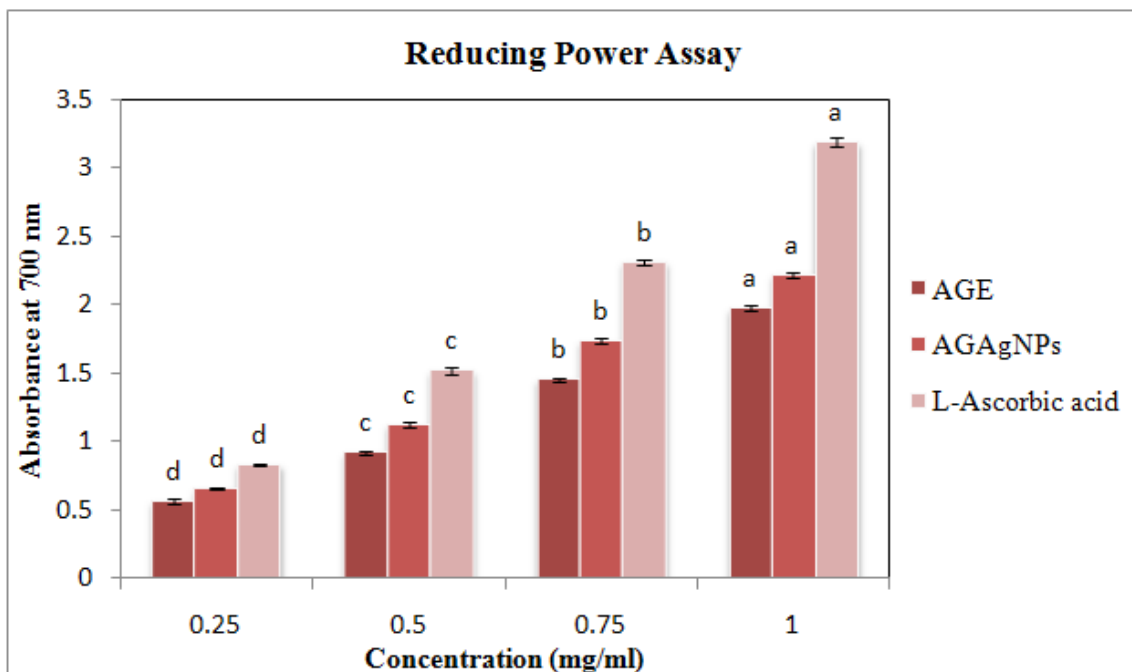


Fig. 9: Reducing of AGE, AGAgNPs and L-Ascorbic acid (Standard). Each bar represents Mean \pm S.D of three separate observations. The results were statistically significant at $p < 0.05$.

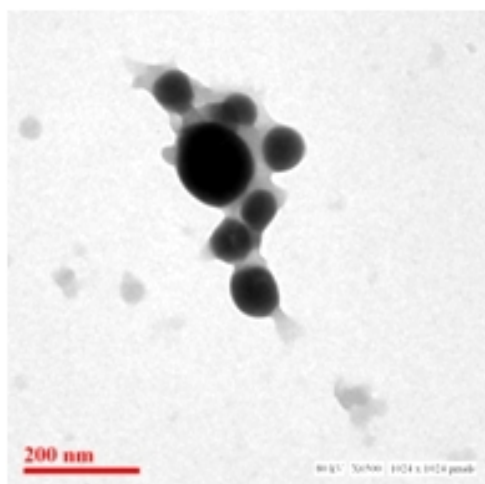


Fig. 4: TEM image for AGAgNPs

Quantification of phenolic and flavonoid content

Polyphenolic compounds derived from plants such as tannic acid has been reported to take part in the bioreduction of silver metal leading to the synthesis of nanoparticles (31). In order to confirm their role in the bioreduction process the total phenolic and flavonoid content in the crude extract and in the synthesized nanoparticles was measured.

Total phenol content: The linearity range of gallic acid was found for different concentrations (15.625 to 500 $\mu\text{g/ml}$) with regression equation as $y = 0.0040x + 0.0159$ obtained from its calibration curve. The total phenolic content of synthesized AGAgNPs was found to be $146.85 \pm 1.44 \mu\text{g}$ of GAE/mg as compared to AG extract ($201.85 \pm 1.44 \mu\text{g}$ of GAE/mg) phenol content (Table 1).

Total flavonoid content: The linearity range of quercetin was found for different concentrations (12.25 to 100 $\mu\text{g/ml}$) with regression equation as $y = 0.0100x + 0.0129$ obtained from its calibration curve. The total flavonoid content of synthesized AGAgNPs was found to be $21.37 \pm 1.15 \mu\text{g}$ of QE/mg as compared to AG extract ($46.04 \pm 0.57 \mu\text{g}$ of QE/mg) flavonoid content (Table 1).

Phenolics and flavonoids are nucleophilic in nature due to the presence of aromatic rings, hence, are responsible for having the chelating potential (31). Lowered amount of phenolic and flavonoid compounds measured in the synthesized AGAgNPs indicates their participation in the nanoparticles formation. Study carried out by Phull *et al.*, reported decreased level of phenolic and flavonoid compounds in the silver nanoparticles synthesized using *Bergenia ciliata* rhizome compared to the extract support our results (32).

In-vitro antioxidant activity

DPPH free radical scavenging assay: The effect of antioxidants on DPPH is attributed to their hydrogen donating capacity (33). DPPH is a commercially available lipophilic radical, which can readily accept electron from the antioxidant compound and converting its color from purple to yellow which is detected at 517 nm. The DPPH radical scavenging activity of AGE, AGAgNPs and standard (BHT) was studied using different concentration viz. 0.25, 0.50, 0.75 and 1 mg/ml. The % radical scavenging activity was found to increase in dose dependent manner in sequence BHT > AGAgNPs > AGE (Fig. 7). Which shows that AGAgNPs contains potential antioxidant properties as compared to AGE, where BHT was used as standard.

Metal chelating activity

In the metal chelating activity, Ferrozine quantitatively chelates with Fe^{2+} forming a red colored complex. In presence of other chelating agents, the reaction gets limited which leads to the decrease in the color intensity of the ferrozine- Fe^{2+} complexes. Chelating activity of the sample to compete with ferrozine for the ferrous ions can be estimated by the measurement of the color reduction (34). Metal chelating activity of AGE, AGAgNPs and standard (EDTA) on Fe^{2+} ions was studied the absorbance of Ferrozine- Fe^{2+} complex decreased linearly in a dose dependent manner (0.25-1 mg/ml). The Ferrozine- Fe^{2+} complex formation was disrupted by the presence of chelating agents in the sample. Thus, the decreased absorbance at 562 nm indicates high levels of iron binding potential. AGAgNPs showed higher chelating ability than that of AGE. However, it was relatively lower than that of EDTA. In this assay both the samples, AGE and AGAgNPs and standard EDTA compounds interfered with the formation of ferrous complex with the reagent ferrozine, suggesting that it has chelating activity and captures the ferrous ion before ferrozine. EDTA is a known metal ion chelator and is thus used for comparison. The result indicates that the sample has good iron binding capacity, suggesting its action as an antioxidant relating to its iron binding capacity (Fig. 8).

Reducing power assay

In the reducing power assay, the reduction of Fe^{3+} to Fe^{2+} has been attributed to the presence of reductants in the sample. Formation of Perl's blue at 700 nm indicates the amount of Fe^{2+} complex. Fig. 9 shows the reducing power of AGE and AGAgNPs and the reference compound, L-ascorbic acid. Here, the AGAgNPs showed more reducing power than that of AGE. However, it was relatively lower than that of L-ascorbic acid. A direct correlation between antioxidant activity and reducing power of certain plant extracts has been observed by earlier authors (35,36). The reducing properties are generally associated with the presence of reductones (37) which have been shown to exert antioxidant action by breaking the free radical chain by donating hydrogen atom. The result suggests that samples are likely to contribute significantly towards the observed antioxidant effect, which increased in dose dependent manner (0.25-1 mg/ml).

Redox potential of phytoconstituents in the plant extract is responsible for its antioxidant activity (38), which could play an important role in satisfying singlet and triplet oxygen, decaying the peroxides or nullifying the free radicals. Nanoparticles have higher surface to volume ratio therefore, higher antioxidant potential of nanoparticles as compared to the extract is anticipated due to the preferential adsorption of

the antioxidant material onto the surface of the nanoparticles from the extract.

Studies carried out by previous researchers reported enhanced antioxidant potential of the green synthesized nanoparticles as compared to the extract support our results (39,40,41).

Conclusion

Present study reports the single step process for the biological synthesis of silver nanoparticles using aqueous extract of *Aloe vera* gel which comes up with ecofriendly, easy and proficient method for the synthesis of innocuous nanoparticles. The synthesized nanoparticles possessed the added advantage of active phytoconstituents incorporated in them. Moreover the synthesized silver nanoparticles exhibited an enhanced antioxidant potential than the crude extract, which illustrate the application of bioactive silver nanoparticles as an excellent antioxidant agent of natural origin.

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Competing Interests

The authors declare that they have no competing interest.

Author's Contribution

AS and KKS designed the objectives and plan of work. JKS carried out the work. JKS and MS wrote the manuscript.

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