Green synthesis of silver nanoparticles from methanolic extract of Plantago amplexicaulis and its biological evaluations

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Abstract

Nanotechnology is one of the modern techniques of material science which have received much importance in the last many years. Nanotechnology is concerned with the production of nanoparticles (NPs) with restricted sizes and shapes through a facile, straightforward, and medicinally active phytochemical route. In this work silver nanoparticles (AgNPs) were synthesized using the Dried fruits of *Plantago amplexicaulis* (*P. amplexicaulis*) plant. The synthesized AgNPs was confirmed by UV-visible spectroscopy which showed a characteristic Surface Plasmon resonance (SPR) band at 416 nm. Moreover, the AgNPs were further confirmed by FT-IR, SEM, and EDX analysis. FT-IR spectra contained the major groups which act as reducing and stabilizing agents for the formation of AgNPs. SEM results revealed that AgNPs has sphere-shaped, monodispersed with density high particles. The average size of 55 nm was calculated using four major peaks 33˚, 39.4˚, 55˚, and 65.7˚ of XRD analysis. These synthesized AgNPs were then tested for anti-microbial and antioxidant activity. Excellent antifungal, antibacterial, and antioxidant activities were shown by AgNPs as compared to plant extract. From the present project, it is concluded that AgNPs showed enhanced properties as compared to the plant crude extract.

1. Introduction

Nanoparticles (NPs) are 1 to 100 meters in size [1]. The NPs have too many applications like Antifungal [2], Antimicrobial [3], Drug delivery [4], etc. NPs synthesis is an international technology made up of a combination of new technologies. Being economic and environmentally friendly, this technology has gained attention [5].

Medical and pharmaceutical applications are derived from NPs such as gold, silver, and platinum. NPs. Silver is of more significance compared to other metals because of its vital role as an antimicrobial agent [6]. In small concentrations, silver has no diverse effect on human cells and can be considered an environmentally friendly antimicrobial agent. silver nanoparticles (AgNPs) have various important applications such in cosmetics, textiles, medical devices, antimicrobial agents, water purifiers, household water purifiers, etc [7]. Silver nanoparticles (AgNPs) among the various metal nets have been considered significant because they are effective antimicrobial agents that are less toxic; and Have huge in vitro and in vivo applications [8].

Conventionally AgNPs are synthesized by chemical methods incorporating the use of ethylene glycol [9] and sodium borohydride [10]. The chemicals used in these chemical methods are either toxic to the humans and environment, or the processes are too costly to be realizable on an industrial scale. To overcome the complications resulting from the chemical methodology there has been a search for a non-toxic, economical, reliable, and “green” methodology for the synthesis of nanoparticles. Amongst all other methods the use of the plant extract as a capping, reducing, and stabilizing agent in the synthesis of NPs has established distinctive attention, due to sterilizing and preservative environment during the process [11].

Plantaginaceae is a family of flowering plants in the order of Lamials. It was the only family of the order Plantagenalis in the older classification, but several phylogenetic studies summarized by the Angiosperm phylogeny group have shown that this toxin should be included within Lamials. *Plantago amplexicaulis* is a therophyte, a type of plant that survives as a seed during adverse conditions. *Plantago amplexicaulis* grows up to 1 m (2 ft 4 in) per year. The species are hermaphrodites (which contain both male and female organs) and pollinate using air. The plant itself is fertile. The flowers are small, unisexual, and usually spread out like new buds. They produce subtle fragrances in certain species. The essence is composed of 3 calyxes and 4 petals of the corolla, forming a tube and 4 membranous lobes. The stamens in the corolla tube consist of 4 stamens, which are many times longer than the petals. In these flowers, the stamens play a seductive role [12]. Keeping in view the importance of nanoparticles this research will be conducted to explore a novel approach for the green synthesis of AgNPs using plant extract and to evaluate them for biological activities.

2. Materials And Methods

2.1. Plant collection and extraction preparation

Plantago amplixicaulis plant was collected locally and identified by a taxonomist at the Department of Botany at the University of Science and Technology Bannu. The fruit of *P. amplixicaulis* plant was ground properly in a blender. The 50g ground was soaked in 150ml methanol for seven days. After seven days the mixture was filtered using Whatman filter paper. The filtrated solution was then placed in a large surface area container for evaporation of methanol. After 2 days the methanol was fully evaporated and a crude extract of *P. amplixicaulis* was left. The extract was then collected in eppendorf tubes and stored for further use.

2.2. Synthesis of silver nanoparticals

The AgNPs were synthesized by the green reduction method, for this, we take 1 ml of silver nitrate ($AgNO_3$) solution having 0.01M concentration in a 10 ml graduated cylinder and diluted it up to 10ml with deionized water (0.001M). Now pour that solution into a conical flask and adjust the pH 11 of the $AgNO_3$ solution by adding NaOH solution dropwise. Now at this pH 11 add 4ml centrifuged extract solution which changes the color of the solution to light brownish yellow. The solution color represents the AgNPs synthesis. Spectrum peak was taken by UV-spectrophotometer [13].

2.3. Characterization of AgNPs

AgNPs concentration in the aqueous solution was definite by using SHIMADZU UV SPECTROPHOTOMETER (UV-1800) in the range of 200 to 800 nm. The purified AgNPs and plants extract were examined for the presence of different phytochemicals by using Fourier Transform-Infrared (FT-IR) Shimadzu (IR Prestige-21) spectrometer (Japan). The crystalline nature of the AgNPs was determined by using the JDX-3532 (JEOL JAPAN) X-ray diffractometer (XRD) with λ-1.54Aº wavelength. The size and

shape of AgNPs were determined by using JEOL Scanning Electron Microscope (SEM) Model JSM-5910 (Japan).

2.4. DPPH free radicals scavenging assay

1.1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging potential of the plant crude and AgNPs was determined using the modified method [14]. The plant extract, AgNPs, and ascorbic acid were dissolving in deionized water (1mg/ml). After the preparation of plant extract, AgNPs and ascorbic acid was diluted to different concentrations of 25, 50, 75, and 100µg/ml. About 200µl samples from the different concentrations and 800µl DPPH were added separately in test tubes. All test tubes were incubated in dark for 30 minutes. The absorbance was recorded at 517nm using a double beam spectrophotometer and deionized water was used as a reference. Free radical scavenging activity was expressed as the percentage of inhibition that was calculated using the equation (i);

$$\%\text{Scavenging} = \text{Ac} - \text{As}/\text{Ac} \times 100 \quad \textbf{(i)}$$

Where Ac is the control absorbance and As is the sample absorbance

2.5. ABTS scavenging assay

The ABTS (2, 2 azobis, 3-ethyl benzothiazoline-6-sulphonic acid) radical scavenging activity will be determined by the modified method [15]. For this essay, we prepare 7mM of ABTS and 2.45mM potassium persulfate solution. Both these solutions were mixed and incubated for 24hrs in dark at room temperature, dark color solution representing ABTS radicals. Various concentrations from stock solutions of plant extracts, AgNPs, and ascorbic acid, i.e. 25µg/ml, 50µg/ml, 75µg/ml, and 100µg/ml, were prepared. About 0.2ml from all running solutions mixed with 0.8ml ABTS. The absorbance was recorded at 745nm using a double beam spectrophotometer. Ascorbic acid was used as a positive control. Free radical scavenging activity was expressed as the percentage of inhibition that was calculated using the equation (i);

2.6. Hydrogen peroxide H_2O_2 Scavenging Activity:

For Hydrogen peroxide activity [16] methods were used. Ascorbic acid, plant extract, and AgNPs solutions were taken as the same with concentrations of 25µg/ml, 50µg/ml, 75µg/ml, and 100µg/ml. First of all 2 mM solution of H_2O_2 was prepared and added to 50 ml in phosphate buffer having pH maintained at 7.4. After this, 0.2 ml running sample solution from each was taken and added to 0.6 H_2O_2 and 0.4 ml phosphate buffer. Properly the tubes were shacked and absorbance was measured at 230 nm after 15 minutes. Free radical scavenging activity was expressed as the percentage of inhibition that was calculated using the equation (i);

2.7. Antibacterial Activity (agar diffusion method)

The bactericidal activity of AgNPs was determined by using the agar well diffusion method [17]. The four bacterial strains, two gram-negative *Klebsiella pneumonia* (ATCC 700603), *Escherichia coli* (ATCC BAA-

2471), and two gram-positive *Acetobactor meriye* (ATCC 35568), and *Staphylococcus aureus (*ATCC 29213) as selected these strains were cultured in nutrients broth incubated for 24hour at 37ºC. Nutrients agar of 7g was weighed and dissolved in 100ml deionize water, autoclaved for 15 minutes at 121ºC, and cooled to room temperature. To each Petri plate, 30ml media was transferred and allowed to solidify. From each bacterial suspension, a good amount was taken by swab and spread gently on Petri plates. Six wells were dug per plate with a sterile cork borer (3–6 mm), and all the wells were properly labeled. DMSO (20µl) was added to one of the wells and serves as a negative control. Antibiotic ciprofloxacin (final concentration of 1mg/ml in DMSO) was added to the central well to serve as a positive control, 20µl from different concentrations (20, 40, 80, and 100 µg/ml) of plant extract and AgNPs was added to the remaining holes. A clear zone of inhibition was measured after 24hours by the graduated ruler.

2.8. Antifungal activity

For the antifungal activity against *Aspergillus niger* and *Aspergillus flavus*, a slightly modified method [18] was followed. Put the sterilized media into each test tube with the help of a graduated cylinder and add 67µl extract and AgNPs to each test tube, from the different concentrations (20, 40, 80, and 100 µg/ml), and kept for drying. Deionized water is used as a negative control and terbinafine is used as a positive control. All tubes were placed at room temperature in a slant position. The fungus is uniformly added to each test tube and sealed by cotton plugs. After all processes, all the tubes were placed at 30ºC in incubators with open water tubes. After 7 days the inhibition was observed by the graduated ruler.

3. Results

3.1. Synthesis of AgNPs

In the present study; the synthesis of AgNPs was studied under different factors. According to the pH study, AgNPs were studied at different pH (pH 8 to 12). No band was observed at lower pH 8, 9, and 10 but with an increase in pH the band become sharp and sharp; finally, a sharp band of 416nm with a maximum absorbance of 1.81 appeared at higher pH 11. (Fig. 1).

The AgNPs were fabricated by the addition of plant extract to the $AgNO_3$ solution (known concentration). Figure 2 shows that on the addition of plant extract concentrations of 1ml, 2ml, 3ml, 4ml, 5ml, and 6ml extract the subsequent solutions start to change the color to dark yellow indicating the synthesis AgNPs. The absorbance was noted by using a UV Vis spectrophotometer. The absorbance increase as the concentration increases and maximum absorbance with a sharp peak of 416nm was noted at concentrations of 4ml.

3.2. FT-IR of plant extract and AgNPs

FT-IR analysis is used for the identification of different functional groups of phytochemicals that cause the stabilization, capping, and reduction of AgNPs. In the present research work plant extract and AgNPs were analyzed by FT-IR and different peaks were reported. These peaks correspond to the different functional groups which are conformed by comparing with standard FT-IR charts. In the present study,

plant extract FTIR analysis gives various peaks i.e. 503, 625, 1320, 1618, 2615, 3250, and 4008 (Fig. 3a), where AgNPs FTIR analysis exhibited the peaks at 3319.75, 2923.64, 2853.12, 1739.94, 1645.86, 1457.16, 1378.78, 1146.54, and 1047.40. (Fig. 3b)

3.3. XRD

Figure 5 indicated 4 Bragg reflections at angles of 33°, 39.4°, 55°, and 65.7° which are corresponded to the planes (1 1 1), (2 0 0), (2 2 0) and (3 1 1) respectively. These reflections can be indexed conferring to the face of face-centered cubic crystal structure of silver ion. That confirmed the crystalline nature of AgNPs of plant extract *P. amplixicaulis* (Fig. 4). The average crystalline size of plant extract induced AgNPs is calculated by using the Debye-Sherrer's formula (iii);

$$D = k\lambda/\beta\cos\theta \qquad \text{(ii)}$$

D is the average crystalline size, k is a geometric factor (0.9), λ is the wavelength of the X-ray radiation source and β is the angular FWHM (full-width at half maximum) of the XRD peak at the diffraction angle θ. For the four major peaks. The average calculated crystalline size was found to be 55 nm.

3.4. SEM Analysis

In a recent study; AgNPs were screened by SEM to determine their surface morphology. The SEM results show spherical monodispersed particles as shown in Fig. 5.

3.5. Biological activities of AgNPs and plant extract

3.5.1. Antioxidant activity

In the present study; plant extract, AgNPs, and ascorbic acid were tested for three antioxidant activities H_2O_2 (Fig. 6a), DPPH activity (Fig. 6b), and ABTS activity (Fig. 6c). In all cases, the higher antioxidant scavenging properties were reported for AgNPs as compared to plant extract. The obtained results indicated that AgNPs and plant extract showed 79.6 and 74.3% ferric reducing potential, 86.11 and 75.5% DPPH scavenging, and 83.3 and 73.2% ABTS scavenging at the highest concentration of 100µg, respectively.

3.5.2. Antibacterial assay

The Table1 showed the antibacterial activity of *P. amplixicaulis* fruit and its AgNPs against both gram + ve and gram –ve bacterial strains. The AgNPs and plant extract showed antibacterial activities at various doses (20, 40, 80, and 100µg/ml). At the highest concentration of 100µg/ml AgNPs and Plant, extract exhibited 21.25 ± 0.35 and 19.55 ± 0.54, against *K. pneumonia*, 22.35 ± 0.53 and 19.9 ± 0.65 against *E. coli*, 19.75 ± 0.62 and 18.3 ± 0.7 against *A. meriye, and* 23.3 ± 0.61 and 20.9 ± 0.31 against *S. aureus*, respectively. DMSO served as negative control and ciprofloxacin (1g/ml) as a positive control.

3.5.3. Antifungal activity

From the current study, it has been found that AgNPs *significantly* inhibited the growth of tested fungal strains and proved a good valuable antifungal source as compared to plant extract. The AgNPs and plant extract at the highest concentration of 100µg/ml exhibited growth inhibition 34.95 ± 0.72 28.6 ± 0.68mm against *A. niger*, while 36.9 ± 0.44mm and33.65 ± 0.15mm inhibition against *A. flavus respectively* (Tale 2). DMSO served as a negative control. No fungal growth was observed in positive control test tubes (terbinafine).

4. Discussion

The UV–vis spectroscopy technique can be used to determine the shape and size of NPs in an aqueous solution [19]. AgNPs have the properties of oscillation of surface Plasmon Resonance (SPR) at the surface of NPs [20]. The formation of AgNPs showed the color change from milky to yellowish-brown color; this property of the AgNPs is due to their SPR. An ideal pH is also required for the synthesis of controlling the shape and size of NPs [21, 22]. In the present study, with the increases in pH, the synthesis of AgNPs increases and reach a maximum of pH 9. Beyond this pH, aggregation takes place. It is concluded from the pH study that AgNPs synthesis is supported by basic conditions while suppressed by acid conditions [13, 23].

Medicinal plants are a rich source of secondary metabolites that act as reducing, capping, and stabilizing agents for NPs. However, the composition of these active secondary metabolites varies from plant to plant depending on the nature, part, type of plant, and method followed for the extraction of these metabolites [24]. In the present study, the AgNPs were fabricated by the addition of plant extract to AgNO3 solution (known concentration) to find out the optimum extract concentration at which NPs get stable with a high SPR band. The AgNPs synthesis shows that on the addition of plant extract concentrations of 4mL extract the subsequent solutions start to change the color to dark yellow indicating the synthesis AgNPs as compared to the other extract concentrations. The absorbance was noted by using a UV Vis spectrophotometer. The absorbance increase as the concentration increases and maximum absorbance was noted at concentrations of 4ml showing the optimum extract concentration. On further increase in extract Concentration, The SPR band tends to decrease, an indication that in the presence of the huge amount of phenolic groups in plant extract, The AgNPs get precipitated, and hence decrease in the peak intensity occurs. One more reason behind this decrease is that all Ag + 1 ions present get reduced and there is a decrease in SPR intensity resulting in the end decrease in the absorption spectrum. To understand how much time is required for the synthesis of AgNPs the pH 11 and concentration 4ml are used for the time study. For this purpose, NaOH was added to 0.001M (0.1mM) solution of AgNO3 to adjust its pH 11 followed by the addition of 4ml plant extract solution to it. Shows that with time, the interaction between different groups of plant extract with Ag becomes enhanced resulting in the increase in SPR band at 416nm after 24 hr. All the results of the present study are in good covenant with the results reported in the literature [25]

The FTIR spectroscopic analysis confirmed the participation of biochemistry, which resulted in reducing the almonds with almonds and hydraulics and ammunition groups, reducing the groups and reducing

committees and stabilizing. [26] said that the reducing agents used to produce NPs were from static points made by Ficus microcarpa leaves. Interestingly, in the plant-mediated synthesis of NPs, the Phyto-components also play important role in the stabilization of NPs which is very crucial for rendering its applicative properties. These results also coincide with reports of earlier findings [27, 28].

For the AgNPs size and crystalline structure study used the XRD method. The XRD analysis is much important to confirm the size and crystallinity of the AgNPs which is synthesized. In the present study, XRD analysis of AgNPs shows four distinct peaks which confirm the crystalline nature of AgNPs. Similar results were also reported by [13, 29]. The four characteristic peaks are typical for the face-centered cubic structure of Ag (ICDD PDF card number 00-004-0783) [30].

The plant extract-induced AgNPs topology and size were studied in SEM analysis, which demonstrated that the AgNPs are sphere-shaped, monodisperse, and are present in huge density. These results were in agreement with previous results [31].

The free radicals are electron-deficient molecules that are responsible for producing various diseases such as Neurological disorders, Parkinson's disease, mild cognitive impairment and aging, etc. [32]. The AgNPs show the highest H2O2, DPPH, and ABTS scavenging as compared to plants while lower scavenging in comparison to standard ascorbic acid. The results are strongly supported by [33] who reported that AgNPs show strong cellular interaction with reactive oxygen species (ROS) and exhibit scavenging that is closer to the standard drug ascorbic acid. The higher % scavenging potential of AgNPs as compared to plant extract suggested the antioxidant nature of AgNPs. The results are best supported by [34]. Our result is also supported by [35].

Like different pathogenic bacteria and fungi cause systemic illness of the mouth, skin, lungs, hypersensitive reaction, blood, liver tinea cruris, and athlete's foot [36–38]. For the therapeutic management of invasive systemic fungal infections only about 10 antimicrobial drugs are permitted in the United States of America by the Food and Drug Administration (FDA) authority [36, 39]. In this study, the significant antimicrobial effects of AgNPs were reported as compared to plant extract. The appreciable antibacterial potential of AgNPs as compared to plant extract depends on the morphology, surface area, particle size, shape, and surface polarity [40]. The Ag ions mechanism of inhibition action on micro-organisms displays that the microbe treatment by metal ions prompts loss of DNA, its capability replication, and translation, as well as other cellular enzymes and proteins that are necessary for the production of ATP (coenzyme, Adenosine Tri-Phosphate,) resulting in living cell inactivation of [41]. It has also been assumed that metal NPs mainly affect the function of membrane-bound enzymes, in the chain of respiration [42].

5. Conclusion

The “green” route in the biological method for AgNPs fabrications is of great attention due to its eco-friendliness, economic prospects, and feasibility with a simple method using *P. amplexicaulis* extract at room temperature. The seed extract of *P. amplexicaulis* has been used as a bioreduction agent for

aqueous Ag + ions. These *P. amplexicaulis* AgNPs have shown good and reliable results s antioxidant and antimicrobial. However, future studies on the antimicrobial and antioxidants effect of these NPs are essential to completely assess their potential use as a novel biocidal material.

Declarations

Conflict of Interest

The authors do not declare any conflict of interest concerning this article.

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Tables

Table 1
Antibacterial activity of plant extract and synthesized AgNPs of *Plantago amplixiculis* against mentioned bacteria

Strains	Samples	20 µl/ml	40 µl/ml	80 µl/ml	100 µl/ml	Ciprofloxacin
K. pneumonia	AgNPs	12.8 ± 0.51	15.3 ± 0.7	17.45 ± 0.61	21.25 ± 0.35	41.3 ± 0.63
	P. Extract	8.35 ± 0.56	10.5 ± 0.62	16.5 ± 0.46	19.55 ± 0.54	45.2 ± 0.68
E. coli	AgNPs	14.7 ± 0.71	16.45 ± 0.35	17.45 ± 0.67	22.35 ± 0.53	38.7 ± 1.72
	P. Extract	8.75 ± 0.54	11.75 ± 0.83	16.65 ± 0.13	19.9 ± 0.65	39.5 ± 0.64
A. meriye	AgNPs	10.7 ± 0.64	13.55 ± 0.62	15.6 ± 0.67	19.75 ± 0.62	43.5 ± 1.75
	P. Extract	9.7 ± 0.71	12.5 ± 0.56	15.35 ± 0.54	18.3 ± 0.7	45.3 ± 0.35
S. aureus	AgNPs	11.55 ± 0.82	13.85 ± 0.73	17.3 ± 0.43	23.3 ± 0.61	36.7 ± 0.91
	P. Extract	10.3 ± 0.51	13.55 ± 0.71	16.45 ± 0.45	20.9 ± 0.31	42 ± 0.74

Table 2
Effect of plant extract and AgNPs against mentioned fungal strains

Strains	Sample	20µg/ml	40µg/ml	80µg/ml	100µg/ml	Terbinafine
Aspargillus niger	AgNPs	21.85 ± 0.7	23.4 ± 0.4	29.25 ± 0.51	34.95 ± 0.72	53.5 ± 0.52
	Plant extract	8.3 ± 0.45	14.6 ± 0.62	22.95 ± 0.45	28.6 ± 0.68	57.95 ± 0.45
Aspargillus flavus	AgNPs	25.75 ± 0.23	29.15 ± 0.69	33.35 ± 0.35	36.9 ± 0.44	48.85 ± 0.35
	Plant extract	22.25 ± 0.25	26.6 ± .61	28.85 ± 0.35	33.65 ± 0.15	53.35 ± 0.34

Figures

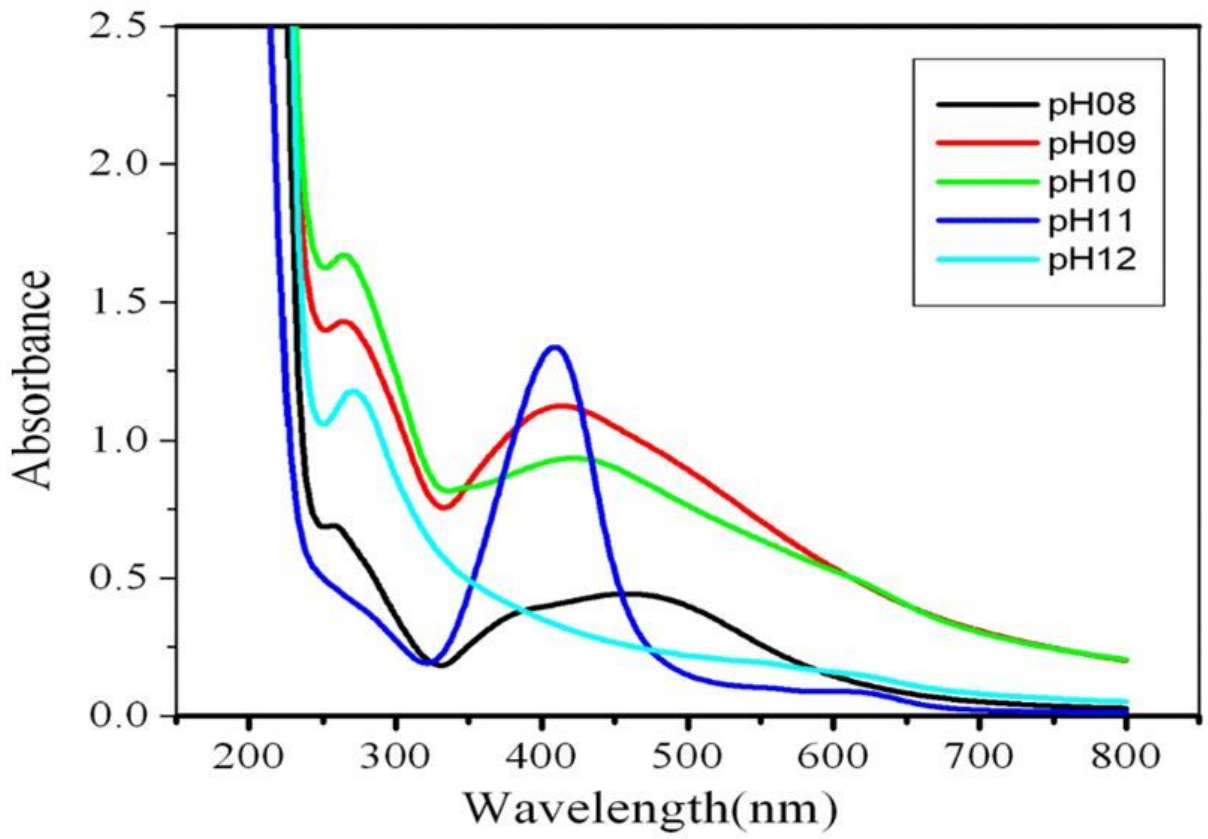

Figure 1

pH effect on the synthesis of AgNPs

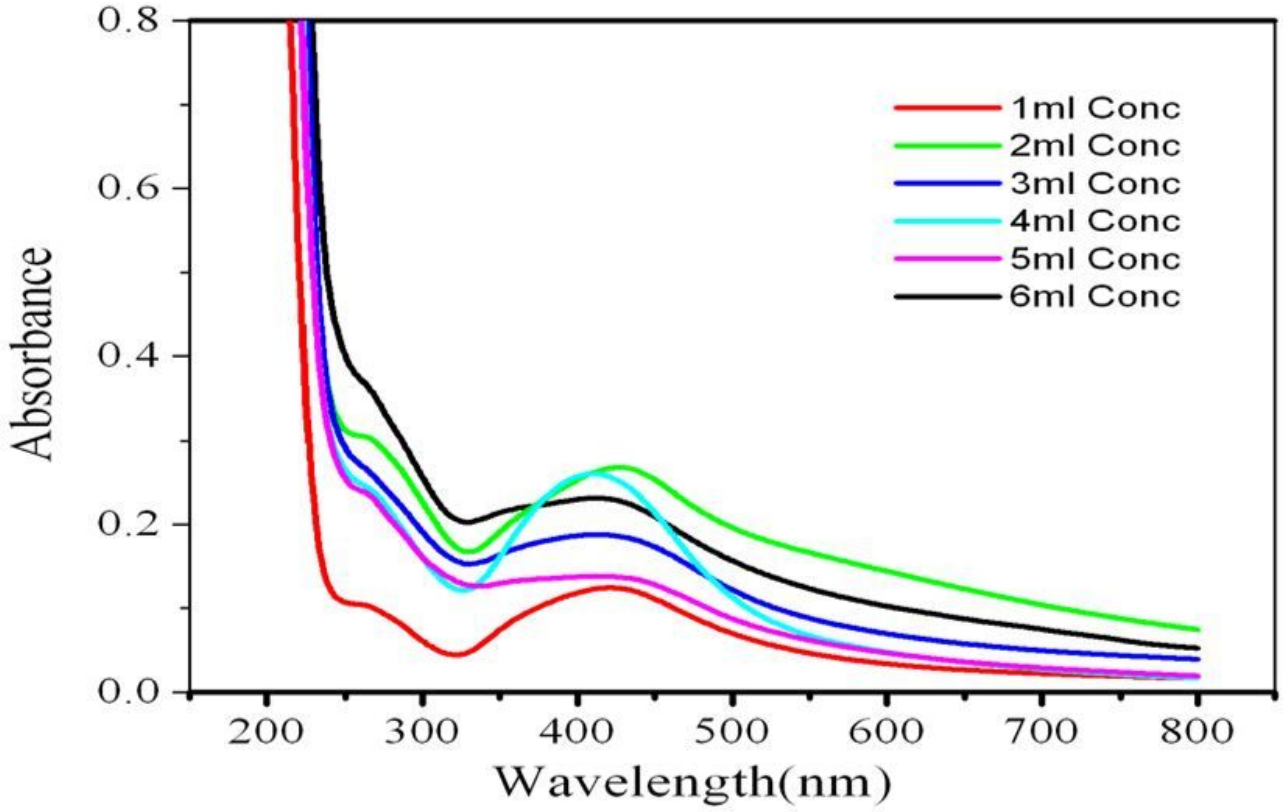

Figure 2

Concentration study of AgNPs

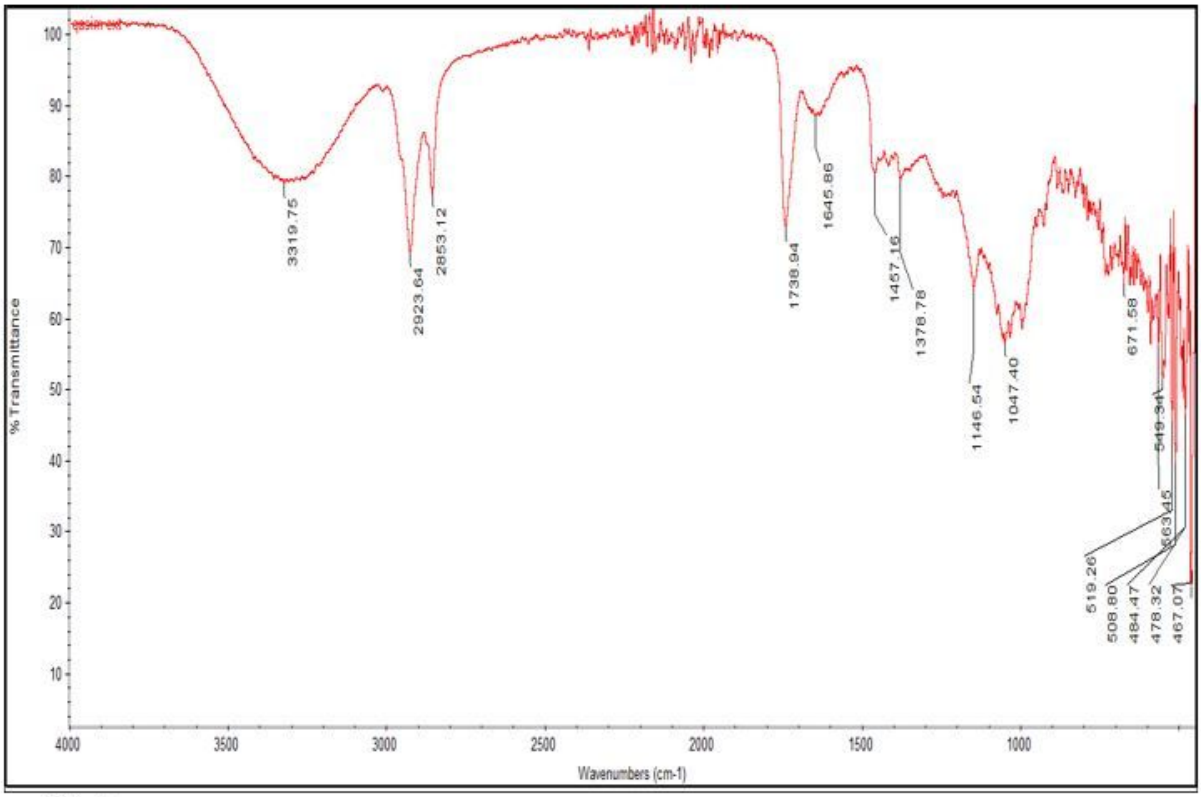

Fig 3a

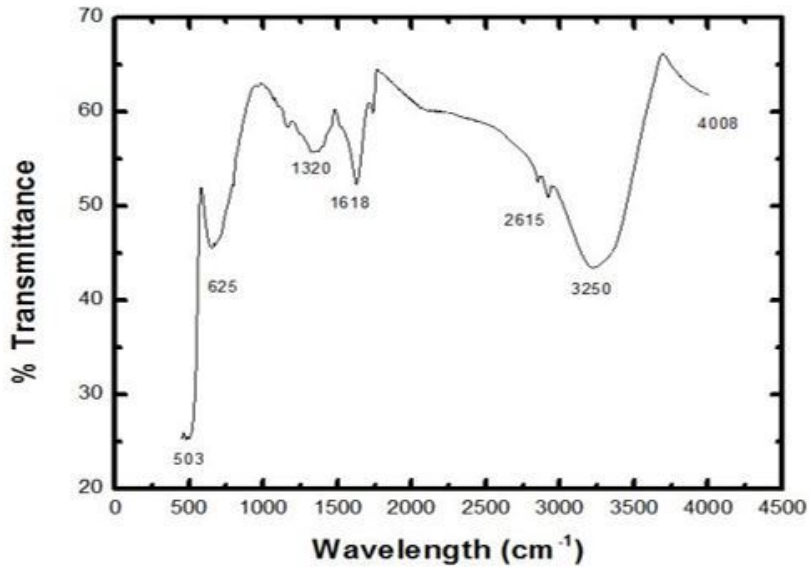

Fig 3b

Figure 3

XRD pattern of prepared AgNPs exhibiting the facts of crystalline NPs.

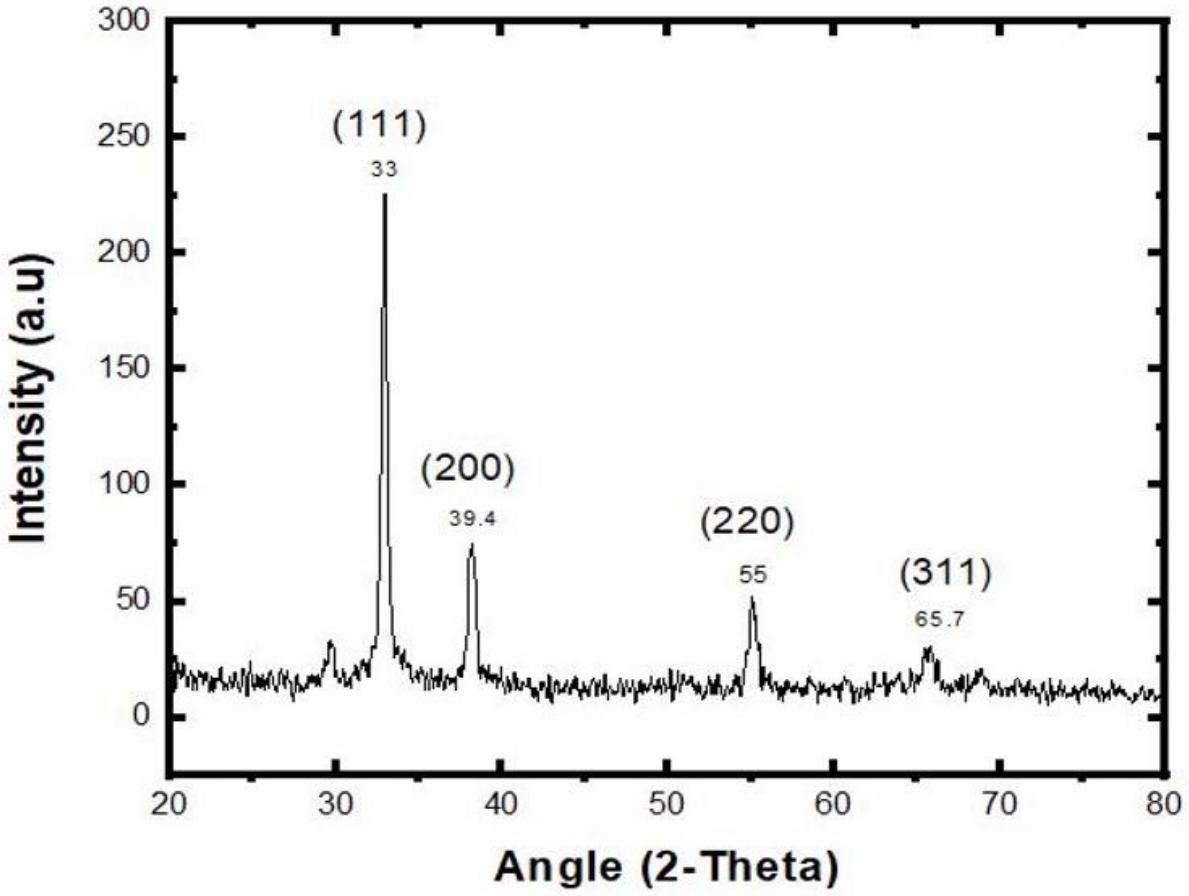

Figure 4

SEM image of the AgNPs of plant P*. amplexicaulis.*

Figure 5

5a and 5b: FT-IR spectrum of plant P*. amplexicaulis* (5a) and AgNPs (5b) AgNPs.

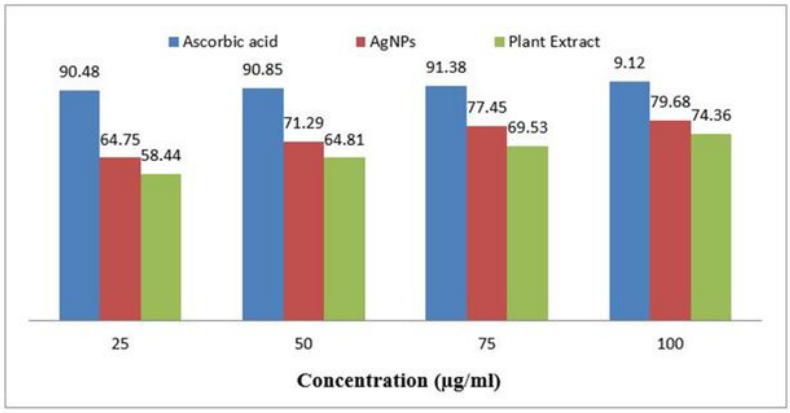

Fig 6a

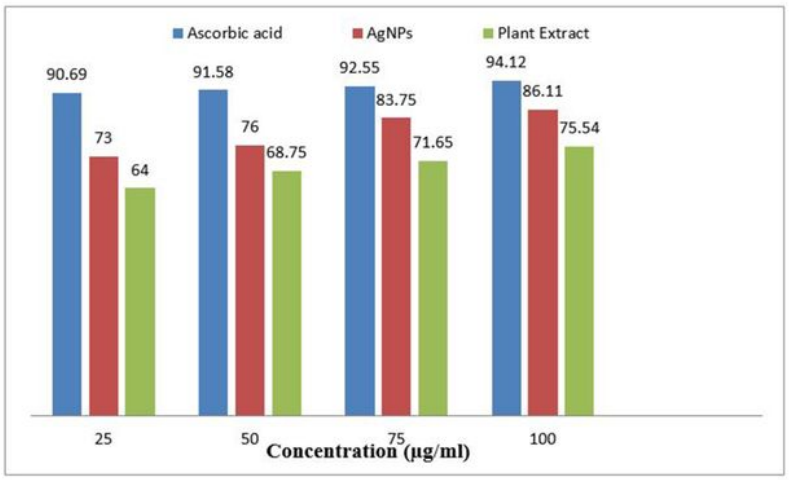

Fig. 6b

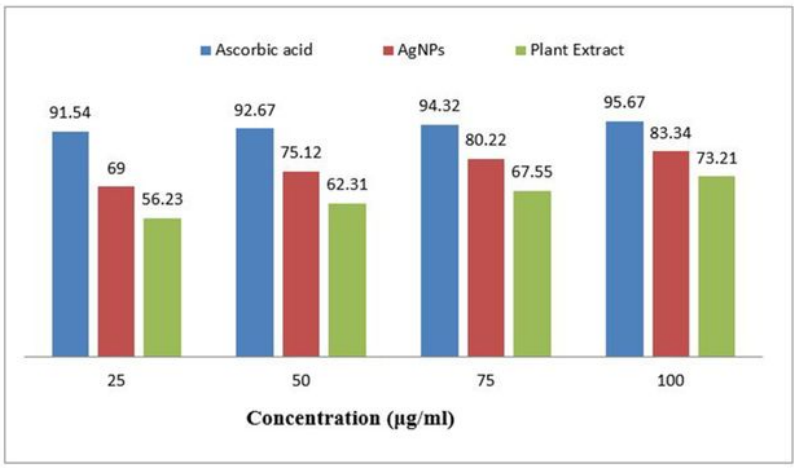

Fig. 6c

Figure 6

a: Comparison of the H_2O_2 scavenging activity among AgNPs and plant extract.

b: Comparison of DPPH free radicals scavenging potential AgNPs and plant extract.

c: Comparison of AgNPs, plant extracts, and ascorbic acid on ABTS free radicals.