

Research Article

Adiantum philippense L. Frond Assisted Rapid Green Synthesis of Gold and Silver Nanoparticles

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Development of an ecofriendly, reliable, and rapid process for synthesis of nanoparticles using biological system is an important bulge in nanotechnology. Antioxidant potential and medicinal value of *Adiantum philippense* L. fascinated us to utilize it for biosynthesis of gold and silver nanoparticles (AuNPs and AgNPs). The current paper reports utility of aqueous extract of *A. philippense* L. fronds for the green synthesis of AuNPs and AgNPs. Effect of various parameters on synthesis of nanoparticles was monitored by UV-Vis spectrometry. Optimum conditions for AuNPs synthesis were 1 : 1 proportion of original extract at pH 11 and 5 mM tetrachloroauric acid, whereas optimum conditions for AgNPs synthesis were 1 : 1 proportion of original extract at pH 12 and 9 mM silver nitrate. Characterization of nanoparticles was done by TEM, SAED, XRD, EDS, FTIR, and DLS analyses. The results revealed that AuNPs and AgNPs were anisotropic. Monocrystalline AuNPs and polycrystalline AgNPs measured 10 to 18 nm in size. EDS and XRD analyses confirmed the presence of elemental gold and silver. FTIR analysis revealed a possible binding of extract to AuNPs through $-NH_2$ group and to AgNPs through C=C group. These nanoparticles stabilized by a biological capping agent could further be utilized for biomedical applications.

1. Introduction

Nanotechnology is the study of materials that have at least one dimension in the range of 1–100 nm. Decrease in size is accompanied by elevated surface-area-to-volume ratio. Electronic and chemical properties of a material are dependent on its size. When the bulk material is reduced to nanoscale, its properties change. Such nanomaterials displaying novel properties have effective and wide utility in biological and biomedical applications. Noble metal nanoparticles such as Au, Ag, Pt, and Pd have been most effectively studied [1, 2].

Physical and chemical methods yield nanoparticles with well-defined shape and size, but these methods are expensive and potentially toxic to environment. This has created a need to develop clean, nontoxic, economical, and environmentally benign methods to synthesize nanoparticles. These concerns have led researchers to develop biological methods for synthesis of nanoparticles.

Castro et al. [3] have reported synthesis of AuNPs, by intracellular or extracellular reduction of tetrachloroauric acid using bacteria. Other biosynthetic routes include fungus, marine sponge, eggshell membrane, whole plant, leaf extract, flower, and tuber extract. Numerous methods available for biosynthesis of AgNPs include bacteria, mushroom, bark, peel extract, and enzyme as reducing agents [1, 4–8].

AuNPs are being considered in a wide range of diagnostic and therapeutic applications, such as cancer detection and cancer treatment, drug delivery, biological imaging, and biomedicine, as well as in electrochemistry. The prominent applications of silver nanomaterials include detection and diagnostics, antimicrobials and therapeutics, catalysis, and biolabeling [2].

Integration of phytochemicals in synthesis of nanomaterials is of extreme importance as it brings about integrity of plant science and nanotechnology, termed as green

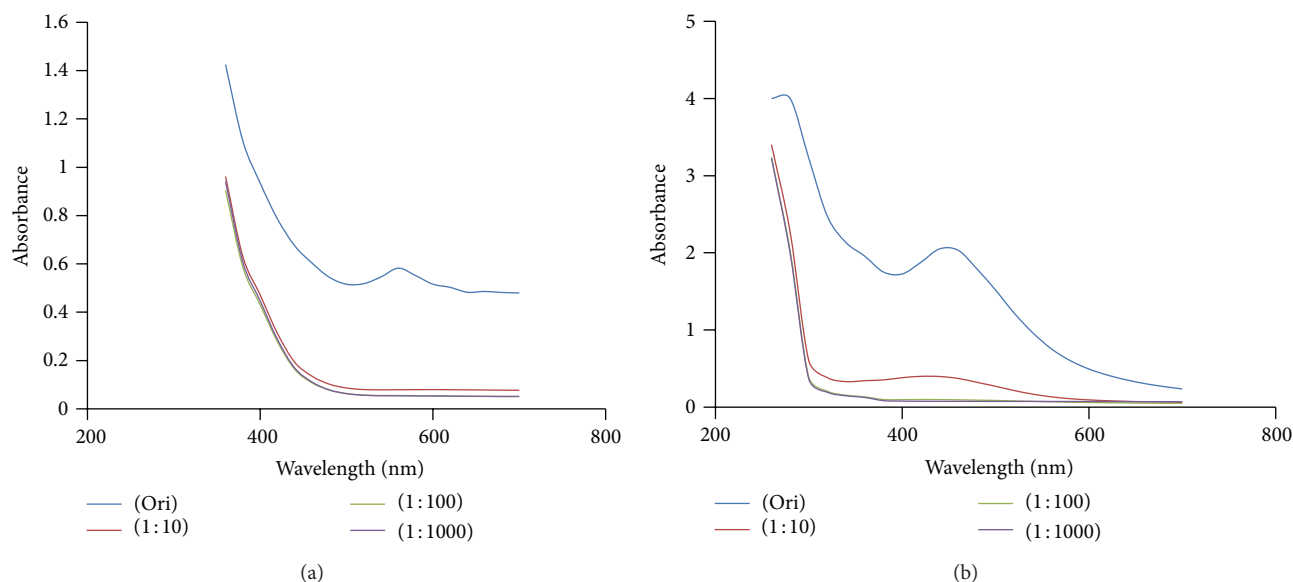


FIGURE 1: Surface plasmon peaks showing effect of varying extract concentrations (original extract, 1 : 10, 1 : 100, and 1 : 1000) on synthesis of AuNPs (a) and AgNPs (b).

technology. Biosynthesis of nanoparticles using dicotyledonous and monocotyledonous plants has been reported [1]. However, pteridophytes known to man for their use in folk medicine remain unexplored for this purpose.

The fern *Adiantum philippense* L. (family Adiantaceae) well known for its antioxidant potential [9] has been ethnomedicinally used in treatment of paralysis, blood diseases, epileptic fits, rabies, dysentery, elephantiasis, pimples, and wounds [10, 11]. It is also known for its antimicrobial activity [11]. Phytochemical analysis of the plant extracts belonging to genus *Adiantum* shows the presence of carbohydrates, phenols, flavonoids, and terpenoids [11–13]. Therefore, presence of these compounds may have a role in making *Adiantum* a potential candidate for bioreduction of gold and silver salts. In the present study, we have used *A. philippense* L. extract for the synthesis of AuNPs and AgNPs.

2. Material and Methods

2.1. Preparation of the Aqueous Plant Extract. Fresh fronds of *A. philippense* L. were obtained from Western Ghats (Sinhagad fort, Pune, India) region and identified from Botanical Survey of India (Western Circle), Pune, India. Fronds with mature sori were washed thoroughly and allowed to dry. Five grams of fronds were chopped into fine pieces and grounded into paste. This paste was extracted in 100 mL double distilled water. The extract was passed through muslin cloth and filtrate was centrifuged at 2500 g for 10 min at room temperature. Supernatant thus obtained was filtered through Whatman Filter Paper No. 1 and used for synthesis of nanoparticles.

2.2. Synthesis of AuNPs and AgNPs. Tetrachloroauric acid salt ($\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$, 49.9%) (SRL Chemicals, India) and silver

nitrate salt (AgNO_3 , 99.9%) (Qualigen Fine Chemicals, India) were procured and used without any further purification. Aqueous stock solutions of tetrachloroauric acid (10 mM) and silver nitrate (100 mM) were prepared in a stoppered volumetric flask and stored in amber color bottles.

Freshly prepared extract of *A. philippense* L. was mixed with aqueous solution of 10 mM tetrachloroauric acid in 1 : 1 proportion. The mixture was allowed to react at 30°C, with vigorous shaking. Synthesis of AuNPs was checked visually by determining the color change from yellow brown to dark blue. Similar procedure was followed for synthesis of AgNPs, indicated by a brick red color formation. Confirmation was done on the basis of a characteristic peak obtained in the absorbance range of 500–600 nm for AuNPs and 400–500 nm for AgNPs.

2.3. Effect of Different Parameters on Synthesis of AuNPs and AgNPs. Effect of different parameters such as concentration of extract (original, 1 : 10, 1 : 100, and 1 : 1000 in double distilled water), concentration of salt (1–10 mM), and pH (2–13) of original extract on synthesis of AuNPs and AgNPs was checked. Criterion for selecting optimum condition was observing for a narrow peak at characteristic wavelength giving maximum absorbance.

2.4. Characterization of AuNPs and AgNPs. Absorption spectrum of AuNPs and AgNPs was recorded on (JASCO V-630) UV-Vis spectrophotometric multiplate reader. Film X-ray diffraction (XRD) was performed using an X-ray diffractometer (Phillips PW1710, Holland) with $\text{CuK}\alpha$ radiation $\lambda = 1.5406 \text{ \AA}$ over a wide range of Bragg angles ($20^\circ \leq 2\theta \leq 80^\circ$). Morphology and size of AuNPs were investigated by transmission electron microscopy (Tecnai G² 20U-TWIN (FEI, the Netherlands), TEM with LaB_6 electron source)

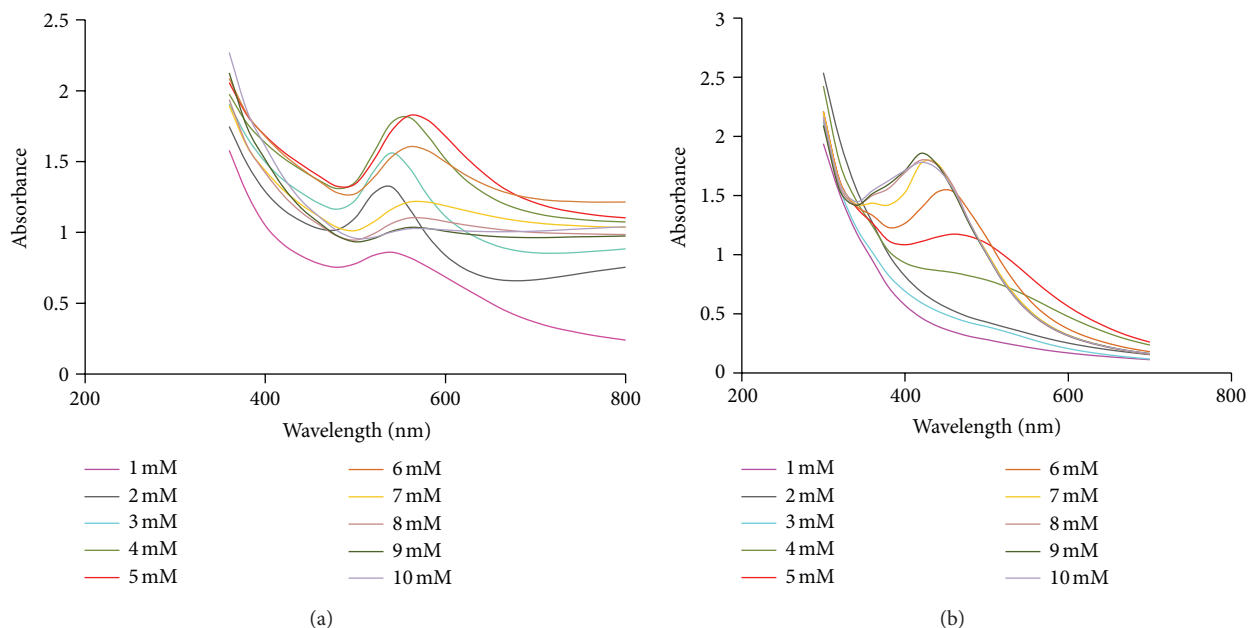


FIGURE 2: (a) Surface Plasmon Resonance of varying tetrachloroauric acid concentrations (1–10 mM) on synthesis of AuNPs. (b) Surface Plasmon Resonance of varying silver nitrate concentrations (1–10 mM) on synthesis of AgNPs.

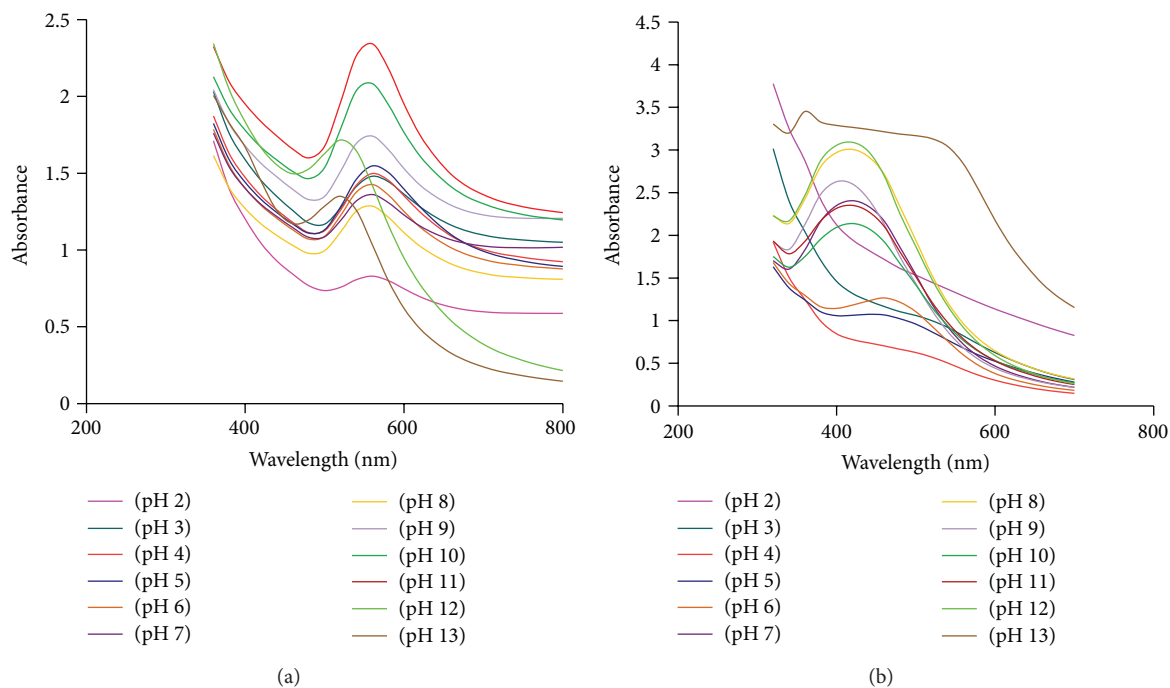


FIGURE 3: (a) Surface plasmon peaks of AuNPs with varying pH values (2–13). (b) Surface plasmon peaks of AgNPs with varying pH values (2–13).

with an accelerating voltage of 200 kV. An elemental analysis of the sample was examined by energy dispersive analyses of X-rays (EDAX) with 6360 (LA) instrument at an Acc voltage of 20 kV. Fourier transform infrared spectroscopic (FTIR) measurements were done using Perkin Elmer (16 PC-FT-IR) spectrophotometer. Particle size distribution was measured

by a dynamic light scattering apparatus utilizing 663 nm red laser at 90° angles.

The samples for transmission electron microscopy (TEM) and energy dispersive scattering (EDS) and X-ray diffraction (XRD) were prepared by placing a drop of AuNPs and AgNPs onto a 1 cm * 1 cm glass slide. Samples were then heat

fixed and subjected for analysis. The AuNPs, AgNPs, and *A. philippense* L. extracts were pelletized separately in IR grade KBr (HiMedia, India) and were scanned over a range of 4000–400 cm^{-1} in FTIR analysis.

3. Results and Discussion

3.1. Effects of Various Parameters on Synthesis of AuNPs and AgNPs

3.1.1. Biosynthesis of AuNPs and AgNPs. High antioxidant potential and ethnomedicinal importance of *A. philippense* L. intrigued us to explore it for biosynthesis of AuNPs and AgNPs. Microbial enzymes, polysaccharides, and plant phytochemicals with antioxidant or reducing properties are usually responsible for reduction of metal compounds into their respective nanoparticles [1, 5, 14–16].

A dark blue color was observed on addition of plant extract to 5 mM tetrachloroauric acid solution indicating AuNPs synthesis. UV-Vis spectroscopy of this solution confirmed the synthesis of AuNPs, as revealed by a characteristic absorption peak at 530 nm [17]. On the other hand, a characteristic brick red color was formed on addition of 9 mM silver nitrate solution to the plant extract, indicating synthesis of AgNPs, which was confirmed by an absorption peak at 420 nm [18]. The time for complete reduction of gold and silver ions in solution to nanoparticles was 10 min.

Shankar et al. [19] reported that reduction of the metal ions and stabilization of AuNP or AgNP are due to the presence of terpenoids or alkaloids present in the geranium extract. On the other hand, AuNP synthesis is caused by the reducing sugar present in the lemongrass extract [20]. Similarly, *A. philippense* L. is known to contain variety of such biologically active phytochemicals like carbohydrates, glycosides, alkaloids, tannins, flavonoids, terpenoids, and saponins. These phytochemicals are responsible for making the extract highly oxidant [12] and thus might play a role in bioreduction of gold and silver nanoparticles.

3.1.2. Effect of Extract Concentration. Various dilutions of plant extract, original 1:10, 1:100, and 1:1000 (diluted in double distilled water), were analyzed for their effect on synthesis of AuNPs and AgNPs. Original extract (5 g plant biomass in 100 mL distilled water) showed effective synthesis of nanoparticles with the characteristic color. However, further dilutions of the extract showed negligible synthesis of nanoparticles. UV-Vis spectra suggest that sharpness of absorption peak is dependent on concentration ratio of extract [21]. Accordingly, sharp peak was observed in original extract concentration (Figures 1(a) and 1(b)). There are similar reports showing effect of biomass quantity on synthesis of nanoparticles [22].

3.1.3. Effect of Metal Salt Concentration. Mixing plant extract with increasing concentration of tetrachloroauric acid/silver nitrate (1 to 10 mM) resulted in synthesis of nanoparticles as indicated by an increasing intensity of the characteristic

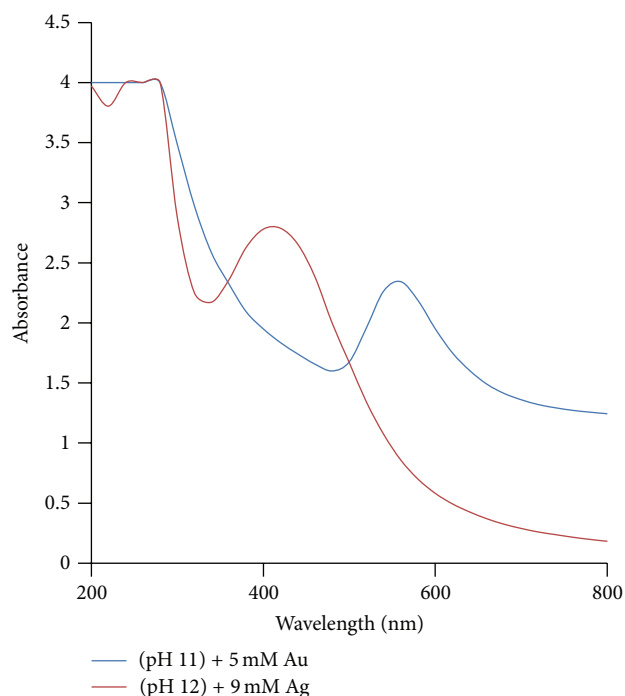


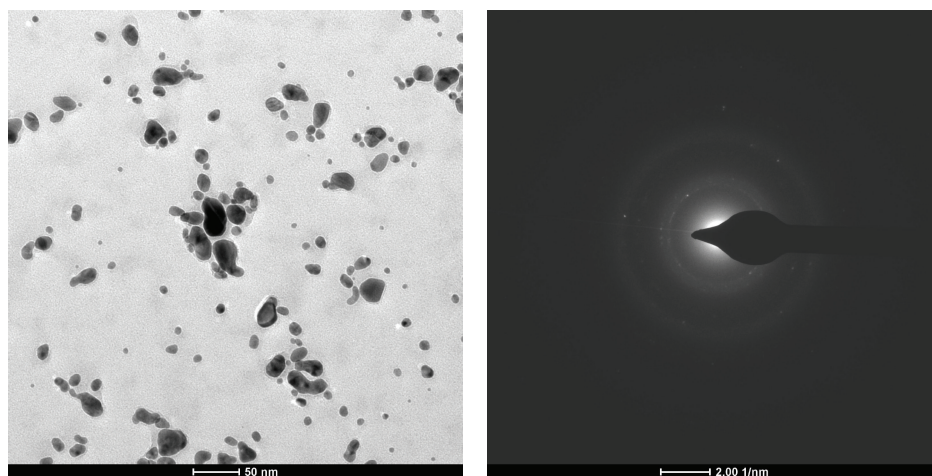
FIGURE 4: SPR at optimum conditions. For Au, original extract at pH 11 and 5 mM tetrachloroauric acid was kept for 10 min. For Ag, original extract at pH 12 and 9 mM silver nitrate was kept for 10 min.

color. In case of AuNPs, peak absorbance increased with tetrachloroauric acid concentration from 1 to 5 mM but decreased at higher metal ion concentrations (Figure 2(a)). Thus, the optimum salt concentration for AuNP synthesis was 5 mM, as revealed by a narrow peak at 530 nm. Similar results have been reported by Ghosh et al. [15] with 1 mM tetrachloroauric acid as optimum concentration for AuNP synthesis.

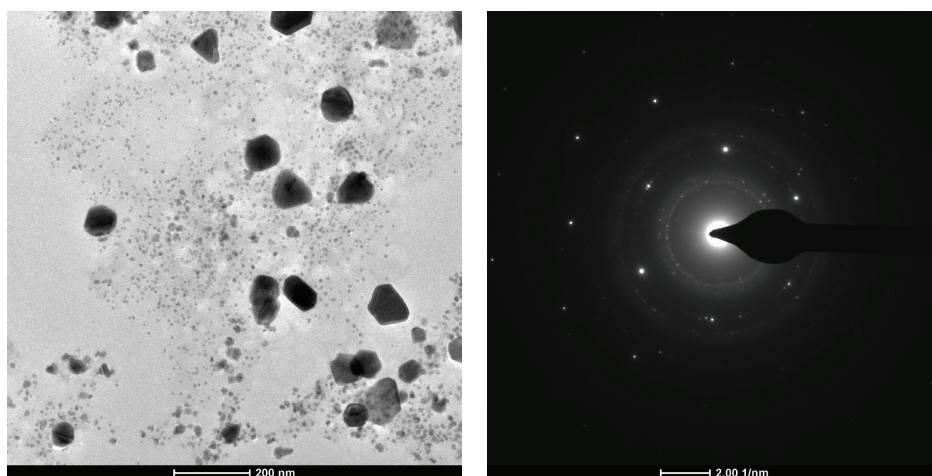
A steady rise in absorbance was also observed for AgNP synthesis using 1 to 10 mM silver nitrate solution. However, there was a marginal difference in absorbance using 9 mM and 10 mM salt concentration as indicated by overlapping peaks in UV-Vis spectra (Figure 2(b)). This indicates that 9 mM was the optimum concentration for AgNPs synthesis. Other researchers have also reported a significant effect of metal salt concentration on the synthesis of silver nanoparticles [5, 23].

3.1.4. Effect of pH. Formation of distinct peak depends on concentration of H^+ ions (pH). Reaction mixtures, containing extract at different pH units (2 to 13) and tetrachloroauric acid solution, showed blue color of varying intensities, indicating formation of AuNPs. UV-Vis spectroscopy further confirmed that a sharp peak obtained at pH 11 was optimum for synthesis of AuNPs (Figure 3(a)).

Similarly for silver, the alkaline pH range showed brick red color of varying intensities indicating the synthesis of AgNPs. UV-Vis spectrum confirmed a sharp peak at pH 12, to be optimum (Figure 3(b)). At lower pH, nanoparticles show lower and broader absorbance which can be due to larger size.

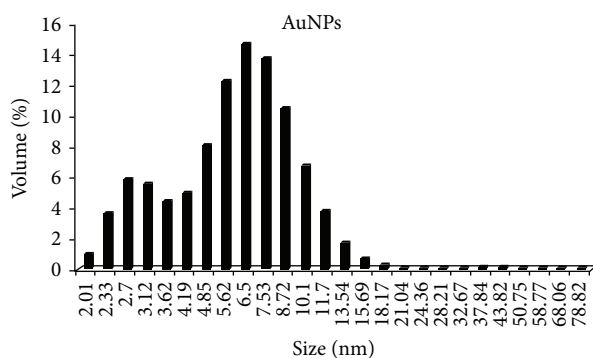


(a)

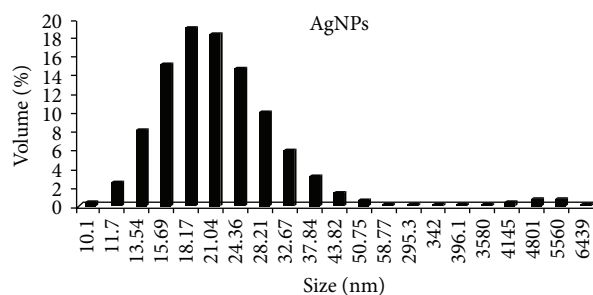


(b)

FIGURE 5: (a) TEM image (left) and SAED pattern (right) of AuNPs synthesized using extract of *A. philippense* L. (b) TEM image (left) and SAED pattern (right) of AgNPs synthesized using extract of *A. philippense* L.



(a)



(b)

FIGURE 6: (a) Histogram of size distribution of AuNPs synthesized by *A. philippense* L. (b) Histogram of size distribution of AgNPs synthesized by *A. philippense* L.

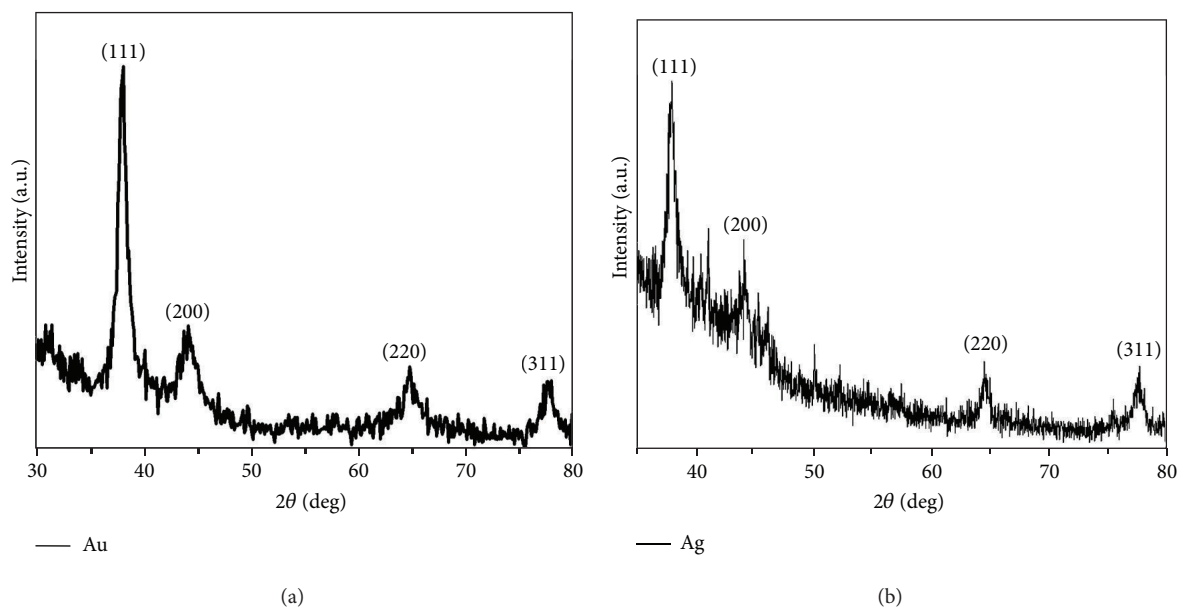


FIGURE 7: (a) XRD pattern of AuNPs. (b) XRD pattern of AgNPs.

Earlier reports also indicate that high absorbance and narrow peaks at alkaline pH favour synthesis of monodispersed and small sized nanoparticles [24]. Alkaline pH prevents agglomeration of synthesized nanoparticles; therefore, small sized particles are formed [4, 23].

Thus, optimum conditions for AuNPs synthesis were 1:1 proportion of original extract at pH 11 and 5mM tetrachloroauric acid, whereas optimum conditions for AgNPs synthesis were 1:1 proportion of original extract at pH 12 and 9mM silver nitrate. Time required for nanoparticle synthesis under optimum conditions was checked by reading absorbance in a UV-Vis spectrophotometer and was found to be 10 min (Figure 4).

3.2. Characterization of AuNPs and AgNPs. Confirmation of AuNPs and AgNPs formation was done by taking UV-Vis spectrum of the resultant solution, in the range of 200–1000 nm. The spectrum (Figure 4) showed a distinct peak, centered at 530 nm, specific for AuNPs [17]. Similarly, a distinct peak observed at 420 nm was specific for AgNPs (Figure 4) [18].

The result obtained from the transmission electron microscopy (TEM) and selected area electron diffraction (SAED) study gives clear indication about the shape, size, and size distribution of the nanoparticles. TEM and SAED pattern revealed that AuNPs were anisotropic and polycrystalline in nature (Figure 5(a)). However, TEM and SAED studies in case of AgNPs showed their anisotropic and monocrystalline nature (Figure 5(b)). Particle size analysis of TEM images was done using ImageJ software. Particle size calculated was also confirmed using Scherrer's formula that makes use of XRD data of the samples. Particle size of AuNPs and AgNPs ranged between 10 and 18 nm. This is in agreement with earlier studies reporting nanoparticles of 10 to 100 nm

obtained using various plant extracts [1, 7, 23, 24]. Small size of nanoparticles obtained in the present study could be a result of alkaline pH used for their synthesis [7, 24].

Particle size distribution was measured by dynamic light scattering (DLS) (Figures 6(a) and 6(b)). Although the histograms show a significant proportion of nanoparticles lying in the size range as calculated by TEM and XRD analysis, other large agglomerates interfere with the accuracy of particle size determination. These agglomerates could be of biological origin present in the plant extract or could be aggregates of nanoparticles [25]. Das et al. [26] reported that the variation in particle size distribution in DLS (99.91 nm) as compared to that observed in HRTEM analysis (15.07 nm) could be due to water absorption on electrostatic stabilized gold nanoparticles.

The obtained XRD data was checked against the standard data for the respective samples, provided by the Joint Committee on Powder Diffraction Standards (JCPDS). Both the particles showed face centered cubic (FCC) structure. In case of AuNPs, the data matched with the JCPDS data card number 04-0784, with lattice constant $a = 4.078 \text{ \AA}$. The observed peaks correspond to the planes (111), (200), (220), and (311), respectively, for the peaks in ascending 2θ order. The results are in agreement with previous reports [4, 8, 23, 26].

The XRD pattern of AgNPs matched with JCPDS data card number 04-0783. Standard lattice constant value for this structure is reported to be 4.086 \AA . Lattice planes (111), (200), (220), and (311) for AgNPs were also observed in the XRD pattern. Broad peak in both the XRD patterns suggests that the particles size was very less. Crystallite size for both the particles was calculated using Scherrer's formula [27] as

$$D = \frac{0.9\lambda}{\beta \cos\theta}, \quad (1)$$

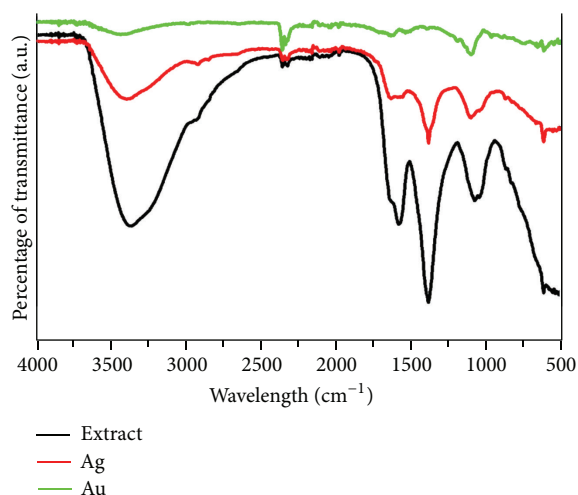
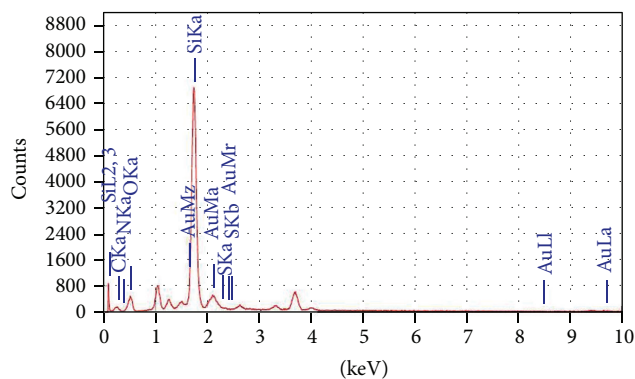


FIGURE 8: FTIR spectra for *A. philippense* L. extract, the AuNPs, and AgNPs.

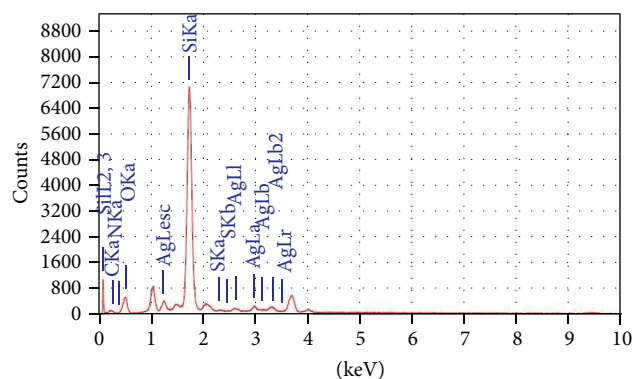
where D is particle size, λ is wavelength of X-ray, β is full width at half maxima for a peak, and θ is the corresponding Bragg angle. The particle size was estimated to be ~ 11 nm for AuNPs and ~ 13 nm for AgNPs (Figures 7(a) and 7(b)). This is in good agreement with the particle size obtained from TEM images.

FTIR spectroscopy was carried out to identify the bio-groups that bound distinctively on the gold and silver surface and were involved in the synthesis of these nanoparticles. The major peaks observed in FTIR of the extract were 3369, 2360, 1585, 1384, 1076, and 514 cm^{-1} . Peak at 3369 cm^{-1} is attributed to OH stretch in phenols. This suggests presence of phenolic compound in the extract [28]. Phytochemical evaluation of *A. philippense* L also reports the presence of phenols [11, 13]. Taraschewski et al. [29] report that the peak at 2360 cm^{-1} results due to CO_2 vibration that may not be necessarily from the sample. Peaks at 1585, 1384, and 1076 cm^{-1} are attributed to C=C stretching, $-\text{NH}_2$ symmetric stretch, and CO vibrations, respectively. The peak at 514 cm^{-1} can be attributed to C-N-C bending [28, 30, 31].

As seen in the case of FTIR for AuNPs, the peak at 1384 cm^{-1} had completely diminished, while the rest of the peaks from extract were present with reduced intensity. This suggests that the extract might be bound to the AuNPs through or by replacing $-\text{NH}_2$ group. In FTIR of AgNPs, the $-\text{NH}_2$ peak remained intact while the C=C stretching peak almost vanished. This may be due to conjugation of extract to the nanoparticle through C=C group (Figure 8). These functional groups of water soluble compounds such as flavonoids, terpenoids, and phenols present in the *A. philippense* L. extract appear to be responsible for synthesis of nanoparticles and their stabilization. Other studies have also reported that proteins or polysaccharides present in the biological material cap and stabilize nanoparticles [1, 17]. These interactions may occur between free amino groups, carboxylate group, or cysteine residues in the proteins [1, 3, 6, 32].



(a)



(b)

FIGURE 9: (a) EDS spectrum of AuNPs synthesized using extract of *A. philippense* L. (b) EDS spectrum of AgNPs synthesized using extract of *A. philippense* L.

Elemental composition of AuNPs and AgNPs and their surrounding was carried out by energy dispersive scattering (EDS). EDS studies for AuNPs and AgNPs showed presence of elemental gold (8.95%) and silver (5.04%) (Figures 9(a) and 9(b)). Detection of carbon and oxygen suggested the presence of carbohydrate compounds containing sulphur that were either capped on AuNPs and AgNPs or were present in the background. Further characterization of the capping agent and its probable involvement in biomedical application needs to be investigated.

4. Conclusion

We have developed a method for synthesis of gold and silver nanoparticles using fern *A. philippense* L., at room temperature, within 10 min. The TEM and EDS analysis results both demonstrate that nanoparticles are successfully synthesized using *A. philippense* L. extract in this study. To the best of our knowledge, use of pteridophytes in nanoparticles synthesis has not yet been reported. Medicinal importance of ferns makes them a suitable candidate for biosynthesis of nanoparticles and application in disease treatment. Use of such medicinally important plants has a promising potential in nanoscience for drug delivery and biomedical application. Exploitation of synthesized capped nanoparticles would

enable us to know the nature of capping agent and utilize them for medicinal and biomedical applications.

Conflict of Interests

The authors declare no conflict of interests.

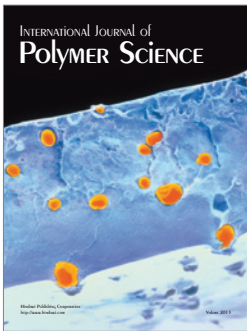
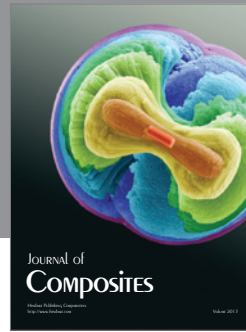
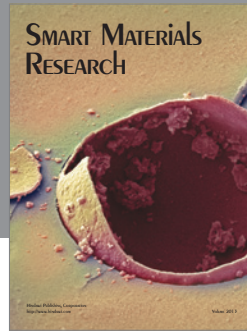
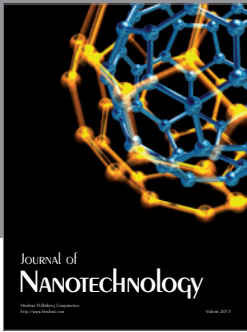
Acknowledgments

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