ORIGINAL ARTICLE

# Green synthesis of silver nanoparticles using flower extract of *Calliandra haematocephala*, screening of antibacterial and antioxidant activities

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## Abstract

**Aim:** Green synthesis of silver nanoparticles (AgNPs) was gained more importance comparative with other methods due to their salient features such as simplicity in procedure, non-toxic by-products, and cost effective; rapidity. Among the different types of nanoparticles, AgNPs have received more focus due to their unique antimicrobial properties. **Materials and Methods:** We successfully synthesized AgNPs from an aqueous flower extract of the *Calliandra haematocephala* plant for the first time. Thus, synthesized AgNPs were characterized by ultraviolet-visible spectroscopy and the surface Plasmon resonance peak was identified to be 451 nm. The key chemical features responsible for the reduction of silver nitrate to form AgNPs and the capping agents present in the flower extract were identified by Fourier-transform infrared spectroscopy. The synthesized AgNPs (0.1, 0.2, 0.3, 0.4, 0.5mM) were screened for antibacterial activity by cup-plate method and antioxidant activities by $H_2O_2$ Scavenging assay, and Phosphomolybdenum Method. **Results:** On phytochemical screening of aqueous flower extract of *C. haematocephala*, it was revealed that various phytoconstituents such as alkaloids, flavonoids, tannins, and saponins, and cardiac glycosides were present. The potent antibacterial and antioxidant activity were identified at 0.5 mM AgNPs solution. **Conclusion:** The overall results confirmed that aqueous flower extract of *C. haematocephala* plant possess good antibacterial and antioxidant activity.

**Key words:** *Calliandra haematocephala*, Capping, Green synthesis, Nano particles, Phosphomolybdenum assay

## INTRODUCTION

A nanoparticles or ultrafine particle are usually defined as a particle of matter that is between 1 and 100 nanometers (nm). This term is sometimes used for larger particles, up to 500 nm, or fibers and tubes that are <100 nm in only two directions.[1] Nanotechnology is an important modern direction of research related to synthesis, and control structure of particles ranging from approximately 1–100 nm in one dimension.[2] Nanoparticles biosynthesis is a bottom-up approach, in which the main reaction is reduction/oxidation. The green synthesis method was gaining popularity enormously due to the high cost and involvement of toxic chemicals in physical and chemical methods. Often, the chemical method leads to the presence of some of the toxic components absorbed on the surface that may cause adverse effects in medical applications. Green synthesis represents a breakthrough over chemical and physical methods, because as it is cost effective, eco-friendly, and can be easily used for large-scale, synthesis. Silver nanoparticles (AgNPs) were gained a lot of interest among metallic nanoparticles, which are of particular medicinal interest due to the antimicrobial properties of the silver metal. The main advantage of using plant extracts to synthesize AgNPs is that in most cases they are readily available, safe, and non-toxic and have a broad variety of metabolites that can help in the reduction of silver ions.

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Hence, in the present study, the aqueous extract of flowers obtained from *Calliandra haematocephala* was studied for the synthesis of AgNPs. *C. haematocephala* is a flowering plant of the genus *Calliandra* in the family *Fabaceae*. *C. haematocephala* is a fast-growing shrub that can grow tall but has a wide canopy. It is a folk medicinal plant of 2–5 m in height, 2–3 m in width. Interestingly enough the branched pinnate, silky leaves close at night. The red powder puff flower is attractive to butterflies and hummingbirds, but only appears from November to April. The buds before the flowers open look like raspberries.[3] The genus *Calliandra* (*Fabaceae*) has 132 species. It is usually cultivated in gardens for ornamental purposes.

The pharmacological activities reported on *C. haematocephala*[4] were anti-inflammatory, anticonvulsant, immunomodulatory, antiulcerogenic activity, insecticidal, and antibacterial, gastro protective activity. Photochemical investigations have been carried out on the constituents of this genus and demonstrated the presence of tannins, flavonoids, and saponins. The leaves of this plant contain large amounts of amino acids that offer fungal resistance. Different types of imino acids such as pipecolic acid, trans-4, and trans-5-hydroxypipecolic acid, trans–cis-4, 5-dihydroxy-pipecolic acid, and trans-4-acetylaminopipecolic acid are present in the leaves of *C. haematocephala* by Romeo.[5] The various phytochemicals present in stems and leaves of C. haematocephala are Gallic acid, methyl gallate, caffeic acid, myricitrin, quercetin, afzelin, and quercetin.[6] The presence of such phytochemicals makes this source rich in biomolecules, which could help in the stabilization/capping and reducing mechanisms, during the synthesis of AgNPs.

## MATERIALS AND METHODS

### Plant Collection

The red-colored fresh flowers of the plant *C. haematocephala* were collected from the gardens of Aditya College of pharmacy, Surampalem in East Godavari District, Andhra Pradesh, India. The plant authentication was confirmed by Dr. T. Raghu, botanist, Maharani College, Peddapuram.

### Preparation of Extract[7]

The fresh flowers are to be cleaned with plain water and pat dry to remove the excess moisture. The cleaned flowers were completely shade dried for 48 h. The dried flowers were collected and stored in an air-tight container at room temperature for further use. The aqueous flower extract of *C. haematocephala* was prepared as follows. 20 g of *C. haematocephala* dried flowers were weighed and boiled with 60 ml of water for about 10 min at 70°C. The boiled extract was collected and settled to room temperature and the extract was filtered using Whatman's filter paper.

#### *Chemicals*

The chemicals and reagents used are of analytical grade and Purchased from S.D. Fine Chemical Limited, India.

### Synthesis of AgNP's[7,8]

For AgNP synthesis, 10 ml of freshly prepared aqueous flower extract of *C. haematocephala* was added to 90 ml of 1 mM aqueous silver nitrate (AgNO3) solution. The reaction mixture was heated at 60 ± 2°C for 10 min with continuous stirring after 10 min, a color change from light pink to dark brown with noticeable precipitate, which showed the reduction of silver ions (A+) to silver atom (A0) indicating the synthesis of AgNP's. different concentrations of AgNPs (0.1, 0.2, 0.3, 0.4, and 0.5 mM) were prepared using 1, 2, 3, 4, and 5 ml of aqueous flower extract and 1 m MAgNO3 solution. The five samples were boiled for about 10–20 min at 60°C, observed the color change from light pink to dark brown with noticeable precipitate. The five samples were cooled to room temperature and incubated in dark conditions for 24 h. The samples were collected and characterized by ultraviolet visible (UV–Vis) spectroscopy at 270 nm.

### Characterization of Synthesized AgNP's

Characterization of plant AgNP's was done using UV–Vis spectroscopy, Fourier-transforms infrared spectroscopy (FTIR), scanning electron microscopy, dynamic light scattering, and zeta potential measurement. UV–Vis absorbance spectral analysis of aqueous suspension of AgNP's was carried out using a spectrophotometer (UV-2450, Shimadzu Corp., and Japan). The absorbance of each sample was taken at 200–700 nm with a resolution of ±1 nm.[9] The chemical compositions and potential biomolecules of the AgNP's were analyzed by FTIR spectrum, recorded using an Alpha FT-IR spectrometer (Bruker, Germany). The AgNP's sample solution was subjected to FTIR using the potassium bromide (KBr) plates and the measurements were taken in the region of 500–4000 $cm^{-1}$.

### Qualitative Phytochemical Analysis[10]

The phytochemical investigation was carried out on aqueous flower extract of *C. haematocephala* for the detection of phytochemical constituents (Gokhale *et al*., 2009).

### Antibacterial Activity[11,12]

#### *Microbial cultures*

The cultures were procured from Microbes Speciality Lab Danavaipeta, Rajahmundry, East Godavari District 533103, Andhra Pradesh, India. These are aseptically maintained in Aditya college of pharmacy laboratory. The gram-positive bacteria are *Staphylococcus aureus* (ATCCBAA 1026), *Bacillus subtilis* (ATCC 11774) the gram-negative bacteria used in the study are *Escherichia coli* (ATCC 10536).

**Table 1:** Phytochemical analysis of flower extract of *C. haematocephala*

| S. No. | Compound | Chemical test | Aqueous extract |
|---|---|---|---|
| 1 | Alkaloids | Mayers | +ve |
| | | Hagers | +ve |
| | | Murexide | -ve |
| 2 | Glycosides | Legal test | +ve |
| | | Borntragers test | +ve |
| 3 | Carbohydrates | Molish test | +ve |
| | | Fehlings | +ve |
| | | Disacchrides | +ve |
| | | Salwinoffs | +ve |
| | | Iodine | -ve |
| 4 | Proteins | Biurette | -ve |
| | | Millons | -ve |
| 5 | Saponins | Foam forming | +ve |
| 6 | Coumarin glycosides | - | -ve |
| 7 | Flavonoids | Schinoda | +ve |
| | | Alkaline sol | +ve |
| | | Fecl3 | +ve |
| | | NaOH | +ve |
| | | Lead acetate | -ve |
| 8 | Volatile oil | - | -ve |
| 9 | Tannins | Fecl3 | +ve |
| 10 | Steroids | Salkowski | -ve |

+: Indicates present, -: Indicates absent

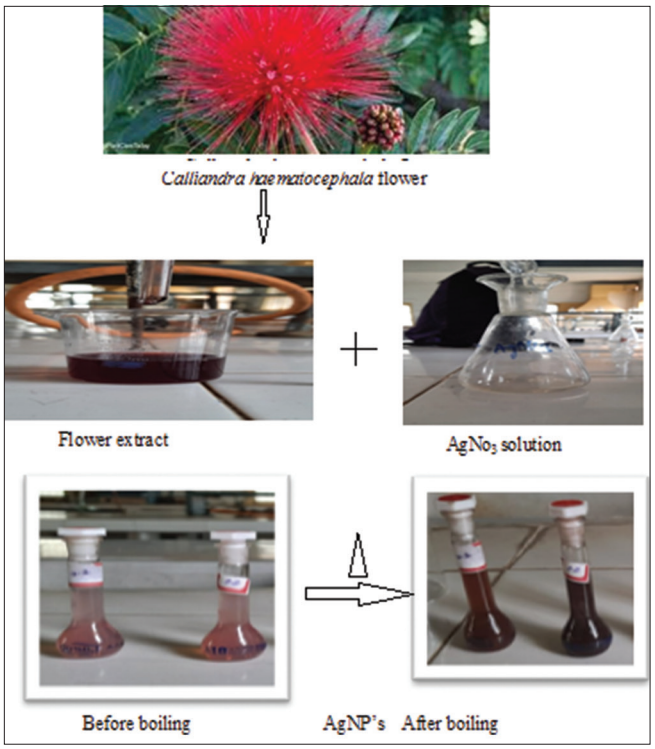


**Figure 1:** Schematic diagram of synthesis of silver nanoparticles from *Calliandra haematocephala*

### *Procedure*

The AgNPs synthesized using the *C. haematocephala* Flower extract were tested for the antibacterial activity using the cup-plate method. The sterilized medium (autoclaved at 121°c for 20 min) was inoculated using 18 h slant cultures of the test organisms and transferred into sterile Petri dishes and allowed to solidify the media. Cups of 8 mm diameters were made on solidified media. Solutions of the synthesized AgNP's at a concentration of (0.1, 0.2, 0.3, 0.4, and 0.5 mM) were placed in the cups using a sterile pipette. In each plate, one cup was used as a control with DMSO and the other for standard. The plates thus prepared were left for 90 min in a refrigerator for diffusion. The plates were incubated at 37°C for 24 h for antibacterial activity and examined for inhibition zones. The experiment was carried out twice and the average diameter of the zones of inhibition was recorded. Gentamycin (40 µg/ml) was used as the standard for antibacterial activity.

## Antioxidant Activity

The antioxidant activity of synthesized AgNP's was studied using $H_2O_2$ assay and phosphomolybdenum assay.

### *Hydrogen peroxide scavenging ($H_2O_2$) assay*

The ability of the plant extracts to scavenge $H_2O_2$ can be estimated according to a method of (Ruchi *et al*., 1989).[13] A solution of hydrogen peroxide (40 mm) is prepared in phosphate buffer (50 mm ph 7.4); the concentration of $H_2O_2$ is determined by absorption at 230 nm using a spectrophotometer. 0.1 ml of synthesized AgNP's (0.1, 0.2, 0.3, 0.4, 0.5 mM) solution was added to 0.6 ml of hydrogen peroxide and 0.3 ml of phosphate buffer and measure the absorbance at 230 nm using UV–Vis spectrophotometer, after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. Repeat the experiment by taking a standard drug as ascorbic acid. The percentage of hydrogen peroxide scavenging is calculated as follows:

% scavenged ($H_2O_2$) = [(Ai-At)/Ai] × 100, Where Ai is the absorbance of the control, At is the absorbance of the test.

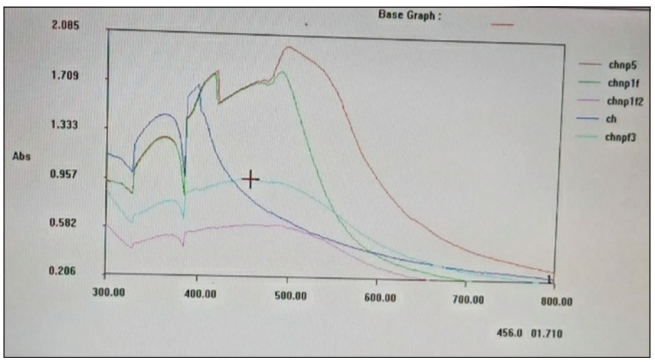


**Figure 2:** Ultraviolet–visible spectra of synthesized AgNP's prepared using 1 mM AgNO3

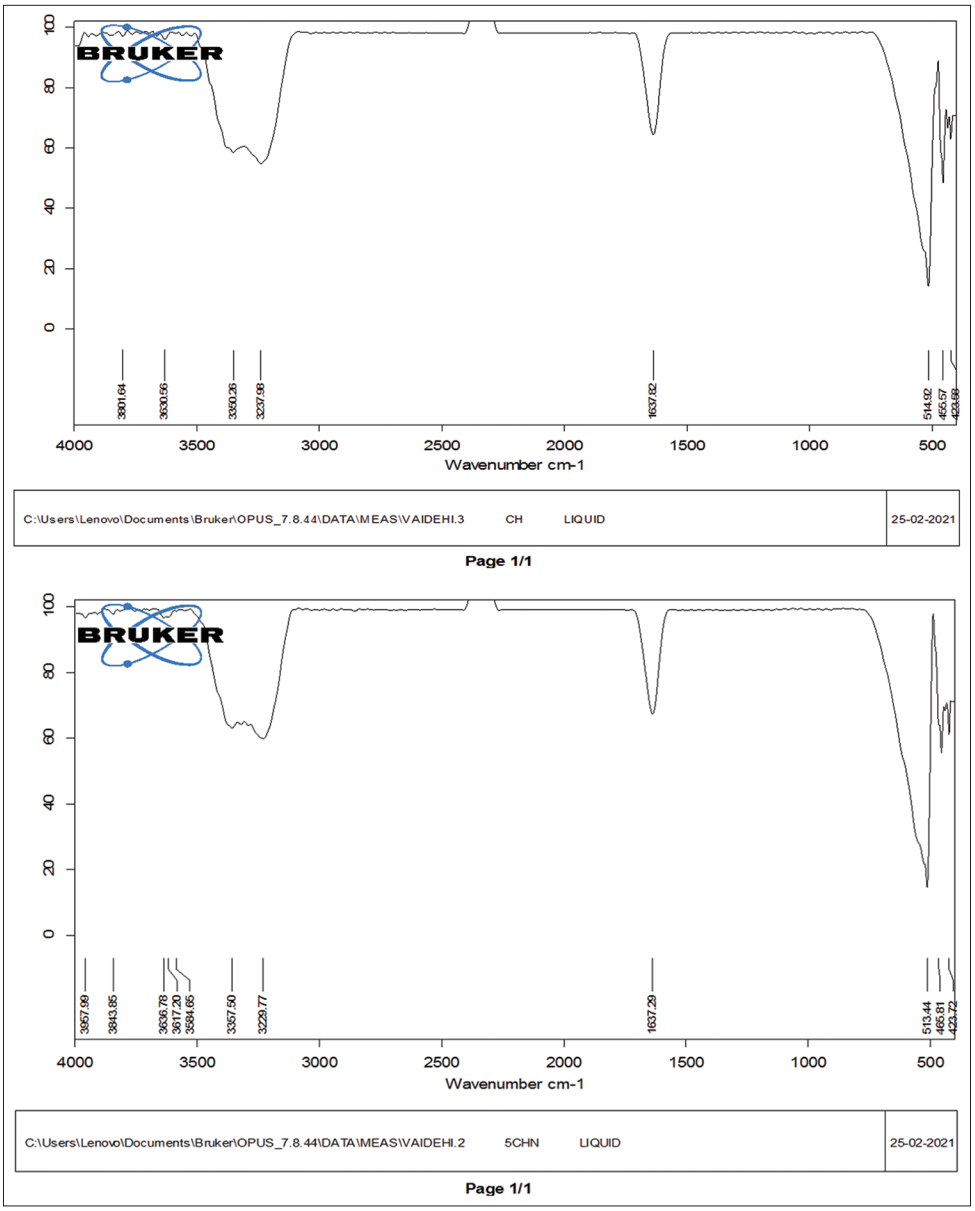


**Figure 3:** Fourier-transforms infrared spectra of (a) *Calliandra haematocephala* flower extract and (b) Synthesized 0.5 mM AgNP

### *Phosphomolybdenum method*

This assay is based on reduction of Mo(VI) to Mo(V) by the analyte of sample and subsequent formation of green phosphate Mo(V) complex at acidic PH. Total antioxidant capacity can be calculated by the method according to (Prieto *et al.,* 1999).[14,15] 0.1 ml of synthesized AgNP's (0.1, 0.2, 0.3, 0.4, and 0.5 mM) solution is combined with 1 ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tube is capped and incubated in a boiling water bath at 95°C for 90 min. After cooling the sample to room temperature, the absorbance of the aqueous solution is measured at 695 nm against blank in UV spectrophotometer. A typical blank solution contained 1 ml reagent solution and the appropriate volume of the same solvent used for the sample and are incubated under the same conditions as the rest of the sample.

## RESULTS AND DISCUSSION

### Results of Phytochemical Analysis

The present phytochemical analysis of an Aqueous Flower Extract of *C. haematocephala* showed positive results for alkaloids, glycosides, carbohydrates, saponins, flavonoids, and steroids. The presence of phytoconstituents is reported in Table 1.

### Characterization of Synthesized Plant AgNP's

### *UV–Vis spectroscopy*

Green synthesis of AgNPs was indicated by the color change of the reaction mixture from light pink to dark brown after the addition of AgNO3 solution [Figure 1]. This color change was observed due to the reduction of Ag+ ions in the aqueous

solution of AgNO3 into AgNP's.[16] The UV–Vis absorption spectra of plant AgNP's were recorded in the range of 300–600 nm [Figure 2]. Synthesized plant AgNP's showed absorption maxima in the range of 420–500 nm due to the surface Plasmon resonance (SPR) phenomenon. A broad SPR band was observed from 456 nm for AgNP's synthesized from *C. haematocephala*. The broad absorption peaks were observed for synthesized plant AgNP's which showed the polydispersed nanoparticles in the solution. The presence of bioactive compounds such as flavonoids, alkaloids, tannins, and amines, in the plant extract resulted in the bio-reduction as well as capping and stabilization of AgNP.

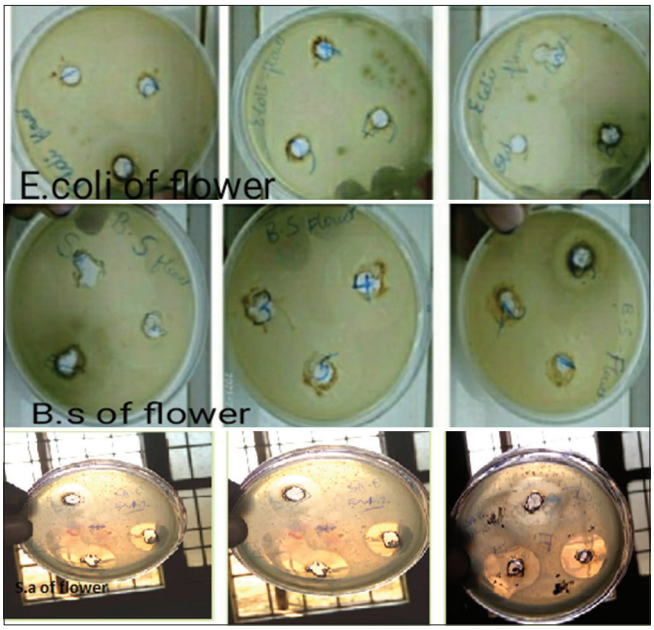


**Figure 4:** Antibacterial Activity of AgNP's against *Bacillus subtilis*

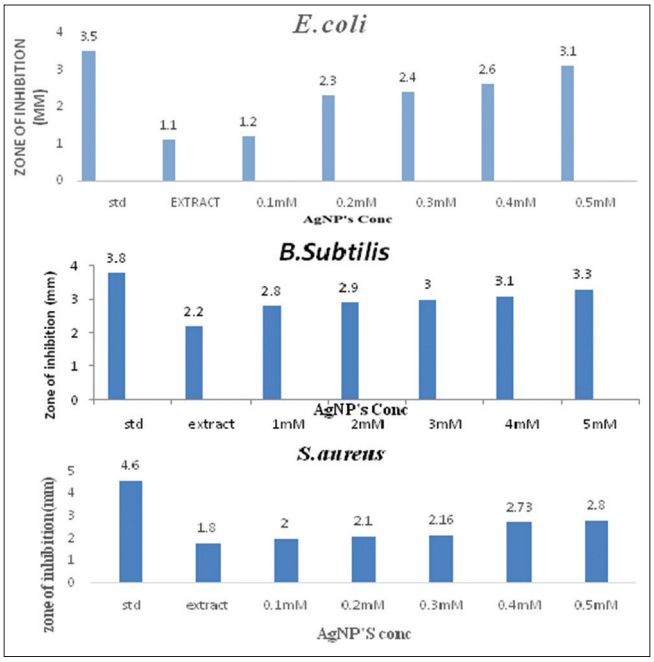


**Figure 5:** Graphical representation of zone of inhibition of AgNp's against *Escherichia coli*

### *FTIR spectroscopy*

The FTIR analysis of synthesized plant AgNP's was carried out to identify the functional groups of the active biomolecules playing roles of reducing as well as capping agents in the synthesis of nanoparticles. The IR spectra obtained by AgNP's and an aqueous flower extract were almost similar with a small shift that corresponds to the orientation of particular bioactive compounds bound to the metal surface. The FTIR spectra of plant AgNP's are shown in Figure 3, in which the spectrum of *C. haematocephala* showed prominent transmittance bands at 3357.50, 3229.77 $cm^{-1}$ (-N-H-), 1637.29 $cm^{-1}$ (-C=O), 513.41 $cm^{-1}$ (-NH-), and 465.81 $cm^{-1}$ (-C-N-). FT-IR spectra revealed that the presence of various biomolecules such as phenols, aldehydes, ketones, alcohols, amines, amides of flavonoids, tannins, and alkaloids is possibly involved in the capping and stabilization of AgNP by reducing the metal salt.[17,18]

### *In vitro antibacterial activity*

The antibacterial activity of AgNP's was evaluated against *S. aureus*, *B. subtilis*, and *E. coli* which are shown in Figure 4, respectively. All the AgNP's solutions (0.1, 0.2, 0.3, 0.4, and 0.5 mM) show inhibitory effect. Figure 5 shows a graph of the antibacterial behaviors of AgNP's extract and standard Gentamycin against *S. aureus*, *B. subtilis*, and *E. coli*.

In both cases, these results confirmed the antibacterial properties of AgNP's.

## DISCUSSION

From the data shown in Table 2 the observations were made as follows.The synthesized AgNP's exhibited and proved to show anti-bacterial activity against the tested gram positive and gram negative micro-organisms.[19,20] The 0.5mM AgNP's solution was found to exhibit good antibacterial activity against *B.subtilis*, *E.coli*, *S.aureus* when compared with standard drug Gentamycin and all other concentrations.

**Table 2:** Antibacterial activity of AgNP's

| S. No. | Name of organism | Std | 0.1 mM | 0.2 mM | 0.3 mM | 0.4 mM | 0.5 mM | Extract |
|---|---|---|---|---|---|---|---|---|
| 1 | *Bacillus subtilis* [g (+) ve] | 3.8 | 2.8 | 2.9 | 3 | 3.1 | 3.3 | 2.2 |
| 2 | *Staphylococcus aureus* [g (+) ve] | 4.6 | 2 | 2.1 | 2.16 | 2.73 | 2.8 | 1.8 |
| 3 | *Escherichia coli* [g (-) ve] | 3.5 | 1.2 | 2.3 | 2.4 | 2.6 | 3.1 | 1.1 |

**Table 3:** $H_2O_2$ Method AgNP's (λ=230 nm; Instrument=UV) Phosphomolybdenum Method

| Concentration | Percentage (%) inhibition | Concentration | Percentage (%) inhibition | Concentration | Percentage (%) inhibition | Concentration | Percentage (%) inhibition |
|---|---|---|---|---|---|---|---|
| 0.1 mM | 46 | 0.1 M | 45 | 0.1 mM | 81 | 0.1 mM | 68 |
| 0.2 mM | 53.23 | 0.2 M | 52.42 | 0.2 mM | 82.08 | 0.2 mM | 72.29 |
| 0.3 mM | 58.06 | 0.3 M | 56.45 | 0.3 mM | 82.92 | 0.3 mM | 74.17 |
| 0.4 mM | 71.77 | 0.4 M | 69.35 | 0.4 mM | 83.75 | 0.4 mM | 77.63 |
| 0.5 mM | 82.26 | 0.5 M | 80.65 | 0.5 mM | 84.17 | 0.5 mM | 79.04 |

λ=670 nm, Instrument=Colorimetry

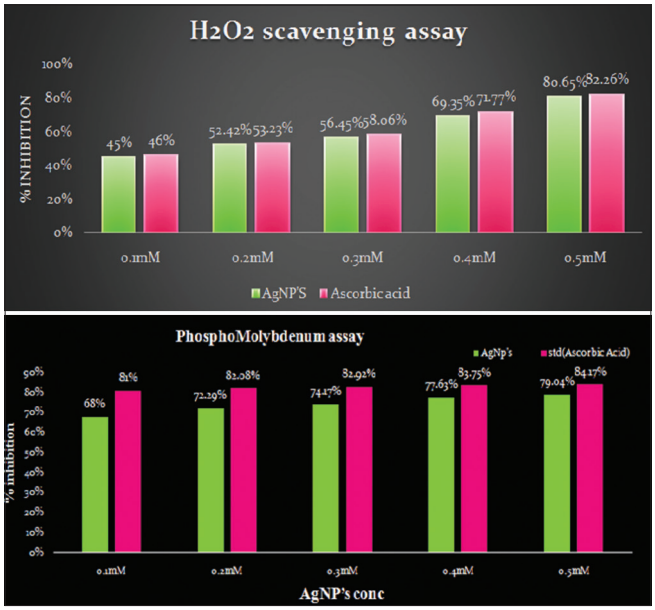


**Figure 6:** Graphical representation of dose-response for hydrogen peroxide scavenging assay

### Antioxidant Activity

For AgNP's for Ascorbic acid For AgNP's For Ascorbic acid.

### Hydrogen Peroxide Scavenging Activity[21,22]

The results confirmed from Table 3 shows that the AgNP's has 60.77% hydrogen peroxide scavenging activity while the standard ascorbic acid has 62.27% hydrogen peroxide scavenging activity.

### Phosphomolybdenum Assay

From The above results, Figure 6 proved that AgNP's has 74.22% of reducing Mo radical in phosphomolybdenum assay, while the standard ascorbic acid has 82.8% reducing Mo radical in phosphomolybdenum assay. The results proved that the AgNP's has antioxidant activity, but it is less compared with standard ascorbic acid, but the synthesized AgNP's also possess good antioxidant activity.

## CONCLUSION

AgNPs were synthesized successfully by the Green-Synthesis approach using aqueous flower extract of *C. haematocephala.* The biological synthesis of AgNPs is a rapid, eco-friendly, cost- effective, and simple method of synthesis. Phytochemical screening was performed on the aqueous flower extract of *C. haematocephala*, and it was revealed that phytochemical constituents such as flavonoids, tannins, phenolics, and alkaloids were present. The synthesized AgNP's were characterized by UV–VIS and IR Spectroscopy. Various Conc. of synthesized AgNP's solutions (0.1 mM, 0.2 mM, 0.3 mM, 0.4 mM, and 0.5 mM) were screened for antibacterial and antioxidant activities. Among the various concentrations, 0.5 mM AgNP's were exhibited good antibacterial and antioxidant activities when compared with standard drug ascorbic acid. The results proved that the AgNP's synthesized from flower extract of *C. haematocephala* is potential free radicals scavengers with effective inhibition activity in a dose-dependent manner. The antioxidant activity of the AgNP's may be attributed to the high phenolics and flavonoids content in the plant. The plant phenolics are strong antioxidants with reducing capacity; thereby, it reduces silver ions to form AgNPs.

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