

## Synthesis of silver nanoparticles from *Anagallis arvensis* and their biomedical applications

Mohib Shah<sup>1\*</sup>, Natasha Anwar<sup>2</sup>, Samreen Saleem<sup>3</sup>, Iqbal Munir<sup>5</sup>, Niaz Ali Shah<sup>4</sup>, Baseerat Fida<sup>1</sup>  
Abbas Khan<sup>2</sup>, Anwar Hussain<sup>1</sup> and Faiz Ul Amin<sup>5</sup>.

<sup>1</sup>Department of Botany, Abdul Wali Khan University Mardan, 23200, Pakistan

<sup>2</sup>Department of Chemistry, Abdul Wali Khan University Mardan, 23200, Pakistan

<sup>3</sup>Department of Botany, Women University Swabi, Pakistan

<sup>4</sup>Tsinghua University, Beijing, China. Postcode: 100084

<sup>5</sup>Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture Peshawar, Pakistan.

\* Corresponding author

Mohib Shah, Prof. Ph.D.

Department of Botany,

Abdul Wali Khan University, Mardan 23200, Pakistan

Tel #: +92-937-830056 Mobile #: +92-314-9047303

E-mail: [mohibshah@awkum.edu.pk](mailto:mohibshah@awkum.edu.pk)

## Abstract

**Background.** Nanotechnology is promising field for generating new applications. A green synthesis of nanoparticles through biological methods using plant extract have a reliable and ecofriendly approach to improve our global environment.

**Methods.** Silver nanoparticles (AgNPs) were synthesized using aqueous extract of *Anagallis arvensis* L and silver nitrate and were physicochemically characterized.

**Results.** The stability of AgNPs toward acidity, alkalinity, salinity and temperature showed that they remained stable at room temperature for more than two months. The SEM and TEM analysis of the AgNPs showed that they have a uniform spherical shape with an average size in the range of 40–78 nm. Further 1-Dibhenyl-2-Picrylhydrazl radical in *Anagallis arvensis* L-mediated AgNPs showed a maximum activity of 98% at concentration of 200 $\mu$ g/mL. Hydrogen peroxide scavenging assay in *Anagallis arvensis* L. mediated AgNPs showed a maximum activity of 85% at concentration of 200 $\mu$ g/mL. Reducing power of *Anagallis arvensis* L. Ag NPs exhibited a higher activity of 330  $\mu$ g/mL at concentration of 200  $\mu$ g/mL. These NPs have cytotoxic effects against brine shrimp (*Artemia salina*) nauplii with a value of 53% LD<sub>178.04 $\mu$ g/mL.</sub>

**Conclusion.** The AgNPs synthesized using *Anagallis arvensis* L. extract demonstrate a broad range of applications.

**Keywords:** Silver Nanoparticles, Green Synthesis, Anti diabetic, Cytotoxic.

## 1. Introduction

In the last two decades, nanoparticle (NPs) research has become one of the most important areas in modern materials science. Nanotechnology deals with exploitation of unique properties of tiny particles at molecular levels i.e. millionth of millimeter. Scientists from different disciplines including chemists, engineers, material scientists, physicists as well as biologists are involved in this emerging field of research. Generally, nanotechnology works with materials, devices and other structures with at least one dimension sized from 1 to 100 nm ([Gardea-Torresdey et al. 2003](#)). The term "nano-technology" was used by Norio Taniguchi in 1974 to designate semiconductor processes involving control on the order of a nanometer. From the mid of 1980s, progress in nanometer scale science and technology was exploded. The term "nano" is derived from Greek origin and means dwarf or extremely small. This term was widely adapted by almost all walks of people. This technology is being applied in almost every field including biomedicine, mechanics, energy science, materials development, magnetics, optics, electronics and information technology. It is multidisciplinary field concerning research and advancement of technology in various fields of science like physics, chemistry, material science and biotechnology ([Taniguchi 1974](#)).

Nanotechnology concerns with the progress of experimental processes for the synthesis of NPs of different sizes, shapes and controlled disparity ([Bhatt & Tripathi 2011](#)). Ultrafine particle or NPs are between 1 to 100 nm in size ([Meshot et al. 2012](#)). The NPs have greater surface area per volume than larger particles and exerting a stronger effect on the surrounding environment and reaction with other substances. Consequently, nanotechnology can make harmless substances assume hazardous characteristics. A reduction in the size of nano-sized particles increases the

particle surface area. Additional chemical molecules may attach to this surface, enhancing the reactivity and increasing toxic effects.

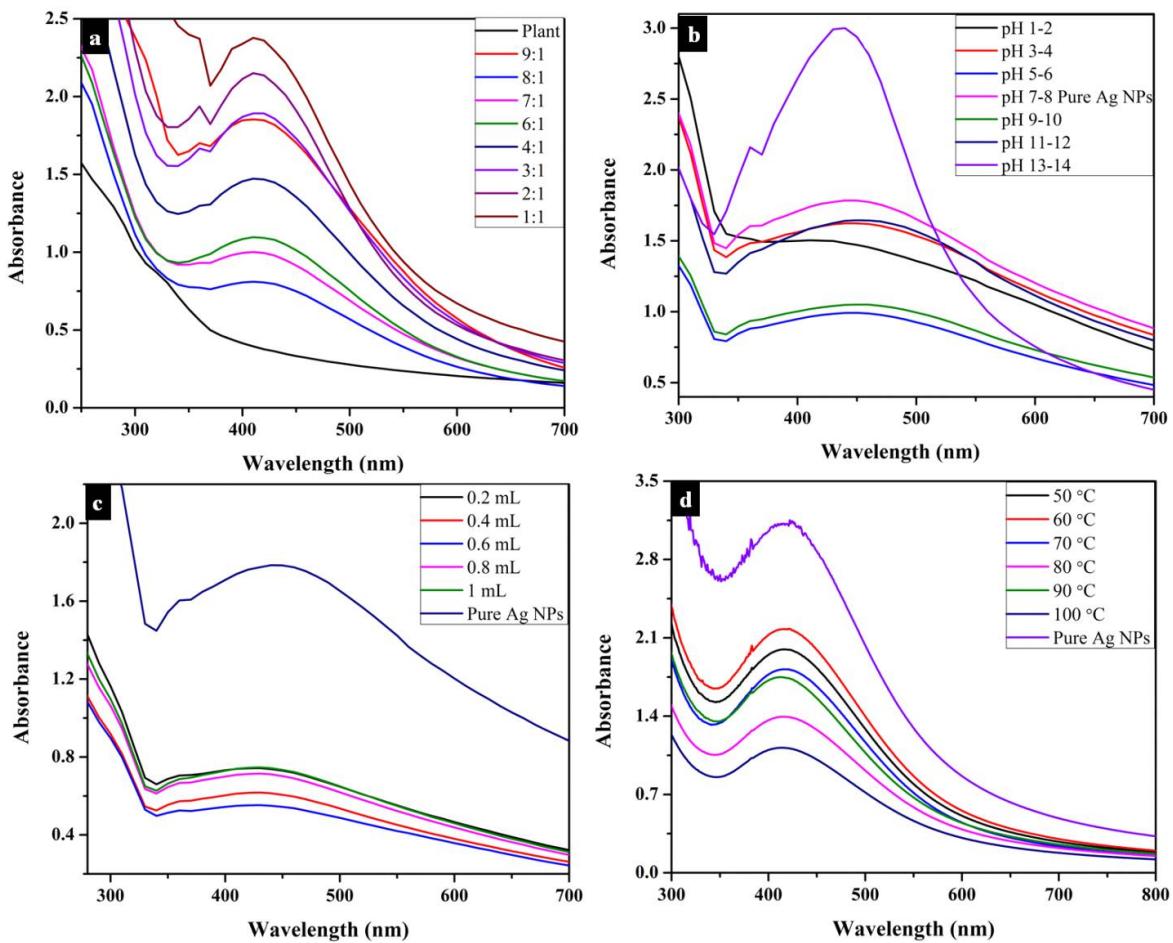
The NPs research is currently an area of scientific interest due to a wide variety of potential application in bio-medicinal, optical and electronic fields. In the recent few decades the field of metal NPs has a wonderful growth. Fe NPs were synthesized for the first time from  $\text{Fe}(\text{CO})_5$  in organic solvents and similar approach was used to make Co NPs. Amorphous Fe NPs may be prepared at low temperature and the carbonyl is decomposed chemically. But great research has been done on silver and gold NPs due to their unique properties (e.g., size and shape depending optical, electrical and magnetic properties) which can be incorporated into antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products and electronic components. Metal NPs can be synthesized using different physical and chemical methods ([Mohanuria et al. 2008](#); [Yu 2007](#)) i.e. citrate reduction method([Turkevich et al. 1951](#)), two phase synthesis and one phase synthesis in organic solvents([Shem et al. 2009](#)). Among the noble metal NPs, silver NPs are a playful product from the field of nanotechnology which has gained boundless interests because of their distinctive properties such as chemical stability, good conductivity, catalytic and most important antibacterial, anti-viral, antifungal and anti-inflammatory activities which can be incorporated into composite fibers, cryogenic superconducting materials, cosmetic products, food industry and electronic components([Ahmad et al. 2003](#); [Klaus-Joerger et al. 2001](#)). They have also lot of application on biomedical side; being added to wound dressings, topical creams, antiseptic sprays and fabrics. Silver functions as an antiseptic and displays a broad biocidal effect against microorganisms through the disruption of their unicellular membrane thus disturbing their enzymatic activities. In our present study AgNPs were synthesized by green way because it play

an important role in our daily life and have wide-ranging applications such as bimolecular detection, catalysis, biosensors and medicine; it is been acknowledged to have strong inhibitory and bactericidal effects along with the anti-fungal, anti-inflammatory and anti-angiogenesis activities (El-Chaghaby & Ahmad 2011). To the best of our knowledge, this is the first report of silver NPs synthesis using an aqueous extract of *Anagallis arvensis* Lor scarlet pimpernel commonly known as blue-scarlet pimpernel (Khoshkholgh-Pahlaviani et al. 2013).

## 2. Results

### Optimization of AgNO<sub>3</sub>/Plant ratios for *Anagallis arvensis* L. stabilized Ag NPs

UV-Visible spectra of AgNPs stabilized with *Anagallis arvensis* L. showed a strong absorbance band near 420nm. Resonance plasmon band in the region of 400nm to 500nm was an indication of the presence of Ag NPs (Ahmad et al. 2011). Optimization study was performed by carrying out a number of reactions while varying the amounts of salt to plant ratio. The UV-Vis spectra for such experiments are shown in Fig. 2a. The best optimized ratio, having good SPR in characteristic region of AgNPs, selected for further studies was 2:1.



**Figure 2.** UV-visible spectra of AgNPs/*Anagallis Arvensis L.* **(a)** UV-visible spectra of Ag NPs/*Anagallis Arvensis L.* having different AgNO<sub>3</sub>/Plant ratios. **(b)** Effect of pH on the stability of Ag NPs. **(c)** Effect of salt (NaCl) on the stability of Ag NPs. **(d)** Effect of temperature on the stability of Ag NPs.

### Stability study of Ag-NPs stabilized with *Anagallis arvensis L*

Stability of prepared AgNPs stabilized with *Anagallis arvensis L.* was checked by varying the pH of the solution, by adding different concentration of NaCl and by varying temperature of the solution. The stability of Ag NPs toward acidity, alkalinity, salinity and temperature showed that they remained stable at room temperature for more than two months.

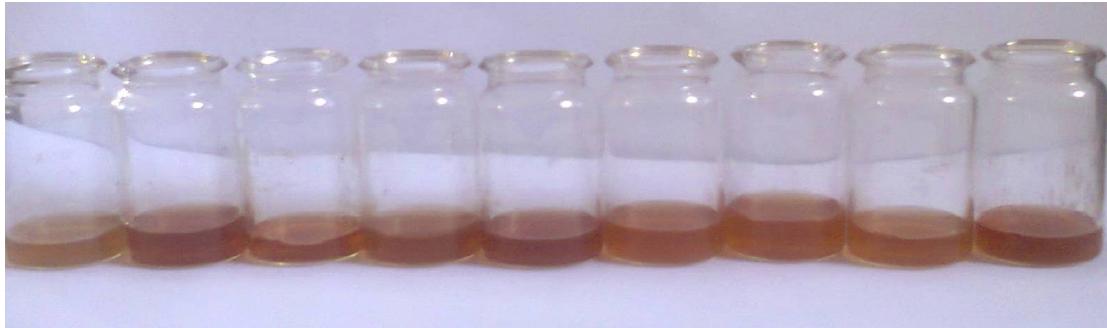


Figure 3a. AgNPs capped with *Anagallis Arvensis L.* having different  $\text{AgNO}_3$ /Plant ratios.



Figure 3b. AgNPs caped with *Anagallis Arvensis L.* showing the effect of salt ( $\text{NaCl}$ ) on the stability of AgNPs.

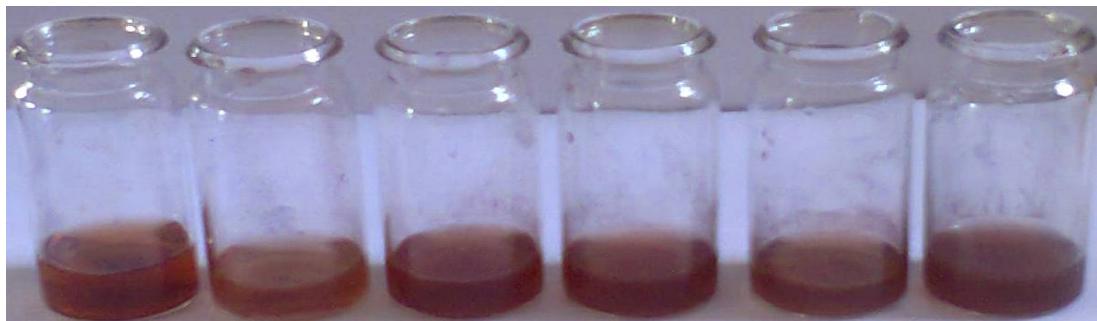


Figure 3c. AgNPs caped with *Anagallis Arvensis L.* showing the effect of temperature on the stability of Ag NPs

## Size of the Prepared Nanoparticles

The size of the nanoparticles were checked by SEM and confirmed by TEM. The SEM analysis of the Ag NPs showed that they have a uniform spherical shape with an average size in the range of 40–78 nm. This was confirmed by TEM analysis which showed an average size of about 50 nm.

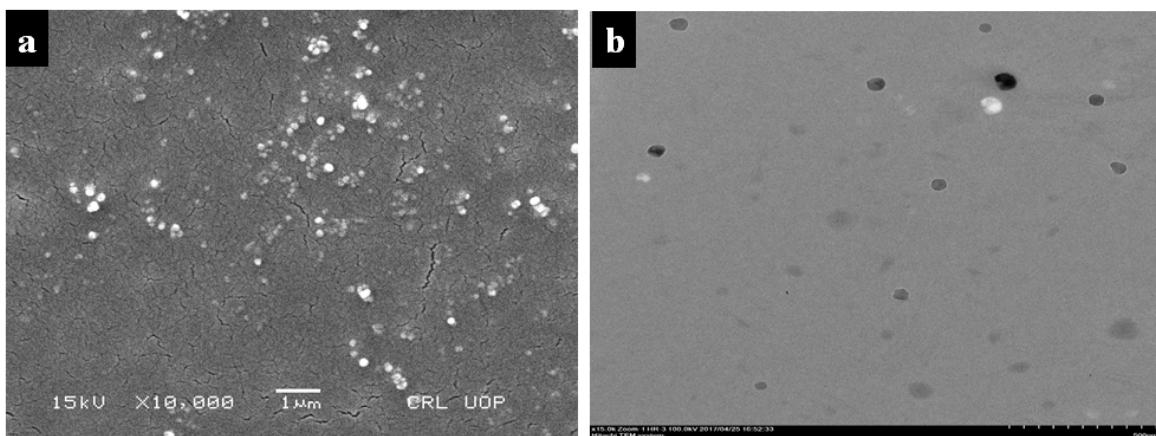


Figure 4. Physical characterization of AgNPs capped with *Anagallis arvensis* L.  
(a) SEM image of AgNPs (b) TEM image of AgNPs.

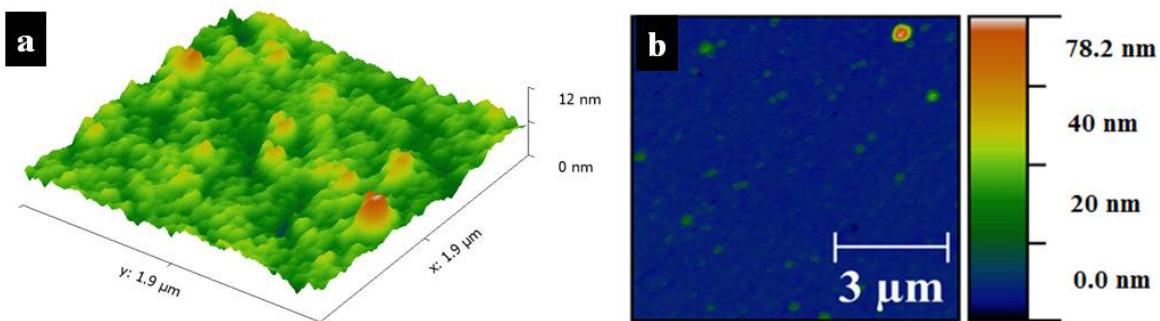


Figure 5. AFM images of Ag NPs.

## DPPH Antioxidant Assay

The antioxidant activity of *Anagallis arvensis* L. aqueous extract and bio-conjugated AgNPs was evaluated using DPPH scavenging assay. The ability of compounds to act as free radical scavengers can be checked by using DPPH assay (Andrade et al. 2013). From (Table 1), it is clear that there was a dose dependent increase in the percentage inhibition (% inhibition) of extract and synthesized Ag NPs.

**Table 1.** Quantitative screening and IC<sub>50</sub> values of antioxidant activity of plant extract and Ag NPs by DPPH assay

Concentration	Plant extract	Plant_AgNPs	Samples	IC <sub>50</sub> (μg/ml)
7.4 (μg/mL)	20.10	30.54		
22.4 (μg/mL)	55.80	65.70	Plant extract	18.29
66.6 (μg/mL)	73.80	81.20	D	12.98
200 (μg/mL)	88.92	92.70	Ascorbic Acid	4.56

**Table 2.** Quantitative screening and IC<sub>50</sub> value of antioxidant activity of plant extract and Ag NPs by hydrogen peroxide assay.

concentration	Plant extract	AgNPs	Samples	IC <sub>50</sub> (μg/ml)
7.4 (μg/mL)	10.10	32.10	Plant extract	60.97
22.4 (μg/mL)	24.30	54.90	AgNPs	14.33
66.6 (μg/mL)	54.30	79.80	Ascorbic Acid	4.56
200 (μg/mL)	73.90	85.40		

## Hydrogen Peroxide Scavenging Activity

$\text{H}_2\text{O}_2$  scavenging activity of the plant extract and Ag NPs capped with plant extract was due to the transfer of electrons to  $\text{H}_2\text{O}_2$  and neutralize it into water.  $\text{H}_2\text{O}_2$  inhibition activity assay plays a significant role for the determination of antioxidant activity (Mittal et al. 2014). Here also a dose dependent increase was observed with concentration.

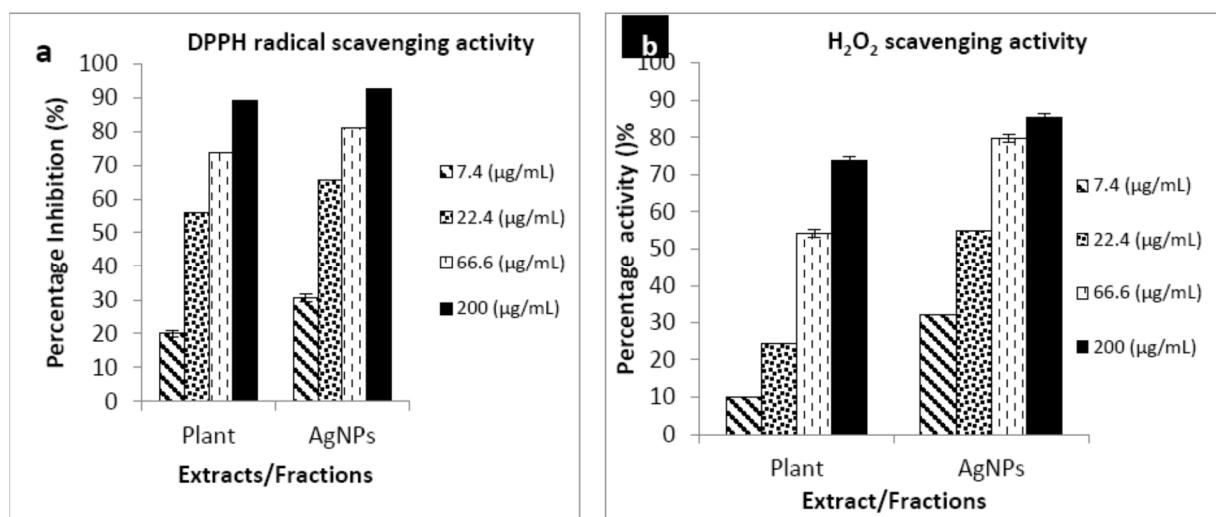


Figure 6. Antiradical and Hydrogen peroxide activity of plant extracts and synthesized AgNPs in comparison to reference drug ascorbic acid.

## Alpha-amylase inhibitory assay

Results revealed that plant extract and Ag NPs showed alpha amylase inhibitory activity of different level. Percent inhibition of salivary alpha amylase activity and  $\text{IC}_{50}$  values are shown in (Table 3) and graphically represented in (Figure 7a).

## Brine shrimp cytotoxic assay

The pharmacological properties of plant extract and Ag NPs can be determined by using brine shrimp cytotoxic activities (Hatano et al. 1989). In this study, four different concentrations;

7.4, 22.4, 66.6 and 200  $\mu\text{g}/\text{mL}$  of synthesized NPs and plant extract were used to find their cytotoxicity using brine shrimp lethality assay (Figure 7b).

**Table 3.** Inhibitory effect and IC<sub>50</sub> value of Ag NPs and crude extract on  $\alpha$ -amylase activity. Brine shrimps cytotoxicity assay of silver nanoparticles capped with *Anagallis arvensis* L. and extract of *Anagallis arvensis* L. Values (mean  $\pm$  SE) are average of three samples of both nanoparticles and extract, analyzed in 3 individual experiments (n = 3).

Alpha amylase inhibitory assay					Brine shrimp lethality assay				
Concentration	Plant	AgNPs	Samples	IC <sub>50</sub> ( $\mu\text{g}/\text{ml}$ )	Concentration	Plant	AgNP s	Sample s	IC <sub>50</sub> ( $\mu\text{g}/\text{m l}$ )
7.4 ( $\mu\text{g}/\text{mL}$ )	18.70	40.90	Plant	39.06	7.4 ( $\mu\text{g}/\text{mL}$ )	29.70	26.80	Plant	243.26
22.4 ( $\mu\text{g}/\text{mL}$ )	42.80	53.50	AgNPs	14.07	22.4 ( $\mu\text{g}/\text{mL}$ )	32.70	30.40	AgNPs	178.04
66.6 ( $\mu\text{g}/\text{mL}$ )	53.20	75.50	Acarbose	23.09	66.6 ( $\mu\text{g}/\text{mL}$ )	36.80	35.00	KMnO <sub>4</sub>	0.96
200 ( $\mu\text{g}/\text{mL}$ )	77.30	84.30			200 ( $\mu\text{g}/\text{mL}$ )	47.00	53.00		

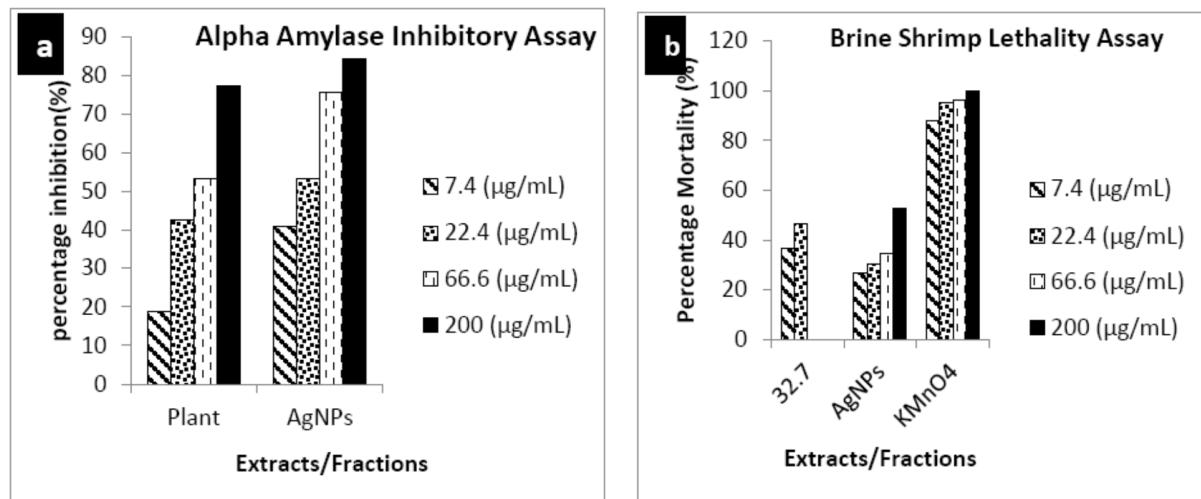


Figure 7. (a) Alpha-amylase and (b) Brine shrimps cytotoxicity assay, of plant extract and biosynthesized AgNPs at different concentrations.

## Reducing power

The ability of extract and Ag NPs to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  (reducing effect) was determined according to the previously described method (Subramanian et al. 2013). The reducing capacity of both plant extract and Ag NPs gives an indication of its potential antioxidant activity (Meir et al. 1995). Ascorbic acid was used for comparison. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes; so that they can act as primary and secondary antioxidants (SivaKumar et al. 2015). The results were expressed as  $\mu\text{g/mL}$  of ascorbic acid, plant extract and NPs (Table 4).

**Table 4.** Reducing power assay and  $\text{IC}_{50}$  value of plant extract and biosynthesized Ag NPs

Concentration	Plant extract	AgNPs	Samples	$\text{IC}_{50}$ ( $\mu\text{g/ml}$ )
7.4( $\mu\text{g/mL}$ )	111.43	121.40	plant	60.97
22.4( $\mu\text{g/mL}$ )	147.92	156.80	AgNPs	14.33
66.6( $\mu\text{g/mL}$ )	215.00	222.80	Ascorbic Acid	4.56
200 ( $\mu\text{g/mL}$ )	322.68	330.30		

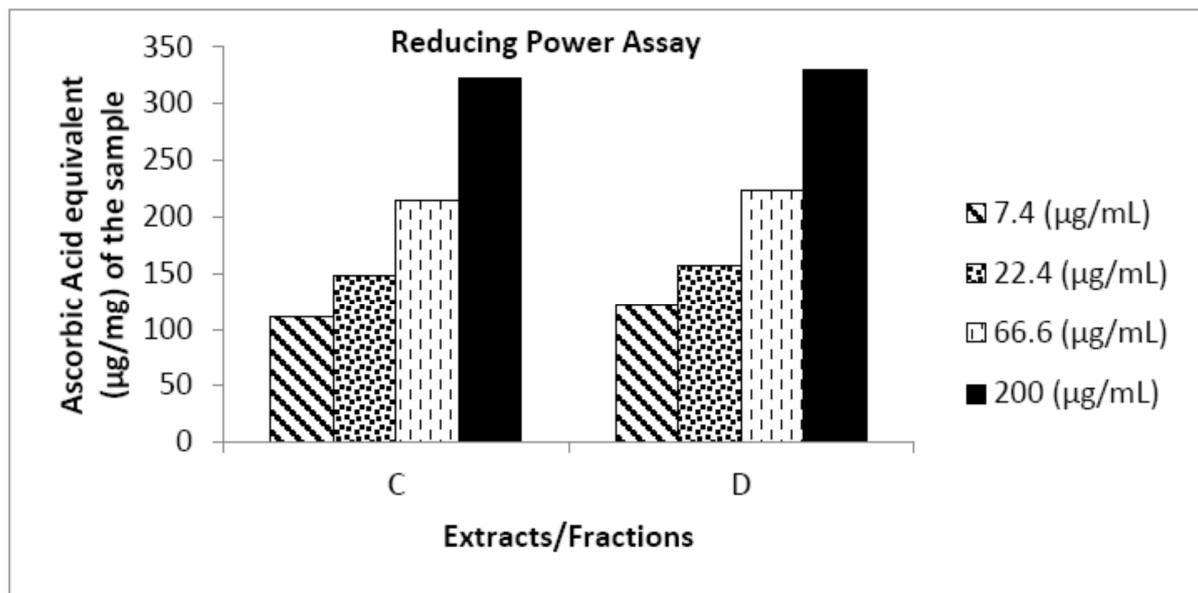


Figure 8. Reducing power activity of plant extract and biosynthesized silver nanoparticles (AgNPs) at different concentrations.

### 3. Discussion

#### Optimization of AgNO<sub>3</sub>/Plant ratios for *Anagallis arvensis* L. stabilized Ag NPs

UV-Visible spectra of Ag NPs stabilized with *Anagallis arvensis* L. showed a strong absorbance band near 420 nm. Resonance plasmon band in the region of 400 nm to 500 nm was an indication of the presence of Ag NPs (Ahmad et al. 2011). Optimization study was performed by carrying out a number of reactions while varying the amounts of salt to plant ratio. The UV-Vis spectra for such experiments are shown in Fig. 2a. The best optimized ratio, having good SPR in characteristic region of Ag NPs, selected for further studies was 2:1.

#### Stability study of Ag-NPs stabilized with *Anagallis arvensis* L.

They were highly stable in basic medium, less stable in neutral medium while unstable in acidic medium. Larger size NPs were synthesized at low pH (acidic), whereas, at higher pH, smaller size NPs synthesis was observed (Fig.2b). The effects of pH on the shape and size of AgNPs was

studied and it was found that NPs aggregation seems to surpass the nucleation process in acidic conditions. At high pH, though, the great numbers of nuclei formation, instead of aggregation led to the synthesis of more of NPs with smaller diameter. At high pH there was high concentration of hydroxyl ions on the surface of NPs, repulsive forces dominated and particles aggregation is reduced. The optimum pH was found to be 13-14 ([Iravani & Zolfaghari 2013](#)).

The stability of AgNPs capped with *Anagallis arvensis* L. decreases with increase in concentration of NaCl ([Fig.2c](#)). It means that stability of AgNPs is inversely proportional to the concentration of the salt used. Adding more concentration of the salt will cause aggregation of AgNPs due to  $\text{Cl}^{-1}$  ions because halide salts are known to affect the properties of AgNPs. They are used to induce aggregation by increasing the ionic strength of the solutions. So adding more amount/concentration of NaCl to NPs can decrease its stability and more aggregation can occur ([Naz et al. 2013](#)). The effect can be attributed to the variation of electrostatic interactions (attraction/repulsion) of  $\text{Ag}^+$  ions with those of NaCl in aqueous media at constant pH. The variation of ionic strength of salt may also affect the micro-environment around the ions and hence the stability of AgNPs.

While studying temperature effect it was observed that as the temperature increases the absorbance decreases as shown in ([Fig.2d](#)). With the optimized ratio which was 2:1 at room temperature, peak arises at 430 nm that indicates monodispersed fine particles and reduction in size of the NPs. Whereas at 50°C to 90°C the absorbance is decreased and asymmetry with broad peak width was observed and at which start off the aggregation of NPs due to denaturation of proteins capping at high temperature. Room temperature was an optimized condition for heating at which maximum synthesis of Ag NPs occur ([Amin et al. 2012](#)).

### Size of the Prepared Nanoparticles.

SEM images show that the NPs were nearly spherical in shape (Andrade et al. 2013)(Fig.4a).which was confirmed by TEM image (Fig.4b) . The major sizes of AgNPs prepared from *Anagallis arvensis* L. leaves extracts were 26, 33 and 39 nm, respectively. The synthesized AgNPs were capped by different plant constituents (polysaccharides, protein, polyphenolic and flavonoidal compounds) that prevented their aggregation. Inherent capping offers the additional advantage of the stability in the green chemical synthesis (Morones et al. 2005). Further AFM images showed the detailed size and morphology of Ag NPs(Logeswari et al. 2015). The area analyzed was 1.9  $\mu\text{m} \times 1.9 \mu\text{m}$ . The particle size of the Ag NPs was found to be 26 nm, 30 nm, 22 nm and 15nm (Fig.5).

### DPPH Antioxidant Assay

The recorded value (% inhibition) for the lowest concentration (7.4  $\mu\text{g/mL}$ ) of the aqueous extract was 20.10 and this value was increased to 55.80 when the concentration was increased to 22.4  $\mu\text{g/mL}$ . However, for AgNPs the percent inhibition values recorded were 30.54 for the concentration of 7.4  $\mu\text{g/mL}$  and 65.70 for the concentration of 22.4  $\mu\text{g/mL}$ . These values indicate that the synthesized bio-conjugated AgNPs have better antiradical potential than the extracts alone. The lower value of  $\text{IC}_{50}$  indicates a higher antioxidant activity. In DPPH radical scavenging, the highest activity was observed in AgNPs(200  $\mu\text{g/mL}$ ). The  $\text{IC}_{50}$  values of both extract and synthesized AgNPs are reported in (Table 1). It can be concluded from the data that synthesized AgNPs displayed better antioxidant activity in comparison to the *Anagallis arvensis* L.extract (Figure 6a)(Parveen et al. 2016).

## Hydrogen Peroxide Scavenging Activity

*Anagallis arvensis* L. showed minimum activity of 10.10 at concentration of 7.4  $\mu$ g/mL and maximum activity of 73.90 at a concentration of 200  $\mu$ g/mL followed by Ag NPs capped with plant extract showing a minimum activity of 32.10 at concentration of 7.4 $\mu$ g/mL and maximum activity of 85.40 at a concentration of 200  $\mu$ g/mL ([Figure 6b](#)). The IC<sub>50</sub> values of both extract and synthesized AgNPs are reported in ([Table 2](#)). It can be concluded from the data that synthesized AgNPs displayed better antioxidant activity compared to *Anagallis arvensis* L. extract([Parveen et al. 2016](#)).

## Alpha-amylase inhibitory assay

Percent inhibition of salivary alpha amylase enzyme by *Anagallis arvensis* L. extract (at concentration ranging from 7.4  $\mu$ g/mL to 200 $\mu$ g/mL) was found to be 18.70to 77.50with IC<sub>50</sub> value of 39.06  $\mu$ g/mL. This inhibition by AgNPs (at concentration ranging from 7.4  $\mu$ g/mL to 200 $\mu$ g/mL) was found to be 40.90to 84.30with IC<sub>50</sub> value of 14.06  $\mu$ g/mL. AgNPs synthesized from *Anagallis arvensis* L.demonstrated high inhibitory activity against  $\alpha$ -amylase than acarbose([Rehana et al. 2017](#)).

## Brine shrimp cytotoxic assay

IC<sub>50</sub> value of extract was found up to 243 $\mu$ g/mL ([Table 3](#)). The lowered IC<sub>50</sub> value suggested the cytotoxic nature of AgNPs compared to plant extract. This enhanced cytotoxicity of NPs having IC<sub>50</sub> of 178.04  $\mu$ g/mL to brine shrimp revealing the presence of toxic constituents. The cytotoxic effect of NPs shows that they can be used as alternative source of anticancer drugs ([Ghareed et al. 2014](#)). These days much attention is being given metallic NPs and their anticancer activity ([Ruby et al. 2012](#)).

## Reducing power

There was dose dependent increase in the reducing power of plant extract as well as Ag NPs. The reducing capacity of the plant extract for the concentration of 200  $\mu\text{g}/\text{mL}$  was 322.68  $\mu\text{g}/\text{mL}$  and for Ag NPs this value was 330  $\mu\text{g}/\text{mL}$ . The reducing power of ascorbic acid was found to be significantly higher than those of plant extract and Ag NPs. This reducing capacity was due to polyphenols flavonoids. The reducing power increased with increasing the phenolic content of the extract, so that's why there was dose dependent increase in the reducing power with concentration ([Figure 8](#)).

## 4. Materials and Methods

Silver nitrate ( $\text{AgNO}_3$ ), hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from Merck. Deionized distilled water ( $\text{ddH}_2\text{O}$ ) was used for all reactions, solution preparations and analysis purpose and was obtained from Millipore Milli-Q system (Millipore, Bedford, MA, USA). Perkin-Elmer spectrophotometer (Lambda 25) was used initially for optimization study of plant/salt ratio for study of better samples. FT-IR spectra were recorded on IR Affinity.1 Shimadzu (Japan). Particle morphology and average size were investigated by using SEM(FEI Quanta FEG 200; FEI Company, Hillsboro, OR) with high and low vacuum. For optimization of reaction samples, pH of the medium was also set using pH meter Milwaukee, MW802, pH/EC/TDS meter. Atomic force microscopy (AFM) analysis for synthesized NPs was done by using the instrument NOVA NT-MDT SOLVER NEXT, Russia. TEM analysis was performed by using HR-3100 advanced 0 kV TEM from Hitachi (Japan).

## Collection of plant material

In the present study *Anagallis arvensis* L. was freshly collected from various localities of district Swabi (Khyber Pakhtoonkhwa, Pakistan). The plant was collected in March, April and June. Samples were brought to the laboratory in polythene bags and were clean thoroughly with fresh water to remove the debris and other micro flora and fauna. The plant samples were identified with the available literature of flora of Pakistan. It is considered one of the precious assets in the medicinal plant market. Owing its importance in the market it was highly desired to use it for green synthesis.

## Preparation of crude extracts of *Anagallis arvensis* L.

Plant leaf extract of *Anagallis arvensis* L. was prepared by taking 5 g of the leaves and properly washed in distilled water. They were then cut into fine pieces and taken in a 250 mL Erlenmeyer flask with 100 mL of sterile distilled water. It was boiled and filtered. The extract was stored at 4 °C and used within a week.

## Synthesis of Ag NPs stabilized with *Anagallis arvensis* L.

*Anagallis arvensis* L. was also used to further explore the green synthesis and stability of AgNPs. 100mL of the prepared solution of plant was taken in a volumetric flask. 1mM AgNO<sub>3</sub> solution was also prepared for the reaction in a separate vial. Both solutions were taken in a vial with different salt/plant molar ratios, such as 1:1, 2:1....10:1 etc., for different reactions. The reaction mixtures were kept on stirring for 4 hours at room temperature. The change in color gives a primary indication of NPs formation. A particular absorption plasmon band in the region of 400 to 500 nm showed the presence of Ag NPs formation. The AgNPs were isolated by

centrifugation at 14000 rpm. The solid AgNPs were collected for FT-IR, SEM, TEM and AFM studies.

### **Biological activities**

To see the potential biomedical use of these green synthesized/stabilized noble metal NPs, DPPH antioxidant assay, hydrogen peroxide ( $H_2O_2$ ) scavenging assay, reducing power, brine shrimp cytotoxic assay and alpha-amylase inhibitory assay were also investigated.

#### **DPPH Antioxidant Assay**

The scavenging activity of DPPH free radical of plant extracts were achieved by using method described by (Kulicic et al. 2004) and adapted by (Obied et al. 2005). Aliquots of 200  $\mu$ L of each test sample (200  $\mu$ g/0.5mL, 66.6/0.5mL, 33.3/0.5mL, 7.4/0.5mL)  $\mu$ g/mL of all extracts were stirred with 2800  $\mu$ L of DPPH 3.2 mg/100 mL solution in test tube. After 1hr of incubation in dark at room temperature, absorbance of each reaction mixture was taken at 517nm by using UV-visible spectrophotometer. In negative control 200  $\mu$ L of respective solvent(methanol) and 2800  $\mu$ L of DPPH solution were used and in blank methanol were used. In positive control, Ascorbic acid is used as same concentrations of plant sample. Each experiment was repeated in triplicates. The DPPH scavenging activity was calculated by using given formula and graphical method was used to calculate the  $IC_{50}$  value.

$$\text{Percentage inhibition} = [(Ac - As)/Ac] \times 100$$

Whereas, Ac is negative control absorbance and As is absorbance of sample. Same method was used to determine the antioxidant activity of Ag NPs stabilized with *Anagallis arvensis* L. extract.

### Hydrogen peroxide scavenging assay

The capacity of the plant extract and silver nanoparticles capped with *Anagallis arvensis* L. to scavenge H<sub>2</sub>O<sub>2</sub> was determined according to the method of (Ruch et al. 1989). A solution of 2 mM H<sub>2</sub>O<sub>2</sub> was prepared in 50 mM phosphate buffer (pH 7.4). An aliquot of 100 µL of the sample was transferred to an Eppendorf tube to have final concentration in reaction mixture of 200, 66.6, 22.2 and 7.4 µg/mL and the volume was made up to 400 µl with 50 mM phosphate buffer (pH 7.4). After addition of 600 µL of H<sub>2</sub>O<sub>2</sub> solution, tubes were vortexed and absorbance of H<sub>2</sub>O<sub>2</sub> at 230 nm was determined after 10 minutes, against a blank. Ascorbic acid was used as positive control. The percentage H<sub>2</sub>O<sub>2</sub> scavenging ability of samples (extract/fraction) was then calculated by using the following equation:

$$\text{H}_2\text{O}_2\text{scavenging activity} = [1-\text{As}/\text{Ac}] \times 100$$

Whereas, As isabsorbance of sample, Ac isabsorbance of control. All experiments were performed in triplicate.

### Brine shrimp cytotoxic assay

The toxicity of *Anagallis arvensis* L. extract and Ag NPs capped with *Anagallis arvensis* L. were determined by brine shrimp cytotoxicity assay as reported by (Hossain et al. 2012). 2.5 mL of plant extract and Ag NPs solution was added to 2.5 mL of sea water containing 10 nauplii. Prepared plant extract and Ag NPs in different concentrations can be checked by implementing the assured volume of the plant extract and Ag NPs in vials have brine solution (Meyer et al. 1982). Selective ten brine shrimp nauplii were added into every sample vial and artificial sea water is added to adjust the final volume of every vial. Each vial consists of the 0.5mL of plant extract and Ag NPs and 4.5mL of manufactured sea water and 10 nauplii of brine shrimp(Hossain et al. 2012). Total volume of test solution is 5mL in each vial. The

experiments were performed in triplicate. All sample vials were kept uncovered under light source for next 24 hrs in incubator at room temperature. After 24 hrs, the number of alive shrimps were counted and noted carefully by using magnifying glass.

### **Alpha amylase inhibition assay**

Alpha-amylase inhibitory activity of extract and Ag NPs were performed according to the previously reported method ([Anjana et al. 2011](#)) with modest modification. In a test tube, reaction mixture containing 350 $\mu$ L phosphate buffer (50mM, pH= 6.8), 70 $\mu$ L alpha-amylase (10U/mL) [SRL] and 140 $\mu$ L of varying concentrations (7.4, 22.2, 66.6, 200  $\mu$ g/mL) of *Anagallis arvensis* L. plant extract and Ag NPs capped with *Anagallis arvensis* L. was pre-incubated at 37°C for 10 min. Then 140 $\mu$ L soluble starch (0.05%) [HiMedia] was added as a substrate and incubated further at 37°C for 15 min. The reaction was stopped by adding 140 $\mu$ L 1N HCl, followed by addition of 700 $\mu$ L iodine reagent (5mM I<sub>2</sub> and 5mM KI, stored in amber colored bottle). The absorbance was read at 620nm using UV-visible spectrophotometer. Each experiment was performed in triplicates, along with appropriate blanks. Acarbose at various concentrations (7.4, 22.2, 66.6, 200 $\mu$ g/mL) was included as a standard. Negative control without extracts was set up in parallel. The result is expressed as percentage inhibition, which was calculated as,

$$\% \text{ Inhibition} = \frac{A_{\text{test}} - A_{\text{negative control}}}{A_{\text{test}}} \times 100$$

Whereas, A is absorbance and the result were also expressed as IC<sub>50</sub> value.

### **Reducing power**

The reducing power of was evaluated according to procedure reported by ([Oyaizu 1986](#)). The reducing potential of the plant extract sample and Ag NPs was determined by using 200  $\mu$ L aliquot of extract prepared in DMSO mixed with 500  $\mu$ L of 2 M phosphate buffer at pH 6.6 and

500  $\mu$ L of 1% potassium ferricyanide [ $K_3Fe(CN)_6$ ]. The mixture was incubated at 50°C for 20 minutes. A volume of 500  $\mu$ L of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the mixture (500  $\mu$ L) was mixed with equal volume of distilled water and 100  $\mu$ L of 0.1% ferric chloride ( $FeCl_3$ ). Absorbance was measured at 700 nm. Blank was prepared by adding 200  $\mu$ L of DMSO instead of the extract and Ag NPs. The reducing power of each sample was expressed as ascorbic acid equivalent. All experiments were performed in triplicate.

## 5. Conclusion

This study demonstrated an ecofriendly, rapid, green approach to synthesis AgNPs by using *Anagallis Arvensis* L. as reducing agent which supplies a simple, cost-effective and ecological method for the synthesis of AgNPs. The AgNPs so-prepared exhibited effective antioxidant, antidiabetic and cytotoxic activities, suggesting AgNPs might be useful as a silver dressing for wounds or as an alternative material. The significances of this study demonstrate a broad range of applications of synthesized NPs.

## Author Contributions

Conceptualization, Mohib Shah, Natasha Anwar and Iqbal Munir; Data curation, Iqbal Munir and Faiz Ul Amin; Formal analysis, Natasha Anwar; Funding acquisition, Anwar Hussain; Investigation, Niaz Ali Shah; Methodology, Samreen Saleem and Niaz Ali Shah; Resources, Abbas Khan; Supervision, Mohib Shah; Validation, Baseerat Fida; Visualization, Anwar Hussain; Writing – original draft, Mohib Shah; Writing – review & editing, Faiz Ul Amin.

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