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Short Communication

In vitro bio-synthesis of silver nanoparticles using flower extract of parasitic plant *Cascuta reflexa* and evaluation of its biological properties

Nida S.Shaikh[a,*], Rahimullah S.Shaikh[a], Sahebrao Kashid[b]

[a] *Department of Chemistry, Govt.Vidarbha Institute of Science and Humanities, Amravati-444604, India*
[b] *Department of Chemistry, Institute of Science, Homi Bhabha State University, Mumbai-400032, Mumbai*

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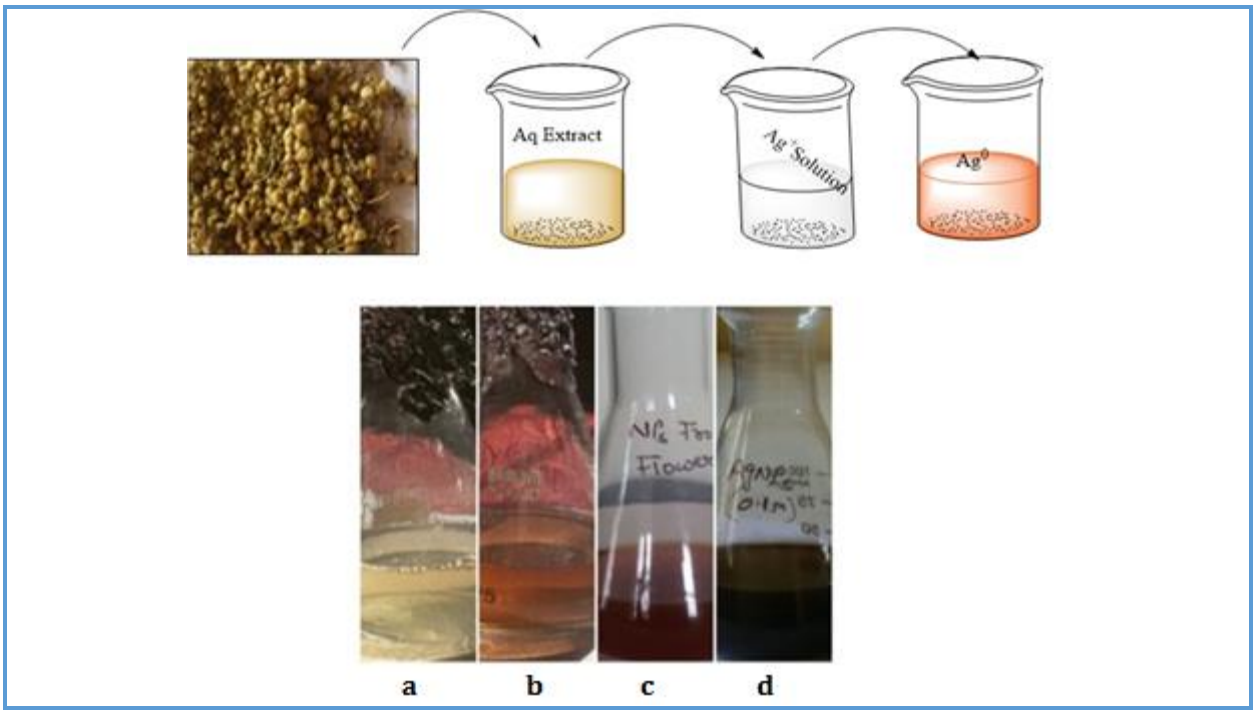
ABSTRACT

This paper deals with the rapid photosensitized biosynthesis of silver nanoparticles using aqueous extract of flowers of Cascuta reflexa. The reaction was carried out in ambient sunlight. The mixing of aqueous solution of silver nitrate and the flower extract shows color transitions from yellow to light brown and finally dark brown colour, indicating the formation of silver nanoparticles. As synthesized the nanoparticles were characterized by various techniques such as UV visible spectroscopy, XRD, FT-IR, TEM. The TEM analysis revealed that the particles were predominantly spherical and size ranging from 20 to 50 nm. The antioxidant properties were tested by FR AP assay method. The antibacterial properties of synthesized Nanoparticles were tested against pathogens such. P.aeruginosa, E.coli, B. subtilus and S.aureus.

Corresponding author, email: nid.aafr@gmail.com (N.S. Shaikh).
Tel.: +91 9764002640..

Graphical Abstract



Introduction

Nanotechnology is playing a vital role in all branches of science and technology. It involves the creation and arrangement of the materials at nanometer scale by reduction. Due to small size and large surface area these materials harbor interesting and important properties which are not found in bulk material [1]. In modern science it has a numerous application in the field of medical due to its antimicrobial activity against pathogenic or non-pathogenic bacteria, fungi, viruses, etc [2] or having antioxidant activity. There are various ways of synthesizing nanoparticles but in the last decade, biosynthesis of nanoparticles has received increasing attention due to a growing need to develop environmentally benign protocols. In recent years the biosynthetic method employing plant extracts has received major attention as a simple and viable alternative to chemical procedures and physical methods in the synthesis of metal nanoparticles [3]. Among various metal nanoparticles silver nanoparticles has gained considerable attention due to the widespread applicability in the fields of medicine [4], preservatives, antioxidants, etc. [5]. Plant extract are more preferable for green synthesis of nanoparticles as they are rich in chemical constituents who offers a reducing and stabilizing capabilities [6]. Polyphenols major constituents of plant material are one of the largest groups of natural antioxidant secondary metabolites [7]. The reduction properties of these antioxidant metabolites have been linked with the higher potential ability of plant extracts to synthesize nanoparticles with improved characteristics [8].

Cascuta reflexa is a parasitic and herbal plant which completely depends on host plants for food and nutrition [9]. The organic substances are transfer from host plant to parasitic plant. It is rich in chemical constituents. It contains dulchitol, an alcoholic substance, an alkaloid,

phenolic compounds, flavonoids, terpenoids and so many other compounds [10]. These constituents are potential candidate for reduction of metal ions and stabilization of nanoparticles [1١]. Aqueous extract of *Cascuta reflexa* was used to synthesize the silver nanoparticles. The aqueous flower extract of *Cascuta reflexa* acts as a reducing and capping agent which reduces the silver ion to silver nanoparticles.

Experimental

Collection of plant

The plant of *Cascuta reflexa* was collected from campus of VMV College, *Amravati District Maharashtra India. Deionized water was used for all purpose.*

Preparation of Plant extract

The flower of *Cascuta reflexa* (Figure 1) were washed with deionized water and air dried. The dried flowers were crushed into fine powder. 10 gm of powdered flower *of Cascuta reflexa* mixed with 100 mL of deionized water. The mixture was then stirred magnetically for 1 hour. The aqueous extract was then separated using Whatman filter paper No.1.

Figure 1. Flower of *Cascuta reflexa*

Synthesis of silver nanoparticles

5 mL of aqueous flower extract of *Cascuta reflexa* (Figure 2**a**) was added to 45 mL of 1 mM silver nitrate solution (Figure 2**b**) in presence of sunlight. Immediately after addition of plant extract in silver ion solution remarkable change in colour was observed. The colour changes first from yellow to light brown and then to dark brown. This characteristic brown colour indicates the formation of silver nanoparticles.

Characterization of silver nanoparticles

The optical property of synthesized AgNps was monitored by UV-Vis spectrophotometer to ascertain the reduction of Ag^{+} ion by flower extract. The absorption peaks were observed at 470, 464, 445, and 420 nm. Deionized water was used as a reference solution. The spectral analysis was done by Shimadzu UV-1800 spectrophotometer at room temperature between the range 190-800 nm. The crystalline nature and size of silver nanoparticles were determined by XRD. The characterization of functional groups present in the silver nanoparticles was investigated by FT-IR spectrophotometer. (FT-IR analysis was done with KBr pellets and recorded in the range of 500-3500 cm^{-1} using Perkin Elmer Spectrum 100). The shape and size of resultant particles were elucidated with the help of TEM on FEI USA Technai G2 analysis.

Antimicrobial activities

Disc diffusion method was used to explore the antimicrobial potential of synthesized silver nanoparticles against pathogens like *P.aeruginosa, E.coli, B subtilus and S.aureus.* The fresh cultures were taken on Muller-Hinton agar. The culture was incubated at 37 °C for 24 hours. Silver in its ionic form is effective against more than 650 pathogens having a broad spectrum of activity, nanoparticles of silver on

the other hand enhances its effectivity allowing its use in a wide range of application.

Ferric reducing antioxidant power assay

Ferric reducing power assay was performed to determine the antioxidant properties of silver nanoparticles. Different concentrations of the silver nanoparticles (10-50 µg/mL) were added to 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$] solution. The reaction mixture was vortexed well and then incubated at 50 °C for 20 min. using vortex shaker. At the end of the incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3,000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of deionised water and 0.5 mL of 0.1% ferric chloride. With increase in concentration absorbance increases which is directly proportional to the increased reducing power.

Results and Discussion

The rapid change in colour from yellow to light brown and then to dark brown after the addition of aqueous flower extract of *Cascuta reflexa* to the 1 mM (10^{-3}) solution of silver nitrate in presence of sunlight indicated the formation of silver nanoparticles. Figure 3 the reaction is fast when addition is done in presence of direct sunlight. The sunlight induces Green synthesis of silver nanoparticles [12]. The reduction of Ag^+ ions in solution was monitored by using UV-Vis spectrophotometer at different time interval i.e. 5 min., 30 min., 1 hr, 24 hr and the peak appeared at 470, 464, 445, and 420 nm [3]. The intensity of the absorbance increased as the reaction proceeds. The maximum absorption spectra appeared at 420 nm suggesting the stability of synthesized AgNps [13]. The Absorption and scattering properties of silver nanoparticles depend upon size, shape and refractive index near the particle surface [14]. Nanoparticles with spherical morphology other than sphere exhibit broader spectra. The UV-Vis. Spectra of the flower extract and silver nanoparticles at different time intervals are shown in Figure 4 Indicating maximum silver nanoparticles formation after 4 hours duration [15, 16]. It is the simplest way to confirm the formation of nanoparticles.

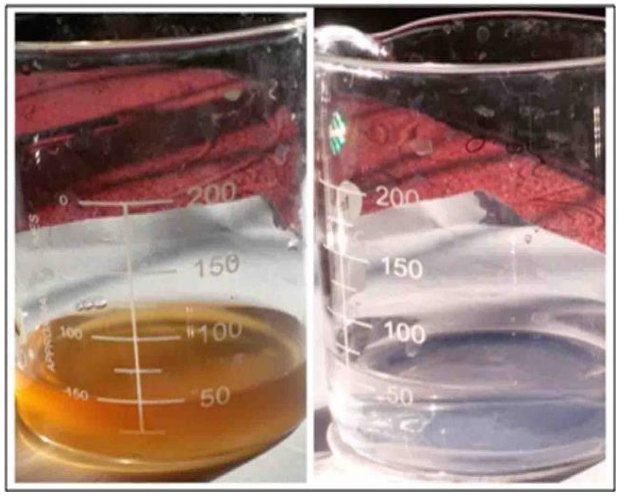


Figure 2. a) Flower extract b) $AgNO_3$

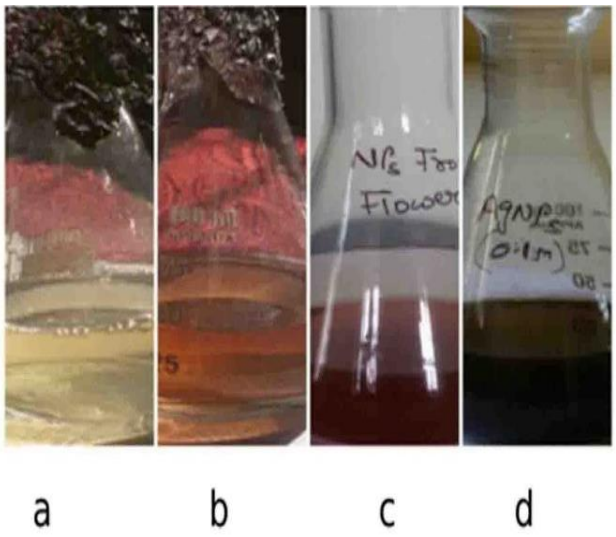


Figure 3. Coloration of silver nanoparticles a) after 5 min, b) after 30 min, c) after 1 hr d) after 24 hrs

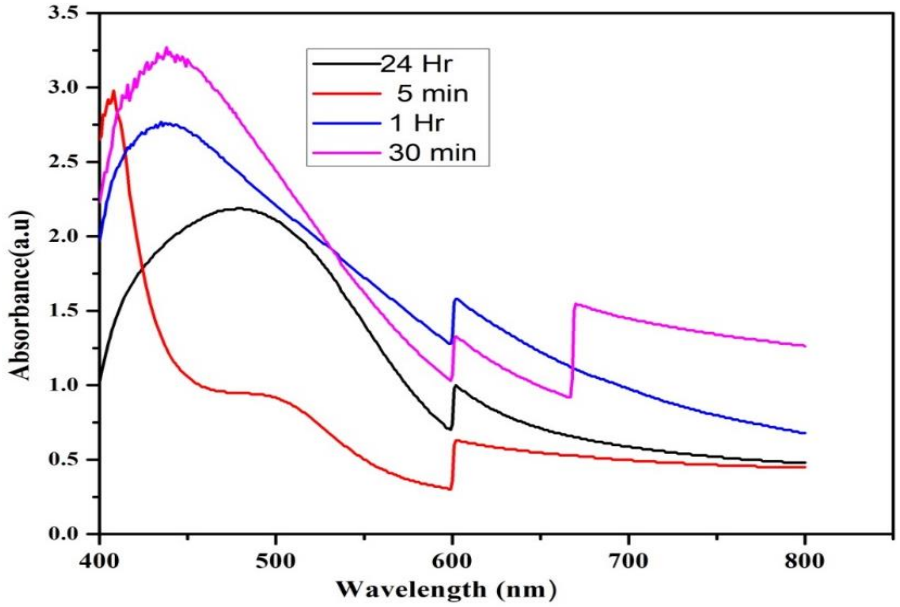


Figure 4. UV-Vis. spectra showing absorbance of synthesized AgNps at different time interval

The XRD pattern was used to determine the crystalline nature, peak intensity position and width of silver nanoparticles (Figure 5). The dried powder of silver nanoparticles was subjected to XRD analysis for their phase structure and exact material identification. The diffractogram was obtained in the range 2θ range of 10 to 60 degree [2]. Bragg's reflection peak for silver nanoparticles appeared at (2θ) 19.5°,24° and 38°. Some study reported five intense peak of silver nanoparticles at 27.5, 31.99 45.99, 67.24, 76.46 [17].

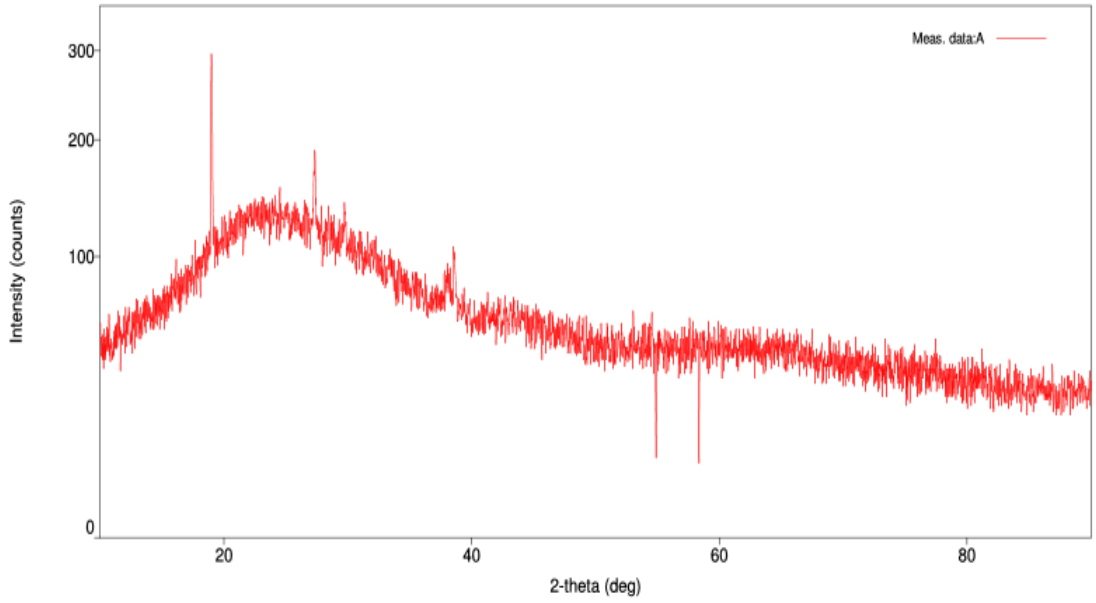


Figure 5. XRD pattern of synthesized silver nanoparticles from flower extract of *Cascuta reflexa*

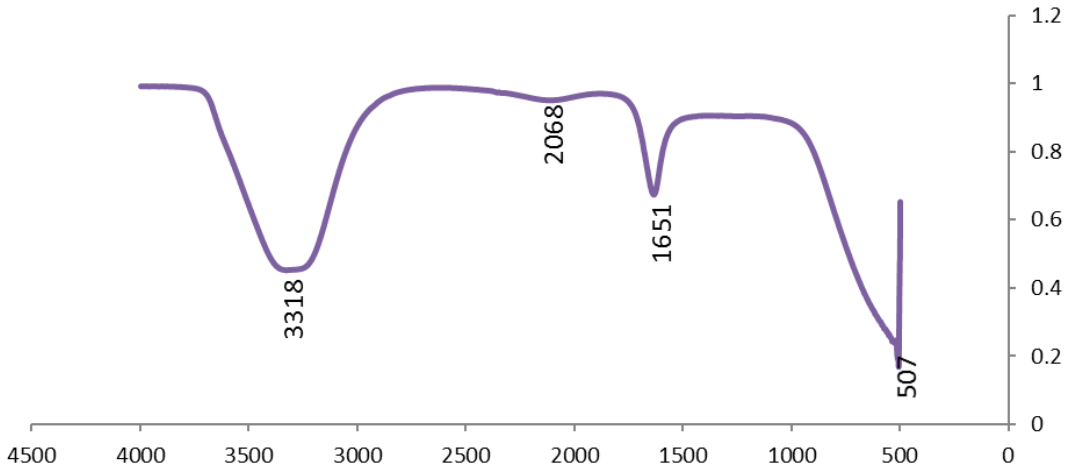


Figure 6. FT-IR spectra of silver nanoparticle

Further investigation on the surface capping agent was done using FT-IR. The FT-IR spectra provided information about the interaction of silver nanoparticles with aqueous flower extract of *Cascuta reflexa*. The results of FT-IR analysis of this study show different stretches of bond shown at different peaks. The strong peak at 3318 cm^{-1} is due to N–H stretch, The band around 2071 cm^{-1} is due to –C≡C–, whereas the sharp peak at 1651cm^{-1} corresponds to amide I arise due to carbonyl stretch in proteins [18] indicating predominant surface capping species which are mainly responsible for stabilization [19]. The broad asymmetric spectra at 2100 cm^{-1} can be assigned to the N–H stretching in the free amino groups of silver nanoparticles.

The shape and size of synthesized silver nanoparticles were confirmed by TEM analysis. The TEM micrographs suggested that the synthesized AgNPs were of spherical in shape [20]. It is used to obtain the measurement of colloidal particle, its distribution and morphology [21]. The images showed the outer coating of minimum 5 nm thickness around the silver nanoparticles (Figure 7**a**) which may be due to the presence of bioorganic compounds in flower extract [22]. The formation of variable size of particles (Figure 7**b**) indicated that the AgNps from flower extract could from polydisperse nanoparticles [23]. Figure 7**d** indicates the SAED pattern of silver nanoparticles which confirms the face-centered cubic (fcc) crystalline structure of metallic silver.

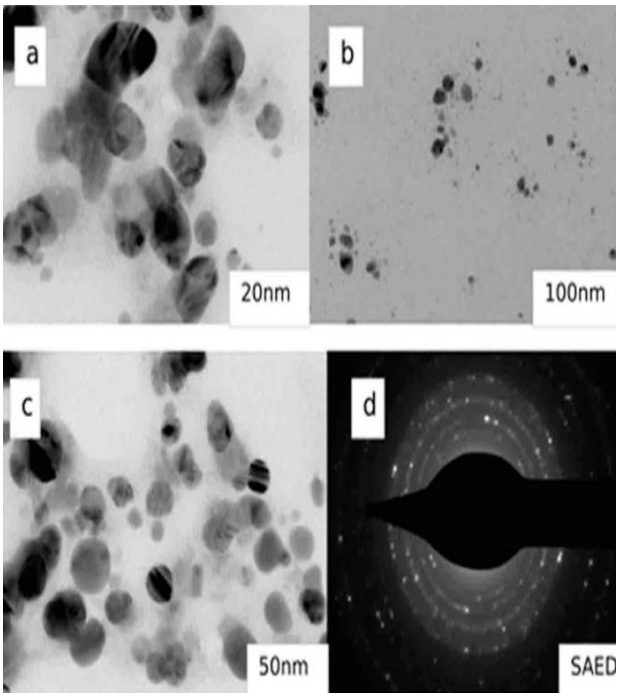


Figure 7. TEM images of synthesized AgNps at different magnification level

The synthesized silver nanoparticles were tested against pathogens such as *P.aeruginosa, E.coli, B. subtilus and S.aureus* by disc diffusion method. Among the pathogens *P.aeruginosa* and *B. subtilus* was highly sensitive to the synthesized silver nanoparticles as given in Figure 8. It is reported as when bacterial cells come in contact with silver nanoparticles it inhibits the growth and reproduction of bacterial cells [24]. The silver nanoparticles are used in industries, pharmacy and in medicine as they have shown inhibitory activities against various microorganisms. By considering the antimicrobial activity of Silver nanoparticles it is used in the treatment of cancer [25]. The AgNps also shows antiplasmodial activity against *P. falciparum* [26].

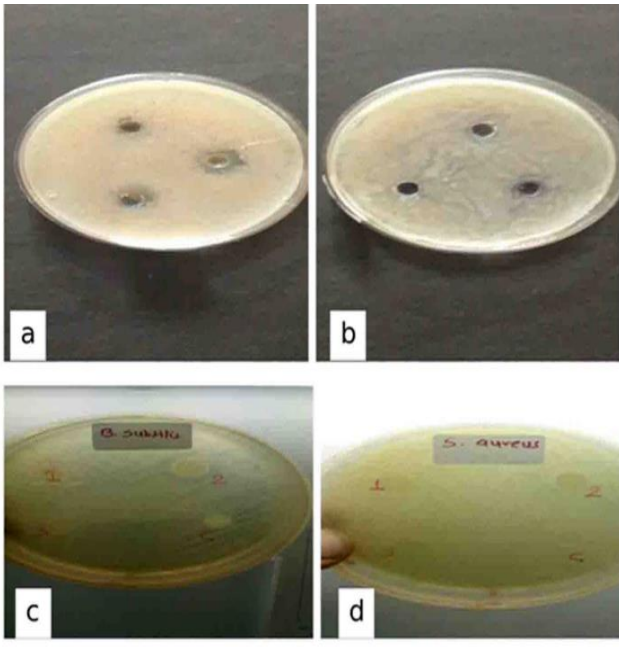


Figure 8. Antimicrobial activity of silver nanoparticles against different bacteria a) *P.aeruginosa,* b) *E.coli*, c) *B.subtilus,* d*) S.aureus*

In FRAP assay the change in absorbance is directly related to the total reducing power of the electron donating antioxidants present in the reaction mixture of Silver nanoparticles containing flower extract of *Cascuta reflexa.* The reducing property is generally associated with the presence of reductants. The antioxidant action of reductants is based on the breaking of free radical chain by donation of a hydrogen atom. Reductants also react with certain precursors of peroxide, thus preventing peroxide formation. The presence of antioxidant molecules in silver nanoparticles act as reductants by donating the electrons and reacting with free radicals to convert them to more stable products and terminate radical chain reaction. The antioxidant activity of synthesized silver nanoparticles was evaluated by ferric reducing power.

Ferric reducing power assay

The presence of antioxidants in the reaction mixture of silver nanoparticles would result in the reduction of Fe^{3+} to Fe^{2+} [27]. The Ferric reducing power (as indicated by absorbance at 700 nm) of Silver nanoparticles increased with increasing concentration (Table 2). The colored solution of Fe^{2+} complex was read at 700 nm against the blank with reference to standard (Figure 9) using UV-Spectrophotometer. Here, ascorbic acid was used as a reference standard, the reducing power of the silver nanoparticles were comparable with reference standard. Higher value of absorbance of AgNps indicated greater reducing power. The absorbance value of ascorbic acid and silver nanoparticles of different concentrations are presented in Table 1 and Table 2. And graphically it is represented in Figure 9**a** and 9**b**.

The present study demonstrates an eco-friendly, cost effective, rapid and safer synthesis of stable AgNps using aq. Flower extract of *Cascuta reflexa.* The green synthesized AgNPs were confirmed by various techniques such as UV-Vis. Spectrophotometer, XRD, FT-IR, TEM etc. The maximum absorbtion peak of synthesized silver nanoparticles appeared at 420 nm.

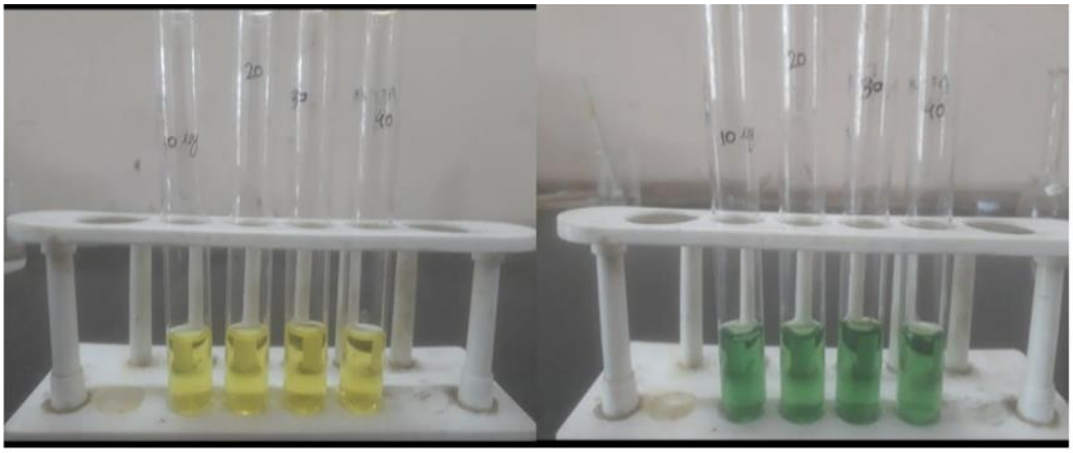


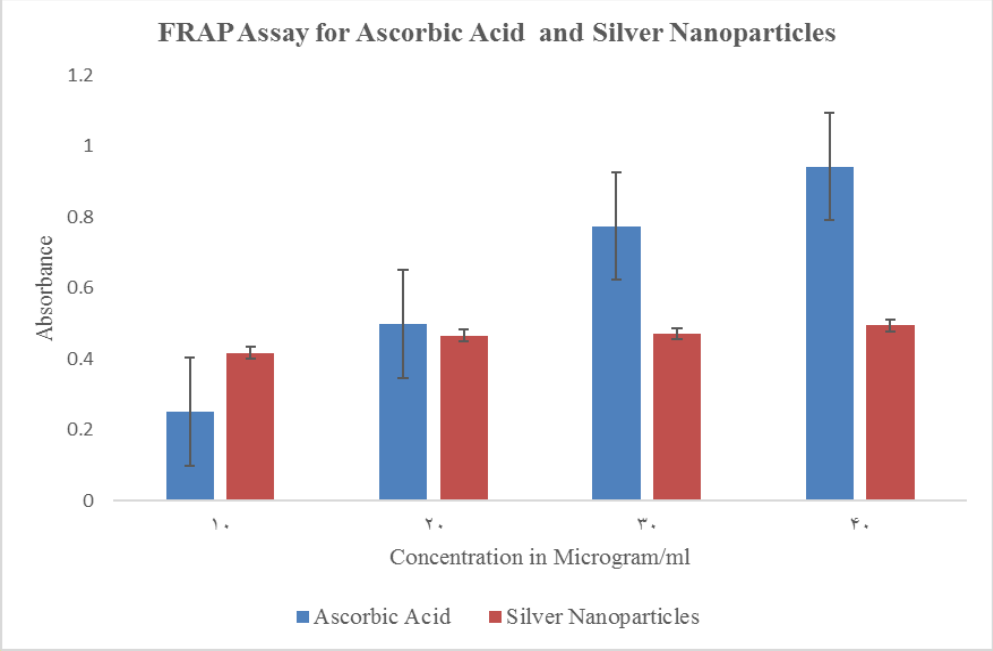
Figure 9. Color solution of ascorbic acid and AgNps with different concentrations

Table 1. Different conc. of standard (Ascorbic Acid) and their absorbance

Ascorbic Acid	Concentration (µg/mL)	Absorbance (A)
1	10	0.251
2	20	0.499
3	30	0.775
4	40	0.943
5	50	1.240

Table 2. Different conc. of AgNps and their absorbance

Sample (AgNPs)	Concentration (µg/mL)	Absorbance (A)
1	10	0.417
2	20	0.466
3	30	0.471
4	40	0.494



TEM analysis revealed the different size of nanoparticles. SAED and XRD pattern confirmed the crystalline nature of AgNPs.

Conclusions

The biosynthesized AgNPs were found to be active against *P.aeruginosa* and *S.aureus* which showed a broad-spectrum antimicrobial susceptibility range and therefore represent promising antimicrobial agents with potential biomedical applications, and also exhibited good antioxidant activity. So it might be useful in making drugs against some bacteria or in the prevention of free radical reducing diseases such as cancer, tumour, etc. The consequence of this work shows a broad range of applications of synthesized silver nanoparticles from aqueous flower extract of *Cascuta reflexa*. Hence, this can be used as a good therapeutic agent against human pathogens. They can be used for successful development of drug delivery in future. It can also be produced commercially at large scale.

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Disclosure Statement

No potential conflict of interest was reported by the authors**.**

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