International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 9 | Special Issue 1- NCBTIL | 2019 | 21-33
National Conference on Biochemistry –Transcending and Integrating Life Sciences (NCBTIL) -2019
Held @ Kristu Jayanti College, K Narayanapura, Kothanur PO, Bengaluru-560077, January 17th & 18th
| Conference Proceedings | Research Article | Biological Sciences |Open Access |MCI Approved|
|UGC Approved Journal|

Ecofriendly synthesis of silver nanoparticles using *Delonix regia* flower extract and its characterization by UV and SEM and *in vitro* study on its toxicity and antioxidant potential

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Received: 10 Dec 2018 / Accepted: 30 Dec 2018 / Published online: 10 Jan 2019
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Abstract

Delonix regia flowers were used for the preparation of aqueous extracts. To avoid the chemical toxicity in the environment, a cost-effective and environmentally friendly synthesis of metal nanoparticles was proposed as an alternative. The present paper involves eco-friendly synthesis of silver nanoparticles using *Delonix regia* flowers and evaluating their toxicity and antioxidant activities. The synthesis of silver nanoparticles was characterized using SEM and UV–Visible spectroscopic analysis. Scanning electron microscopy revealed the presence of silver nanoparticles of *Delonix regia* flowers with the smallest particle size in the range of around 40-90 nm and exist in spherical shape. The investigated samples were evaluated as antioxidant agents by DPPH assay and iron reducing power assay.

The results of antioxidant tests indicate that these nanoparticles possess significant antioxidant potentials compared to the results obtained by control samples. In addition, a toxicity evaluation of these AgNP containing solutions was carried out on seeds of Moong Bean (Vigna radiata). Results showed that seeds treated with AgNP solutions exhibited better rates of germination and catalase activity. Based on the results obtained it can be frequently said that the plant resources can be efficiently used in the production of silver nanoparticle and it could be utilized in several fields like scientific uniqueness, nanotechnology and so on.

Keywords

Silver nanoparticles, *Delonix regia*, UV–visible, SEM, Toxicity, antioxidant activity

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1. INTRODUCTION

In current science nanotechnology is a rapidly growing field for the researchers. Nanotechnology is a science of producing and utilizing nano-sized particles. A number of approaches are available for the synthesis of silver nanoparticles, such as thermal decomposition, electrochemical, microwave assisted process and green chemistry. The term green technology is used for an interdisciplinary field when various plant materials are used for the biosynthesis of nanoparticles, as it does not involve the use of hazardous chemicals, low material conversions and high energy requirements. Green syntheses of nanoparticles provide advancement over other methods as they are cost-effective, environment friendly, simple one step and relatively reproducible and results in more stable materials (Mittal, Batra, Singh, & Sharma, 2014).

So, a growing need to develop an environmentally friendly process for nanoparticle synthesis without using toxic chemicals is gaining importance (Logeswari et al., 2013). Products from nature, such as extracts of various plants or plants parts, biodegradable polymers (chitosan, etc.),tea, coffee, sugars , banana, simple amino acids, as well as wine and glucose, have been used as reductants and as capping agents during syntheses of nanoparticles and could be considered attractive for nanotechnology (Kharissova et al., 2013; Ahmed et al., 2014: Ahmed and Ikram, 2015).

Nanoparticles possess very high surface to volume ratios and thus, can be utilized in the scientific fields. They also exhibit unique properties and impart enhancements to various engineered materials like enhanced magnetic, electrical activity, and optical properties (Okafor et al., 2013). Silver is one of the basic elements that make up our planet. It is a rare, but naturally occurring element, slightly harder than gold and very ductile and malleable.

The studies performed by Larue et al., in 2014, estimated silver as one of the most commercialised nano-material with five hundred tons of silver nanoparticle production per year and is estimated to increase in next few years.

Silver nanoparticles have gained global popularity and have been extensively studied for many decades because of its wide range of application in crucial areas such as therapeutics, solar energy conversion, water treatment, , biomedical science ,surface coating ,areas of catalysis and cosmetic production (Sani et al., 2017).

Silver nanoparticles can be synthesized by physical as well as chemical methods (Tippayawat et al., 2016). Physical approaches include evaporation-condensation and laser ablation Chemical methods include chemical reduction of silver ions by using chemical substances like hydrazine, sodium borohydride, trisodium citrate, ascorbic acid and polyols etc. In these methods some chemical reagents are used additionally which may be hazardous to the environment. A number of methods like ion sputtering, chemical reduction, sol gel, etc are available for the syntheses of silver nanoparticles. (Bindhu and Umadevi, 2015, Mahdi et al., 2015, Padalia et al., 2014, Sre et al., 2015); but many of these methods involve the use of either hazardous chemicals or high energy requirements, which are rather difficult and also include wasteful purifications (Ahmed et al., 2016).

To avoid the toxicity of these chemicals and protect our environment, green synthesis approach started as an alternative method. Biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction/oxidation. As the physical and chemical methods were costly and hazardous to the environment and may have adverse effect in the medical applications, a demand for green syntheses rose over all the other processes. Thus, scientist started using cost effective, environment friendly plant extracts (phytochemicals) for nanoparticles synthesis,In this approach there is no need of high pressure, energy, temperature and toxic chemicals.

A lot of research has been done on biological syntheses of silver nanoparticles using microorganisms including bacteria, fungi and plants. Among the various biological methods, microbe mediated synthesis of silver nanoparticles is not of industrial feasibility as high aseptic conditions are required for their maintenance. The use of plant extracts for synthesis of silver nanoparticles is potentially advantageous over microorganisms because in case of plants elaborate process of maintaining cell cultures are available and also are less biohazard. Therefore, it is the best platform for syntheses of silver nanoparticles. The use of plant extracts generally reduces the cost of isolation of micro-organisms and their culture media thus enhance the cost competitive feasibility over silver nanoparticles synthesis by microorganisms.

The present study aims to apply a green synthesis technique for the synthesis of silver nanoparticles as an

alternative to conventional methods. In this regard, leaf extract of Delonix regia a flowering tree (commonly known as Gulmahor) a species of family Fabaceae, was used for bioconversion of silver ions to nanoparticles and the silver nanoparticles formed were characterized by using SEM and UV spectrophotometric analysis . This plant is commonly available in India and plant parts have been used as a traditional medicine. Recent research on gulmohar has shown many medicinal properties like antidiabetic activity, anti-Inflammatory activity antimicrobial, anti-diarrhea, hepatoprotective, anti-inflammatory, antioxidant, antibacterial, antipyretic etc. Gulmohar also have economic importance. The present study also involved its toxicity studies and antioxidant potentials.

2. EXPERIMENTAL METHOD

Generally, there are two approaches which are involved in the syntheses of silver nanoparticles, either from ''**top to bottom**'' approach or a ''**bottom to up**'' approach. In bottom to top approach, nanoparticles can be synthesized using chemical and biological methods by self-assemble of atoms to new nuclei which grow into a particle of nanoscale. Wheras in "top to bottom" approach, suitable bulk material breaks down into fine particles by size reduction with various lithographic techniques **(Figure 1).**

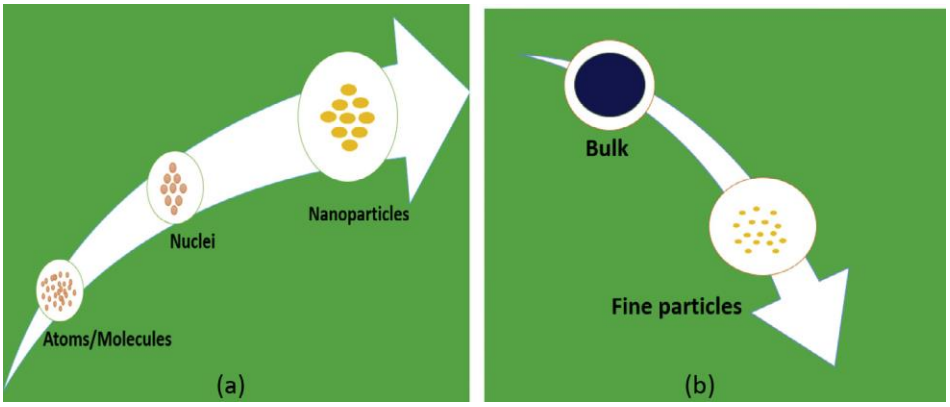


Fig. 1 Protocols employed for synthesis of nanoparticles (a) bottom to top approach and (b) top to bottom approach. (Courtesy: Shakeel Ahmed et al., 2016)

2.1. Preparation of the *Delonix regia* flower Extract:

Delonix regia flower were collected locally and rinsed throughly first with tap water followed by double distilled water to remove all the dust and unwanted visible particles, cut in to small pieces and dried at room temperature.

Delonix regia flower aqueous extract were used for the reduction of silver ions (Ag+) to silver nano particles (Ag0). The aqueous extract of *Delonix regia* flower were prepared by placing 5g of washed dried fine cut flowers in 250ml round bottom flask along with 200ml of double distilled water. The mixture was then boiled for 4 hours until the colour of the aqueous solution appeared as orange **(Figure 2).** Then the extract was cooled to room temperature and filtered with WhatmanNo.1 filter paper.

Fig. 2: Pure *Delonix regia* flower Extract

2.2. Synthesis of Silver Nanoparticle:

1mM aqueous solution of silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles. The *Delonix regia* flower extract of 10ml was added to 90ml of 1mM silver nitrate solution and kept at room temperature. As a result, a dark brown solution was formed indicating the formation of silver nanoparticles **(Figure 3)** and it was further confirmed by UV-Vis spectral analysis.

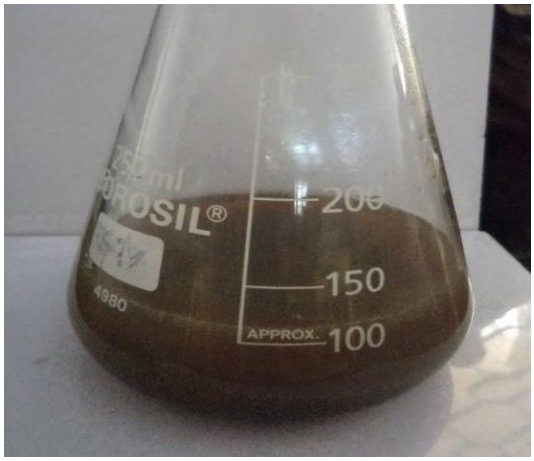


Fig 3: Formation of Silver nanoparticles

2.3. Separation of Silver nanoparticles:

The synthesized Silver nanoparticles we are separated by means of centrifugation at 3000 rpm for 15 minutes.

2.4. Characterization of Silver nanoparticles:

2.4.1. UV-Visible Spectral Analysis:

Characterization of silver nanoparticles was first carried out using UV-Visible absorption spectrometer with a resolution of 1nm between 200 and 800nm possessing a scanning speeds of 200nm/minutes. Absorption spectra of silver nanoparticles formed **(Figure 4)** in the reaction media have absorbance peak at 350nm.

2.4.2. SEM:

The Scanning electron microscopy image of *Delonix regia* stabilized silver nanoparticles was shown in **Fig.5**

2.5. Toxicity Assay:

2.5.1. Seed Germination:

Four different concentrations of (25%, 50%, 75% and 100% (v/v)) of Ag NP dispersions were prepared in distilled water. The germination test was carried out in sterile Petri dishes of 12 cm diameter by placing a Whatman® no. 3 filter paper on them. Fifty seeds of each receptor crop, Moong Bean (*V. radiata*), were placed in the respective Petri dishes. The seeds were surface sterilized with 0.1% HgCl2 solution and rinsed three times with distilled water. The solution of each concentration was added to each Petri dish of respective treatment daily in such an amount just enough to wet the seeds. The Petridish were then placed in seed germinator in dark room and maintained at 25°C. Seeds with root tip 1 mm and higher were considered as germinated. Percent germination and length of root (in mm) obtained following each 24 h up to 72 h after the germination of seeds was observed were calculated.

2.5.2. Catalase activity

2.5.2.1. Standard curve for H_2O_2

Different amounts of hydrogen peroxide, ranging from 10 to 160 µmoles, were taken in small test tubes and 2 ml of stop solution. (Dichromate in acetic acid, 1:3, and v/v) was added to each. Addition of the reagent to hydrogen peroxide instantaneously produced an unstable blue precipitate of perchromic acid. On subsequent heating for 10 min in a boiling water bath, the colour of the solution changed to stable green due to the formation of chromic acetate. After cooling at room temperature the volume o the reaction mixture was made 3 ml and the optical density was measured at 570nm.

2.5.2.2. Determination of catalase activity by dichromate-acetic acid solution:

Catalase activity was determined in germinating seedlings which were treated with different solvents - distilled water (control), *Delonix regia* flowere extract, silver nitrate solution and silver nanoparticles and expressed as µmol H_2O_2 consumed min^{-1} mg^{-1} protein.

2.6. Antioxidant activity

2.6.1. Determination of Total antioxidant capacity:

The antioxidant activity of the extracts was evaluated by the Phosphomolybdenum method according to the procedure of prieto *et al.* The assay is based on the reduction of Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. The flower extract 0.3ml (50 - 250ug/ml) was mixed with 2.5ml of Phosphomolybdenum reagent (0.6M sulphuric acid, 28mM sodium sulphate and 4mM Ammonium molybdate were mixed together in 250ml distilled water). The absorbance of the reaction mixture was measured after 15minutes at 695nm on UV-double beam spectrophotometer. The L-ascorbic acid was used as reference standard. For standard L-Ascorbic acid was used as a control and prepared by

dissolving 2mg of L-Ascorbic acid in 10ml. The antioxidant activity of sample was expressed as %.

$$\text{Total antioxidant assay (\%)} = \frac{\text{A Control} - \text{A Sample}}{\text{A control}} \times 100$$

2.6.2. Determination of DPPH radicals scavenging activity:

The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH. Determination of DPPH radicals scavenging activity was estimated with the method used by Kato. 1mM solution of DPPH in ethanol and also 1mg/1 ml extract solution in ethanol was prepared and 1.5ml of this solution was added to 1.5 ml of DPPH. The absorbance was measured at 517 m against the corresponding blank solution which is prepared by taking 3ml ethanol and control O.D. was prepared by taking 3ml of DPPH. The assay was performed in triplicates. Percentage inhibition of free radical DPPH was calculated based on control reading by following equation.

% DPPH radical scavenging

= Control OD- Sample OD /Control OD X 100

A con = absorbance of the control reaction.

A test = absorbance in the presence of the sample of the extracts.

2.6.3. Iron Reducing power assay:

0.75 ml of extract and 0.75ml phosphate buffer (0.2mole pH 6.6) different fraction was added to 0.75ml Potassium ferrocyanide (1mM) solution. The mixture was shaken thoroughly and incubated for 20 minutes at room temperature. Arrest the reaction by adding 0.75ml 10% TCA, centrifuge at 3000rpm for 10 minutes. 1.5ml supernatant and 1.5ml distilled water added to 0.1ml $FeCl_3$ (0.1%), wait for 10 minutes. Finally the mixture was tested spectrophotometrically at 700 nm against an appropriate blank of 0.75 ml of potassium ferrocyanide solution.

3. Results and Discussion

The green synthesis of silver nanoparticles through plant extracts were carried out. Silver nitrate was used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol. The appearances of brown colour in the reaction vessels suggest the formation of silver nanoparticles. In this present study, *Delonix regia* flower extract was used for the synthesis of silver nanoparticles. The aqueous AgNO3 solution turned to brown colour after 24h incubation with the addition of flower extract, indicating the formation of AgNPs in the reaction solution probably as a result of the excitation of surface plasmon resonance (SPR) bands. The control tubes (AgNO3) showed no change in colour when incubated in a similar condition. These silver nanoparticles were confirmed by Spectral techniques as given below:

3.1. UV-Visible Spectroscopy

UV-Vis spectroscopy analysis showed the absorbance band of silver Nanoparticles synthesized using *Delonix regia* flower extract at 400 nm. The broadening of the peak indicates the uniform distribution and polydispersion of silver nanoparticles raised from the leaf extract of *Holoptelea integrifolia.* The size and shape of the nanoparticle was determined by the frequency and width of the surface plasmon absorption produced. The surface Plasmon resonance (SPR) at 350nm (Figure 4) similar to those reported in literature (Obaid et al., 2015) indicates the stable silver nanoparticle formation, using the flower extract of *Delonix regia* were confirmed by UV Vis spectral analysis.

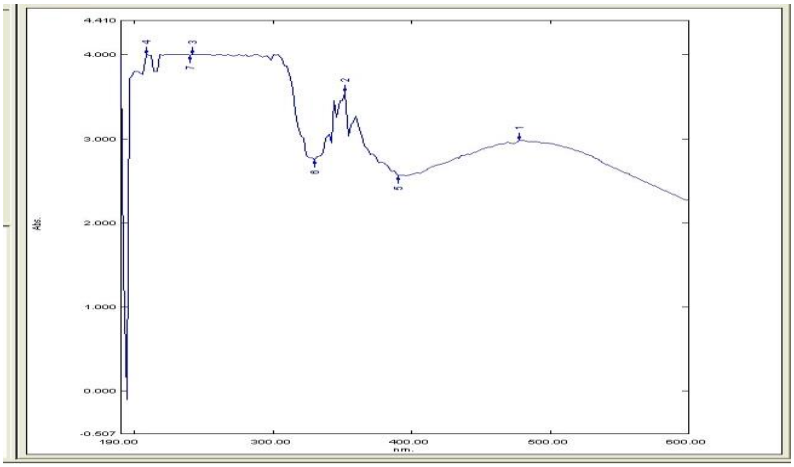


Fig.4. UV-Vis absorption spectrum of silver nanoparticles synthesized by treating 1mM aqueous AgNO3 solution with *Delonix regia* flower extract after 24 hrs.

3.2. SEM Analysis

SEM analysis was carried out to understand the size of the Ag-NPs, which showed the synthesis of higher density polydispersed spherical Ag-NPs of various sizes. The SEM image showing the high-density silver nanoparticles synthesized by the flower extract further confirmed the development of silver nanostructures. Most of the nanoparticles were scattered, as observed under SEM. Scanning electron microscopy analysis result showed that the synthesized silver nanoparticles exist in spherical shape with average size of 40-90nm. **(Figure 5)**

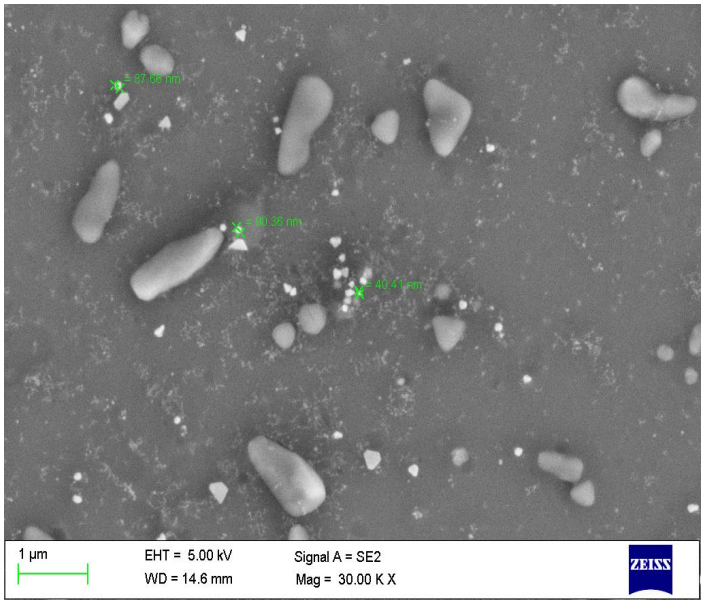


Fig 5: SEM image of silver nanoparticles synthesize by *Delonix regia*

3.3. Silver nanoparticles' toxicity analysis

3.3.1. Effect on seed germination

Toxicity analysis of the AgNPs prepared was carried out on Moong bean (*V. radiata*) seeds and their resultant shoot lengths were recorded. Seeds were considered to have germinated by observing the emergence of radicles. Maximum percentage germination of seeds was obtained after 24 h. Results obtained varied significantly with each treatment. Shoot length of seedlings treated with AgNP were significantly higher than distilled water-treated controls, flower extract treated, and silver nitrate treated seedlings. Shoot length of the seedlings was increasing as the concentration of AgNPs was increase. (Figure 6). The germination percentage (%) in gram seeds after 24h was calculated and the results were shown in table 1 and (Figure 7).

Delonix regia flower extract AgNPs aided in plant germination and growth by sequestering nutrients for them and could hence be implemented for agricultural purposes. This was also accompanied by an increase in the shoot length.

Fig 6: Germinating moong beans

Table 1. Germination Percentage (%) in gram seeds after 24h

Concentration	Control	AgNPs	Flower Extract	AgNO3
25%	80	70	69	69
50%	82	75	72	69
75%	79	80	75	72
100%	80	84	79	77

Fig 7: Graph Showing Germination Percentage (%) in Gram seeds after 24h

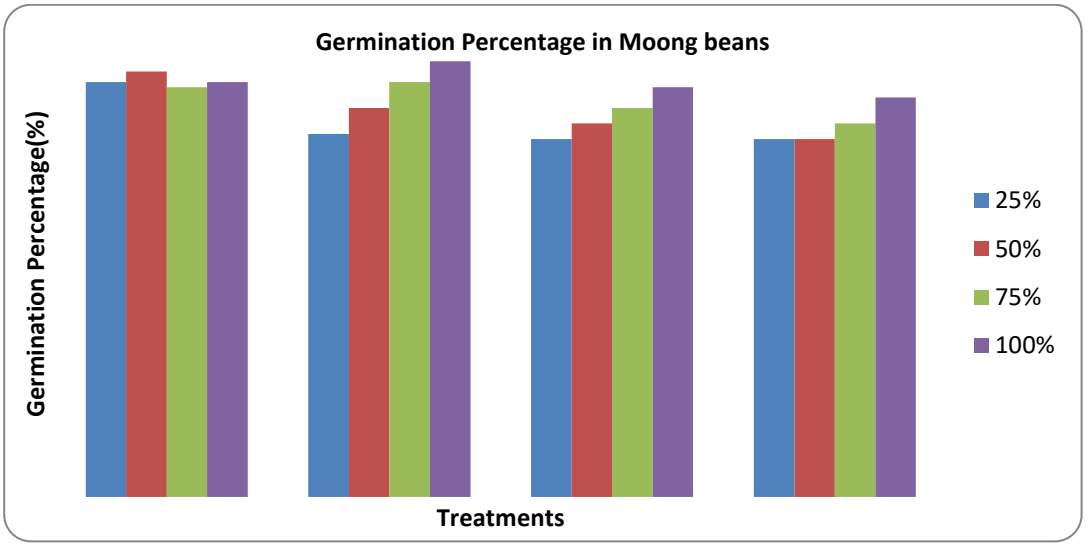
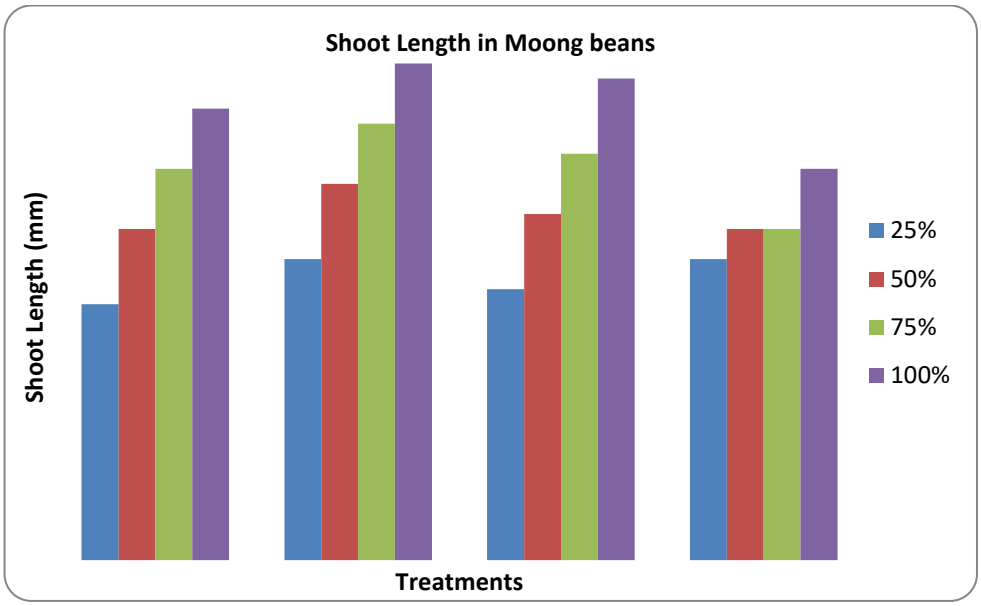


Table 2. Shoot Length (mm) in gram seeds after 24h

Concentration	Control	AgNPs	Flower Extract	AgNO3
25%	17	20	18	20
50%	22	25	23	22
75%	26	29	27	22
100%	30	33	32	26

Fig 8: Graph showing shoots length (mm) in gram seeds after 24h



3.3.2. Catalase activity

Catalase are considered as first-defence system against oxidative stress whose activity may be altered by toxic stress. Catalase activities significantly increased with respect to an increase in size and concentration of AgNPs(Table 4 and **Figure 10**).

Fig 9: Standard curve values for the H_2O_2.

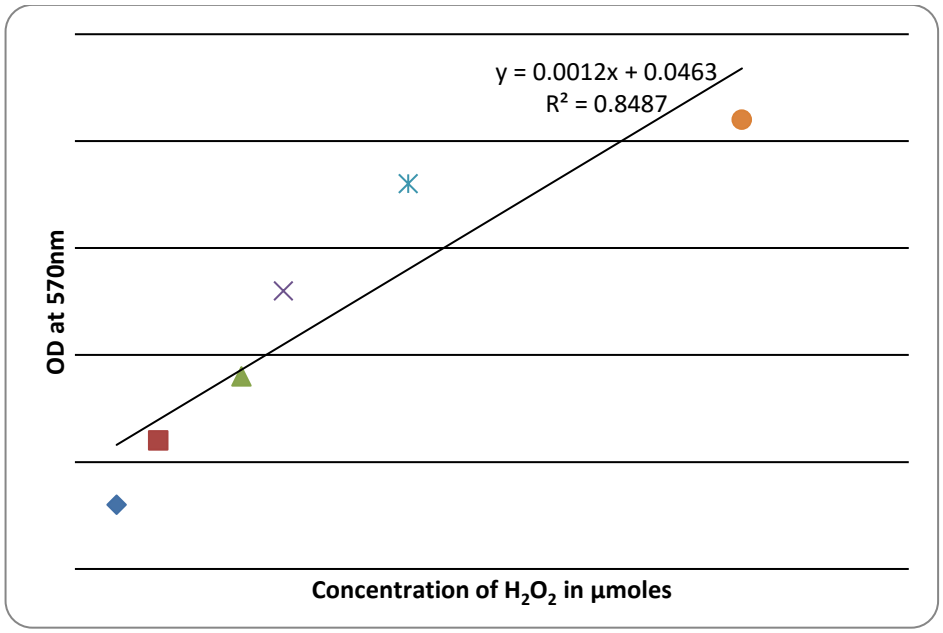
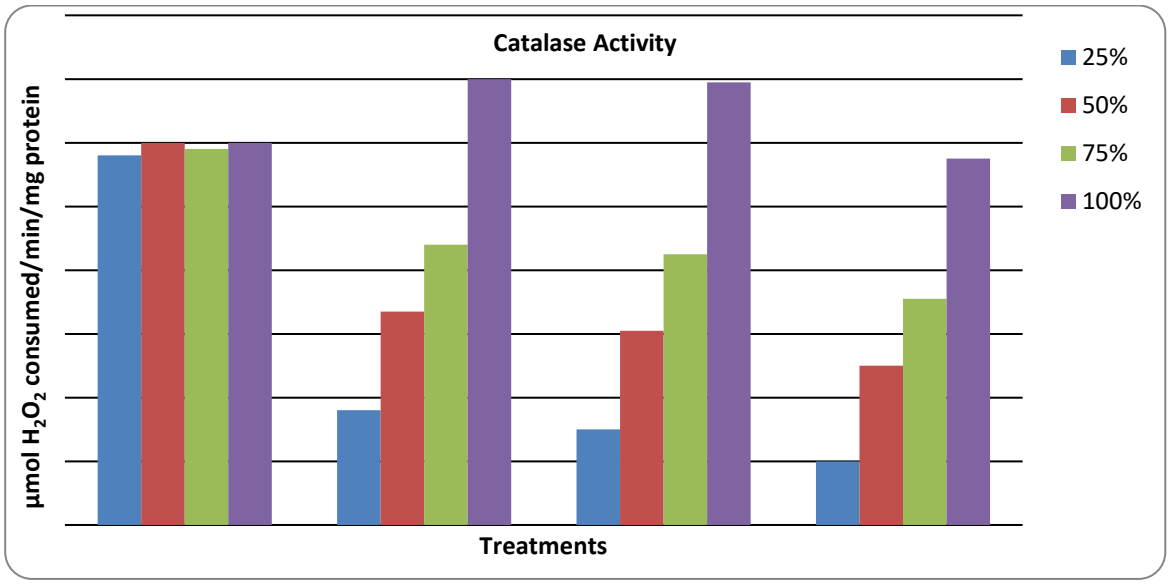


Table 4: Catalase activity of the samples.

Concentration	Control IU/mg	AgNPs IU/mg	Flower Extract IU/mg	$AgNO_3$ IU/mg
25%	0.116	0.036	0.03	0.02
50%	0.120	0.067	0.061	0.05
75%	0.118	0.088	0.085	0.071
100%	0.120	0.14	0.139	0.115

Fig 10: Graph showing Catalase Activity of the samples



3.4. Antioxidant activity

3.4.1. Total antioxidant activity:

Total antioxidant assay activity of *Delonix regia* flower extract at different concentrations was determined and shown in Table 5. Total antioxidant capacity of *Delonix regia* is expressed as the number of equivalents of ascorbic acid. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of AgNPs. The observed scavenging effect of flower extract, AgNPs and standard on the total antioxidant activity decreases in the following order: AgNPs >L ascorbic acid > plant extract. Among this AgNPs possess highest antioxidant potential as compared with ascorbic acid (Figure 11 & 12).

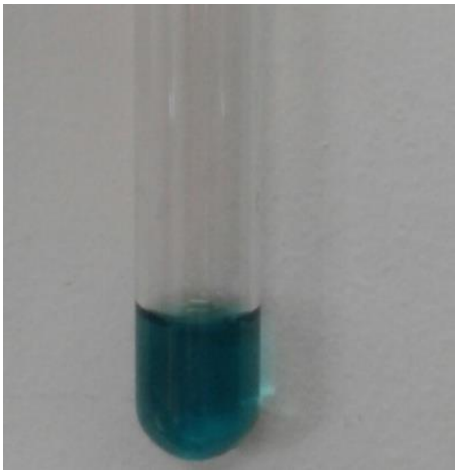


Fig 11: Antioxidant activity of *Delonix regia* extract

Table. 5. Total antioxidant assay activity of *Delonix regia* extract at different concentrations

Concentration (µg/ml)	% of antioxidant activity		
	AgNPs	Ascorbic Acid	Flower Extract
40	20	15	10
80	30	25	20
120	45	37.5	27.5
160	62.5	52.5	42.5
200	72.5	65	52.5

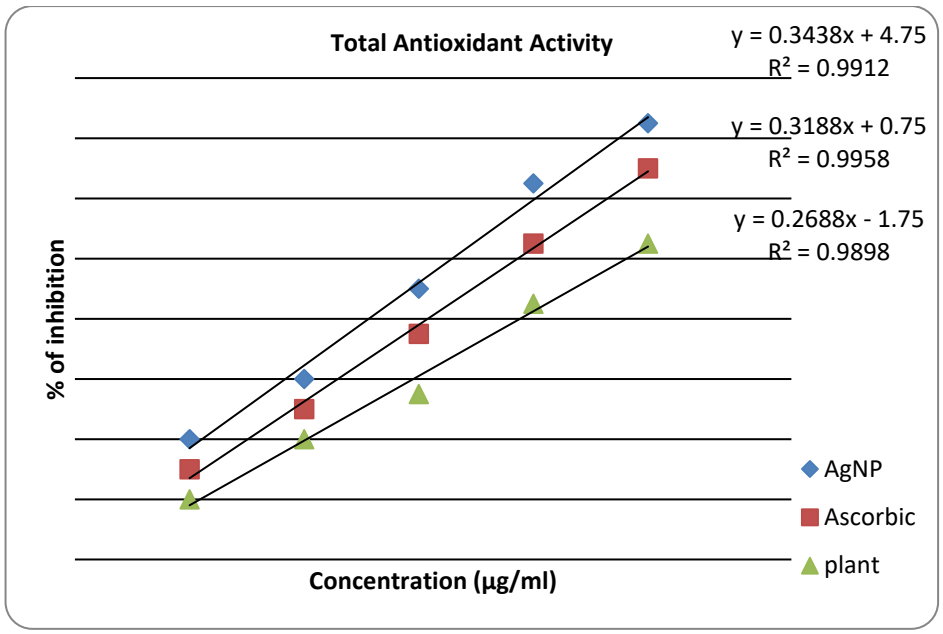


Fig. 12: Total antioxidant assay activity of *Delonix regia* extract at different concentrations

3.4.2. DPPH radicals scavenging activity

Free radical scavenging activity of *Delonix regia* flower extract at different concentrations is given in Table 6. The antioxidant activity was found to be highest for the 100µg/ml of flower extract that was used in the DPPH assay. Free radical scavenging activity of the AgNPs on DPPH radicals was found to increase with increase in the concentration. Among this AgNPs possess greatest scavenging activity as compared with ascorbic acid (Figure 13 &14).

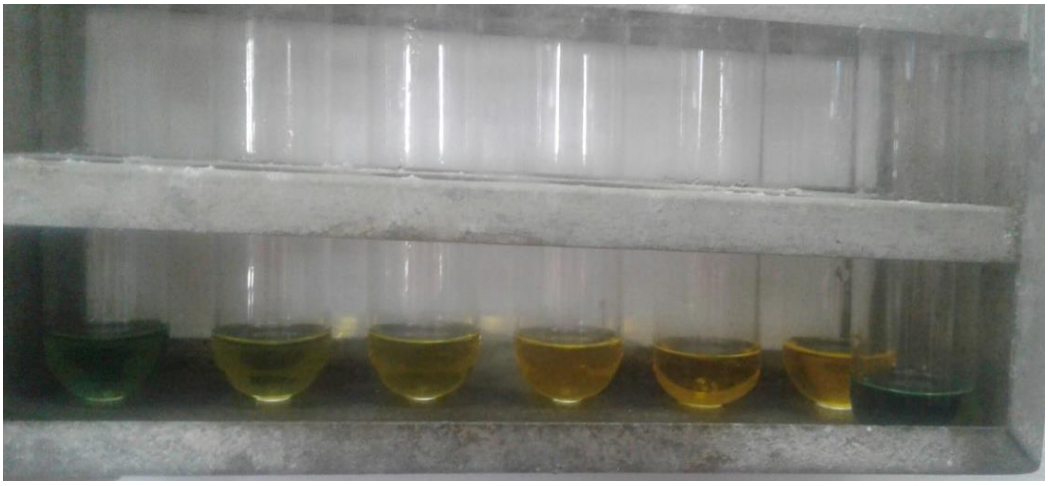


Fig 13: DPPH Assay

Table 6. DPPH Radical Scavenging Activity of *Delonix regia* extract at different concentrations

Concentration (µg/ml)	% of Inhibition		
	AgNPs	Ascorbic acid	Flower extract
20	12.5	10	5
40	22.5	17.5	10
60	32.5	27.5	25
80	47.5	42.5	35
100	62.5	55	50

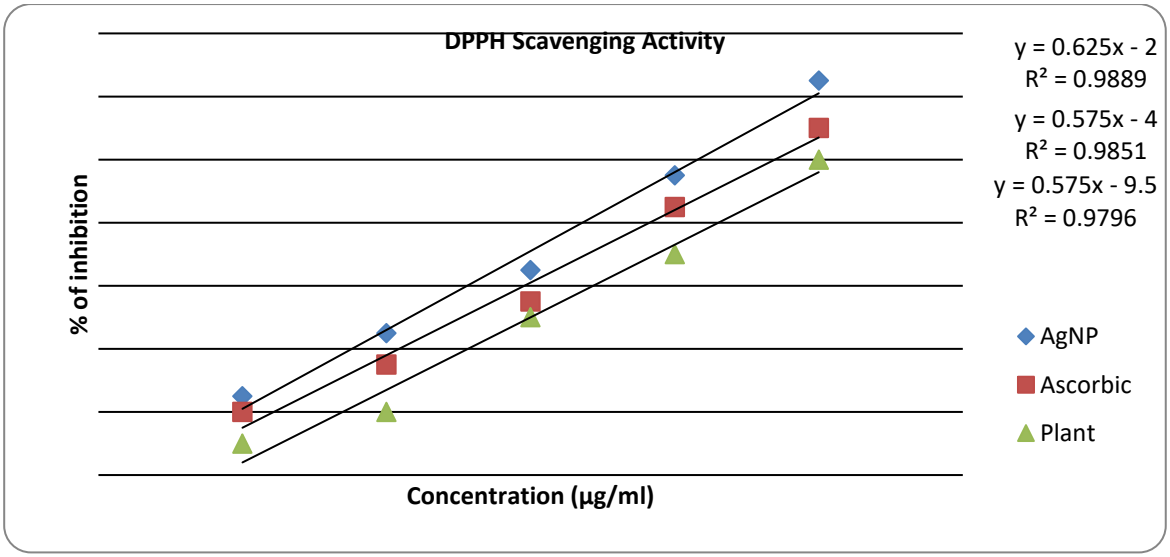


Fig 14: DPPH Radical Scavenging Activity of Delonix regia extract at different concentrations

3.4.3. Iron Reducing power assay:

The iron reducing power activity of *Delonix regia* and AgNPs were increased markedly with the increase of concentrations. The observed scavenging effect of flower extract, AgNPs and standard on the iron reducing power activity decreases in the following order: AgNPs >L ascorbic acid > plant extract. The iron reducing power capability is high in AgNPs **(Figure 15).** These results suggested that AgNPs had superior iron reducing power effect. The iron reducing power assay of AgNPs, plant extract and ascorbic acid represented in Table 7. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

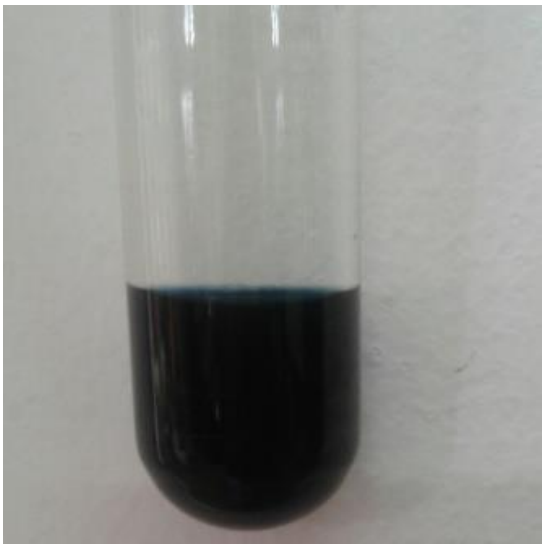


Fig 15: Reducing power assay of *Delonix regia* extract

Table 7. Reducing Power assay activity of *Delonix regia* extract at different concentrations

Concentration (µg/ml)	Optical Density		
	AgNPs	Ascorbic acid	Flower extract
20	1.5	1.3	1
40	1.9	1.7	1.4
60	2.4	2.2	1.9
80	3	2.8	2.4
100	3.5	3.3	2.9

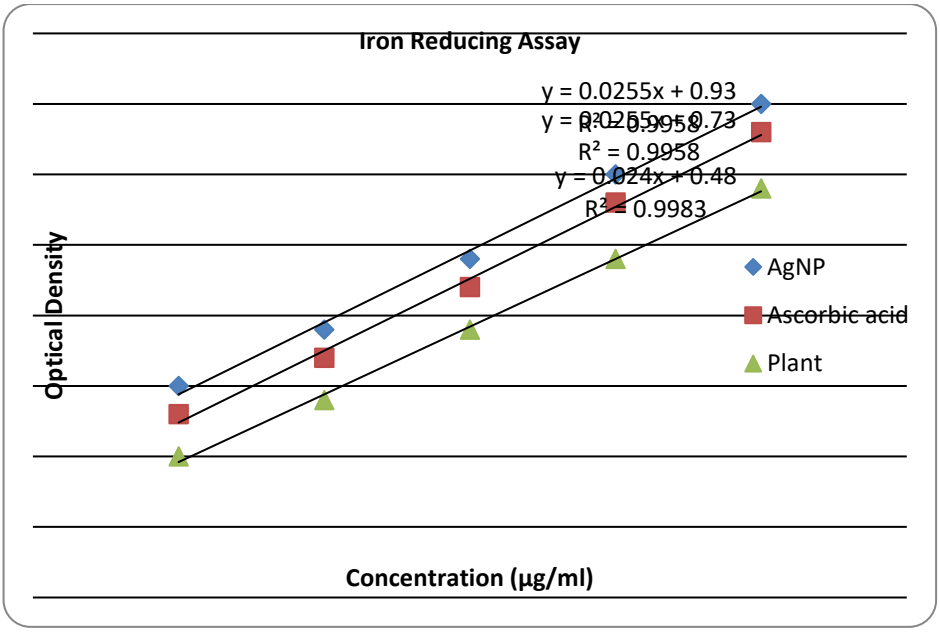


Fig. 16: Reducing power assay of *Delonix regia* extract at different concentrations

4. CONCLUSION

The present study was concluded the bio-reduction of silver ions through *Delonix regia* flower extract and testing for their toxicity and antioxidant activity. The bio-reduction of aqueous Ag+ ions by the flower extract of the *Delonix regia* has been demonstrated. The aqueous silver ions exposed to the extract, the synthesis of silver nanoparticles were confirmed by the change of colour of flower extract. Absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak at 350 nm also confirmed. These silver nanoparticles were further confirmed by using SEM. The SEM analysis showed the particle size between 40-90nm as well the spherical structure of the nanoparticles.

On the basis of the results of this study, it clearly indicates that *Delonix regia*, ascorbic acid and AgNPs possessing antioxidant activity against various pro oxidant systems. The free radical scavenging activity of AgNPs was found to be higher than that standard confirmed in the present investigation. From the above assays, the possible mechanism of antioxidant activity of AgNPs includes reductive ability, total antioxidant ability.

Besides, they also aided in plant germination and growth by sequestering nutrients for them and could hence be implemented for agricultural purposes. Catalase is considered as first-defence system against oxidative stress whose activity may be altered by toxic stress. Catalase activities significantly increased with respect to an increase in size and concentration of AgNPs.

These obtained silver nanoparticles are advantageous in medical and pharmaceutical purposes. It also has potential applications in the biomedical field and can be produced commercially at large scale.

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