Synthesis of Silver Nanoparticles by *Phyllanthus emblica* Plant Extract and their Antibacterial Activity

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Abstract
The silver nanoparticle was successfully synthesized by using the help of *Phyllanthus emblica* plant extract as a reducing agent and aqueous silver nitrate as the precursor. Moreover, physical and chemical methods are widely used for the synthesis of nanoparticles, but these methods have expensive and not ecofriendly. This study highlights the green, rapid, facile, cost-effective, and eco-friendly synthesis and synthesized nanoparticles also investigate their antibacterial activity. Synthesized silver nanoparticles are analyzed by different techniques of modes like XRD, UV-Visible spectroscopy, TEM, FTIR, and photoluminescence (PL). The prepared AgNPs show characteristic absorption peak in UV-Visible spectroscopy due to SPR (surface plasmonic resonance) band between 400 to 450 nm wavelength, which was confirmed by TEM (transmission electron microscopy) image. X-ray diffraction (XRD) results showed the crystalline nature of AgNPs as well as the size of nanoparticles calculated with the help of TEM (20-25 nm) and XRD (25 nm). ATR spectroscopy identified the functional groups that are involved in the reduction of silver ion to AgNPs and the PL spectrum indicates higher emission in the green region and low emission peak in the UV region. Antibacterial activity of AgNPs analyzed against with the help of *E.Coli* bacteria and the result shows that a higher concentration of AgNPs is increasing as well as a zone of inhibition increased. This method is environmentally friendly, of low cost,
and less expensive method for the fabrication of AgNPs in abundance which can be further helpful for biosensor devices as well as for other applications such as pollutant degradation, pharmaceutical, and hydrogen production, etc therefore can promote the application of green technology for the production of AgNPs.

**Introduction**

The specific characteristics features of metal nanoparticles participate in the field of energy, optics along with biomedicine as well as disaster management with other health care issues. Among the various nanoparticles like Ag, Au, Pt, and Pd, etc. are their broad range of applications like antibacterial agent, photocatalyst in a photocatalytic reaction as well as a biosensor technology. Generally, researches are prepared of silver nanoparticles by a number of the process such as chemical reduction, electrochemical reduction and photochemical reduction but such type of convention method requires more amount of chemicals as well as a large amount of energy for nanomaterial synthesis process so such type of synthesized products are more hazardous for the environment. Different types physical and chemical methods apply for preparation of silver nanoparticles for various shape and size of nanoparticles, and such type of technique are involved in UV irradiation, microwave irradiation, chemical reduction photochemical method and sonoelectrochemical method. However extremely all previous methods in engaging more than one step as well as advanced energy, low conversions, the trouble of purification, and required critical chemicals. All chemical synthesis methods of nanomaterial show the poisonous chemicals, but the synthesis of nanomaterial by the green method can be removed from this issue, so it requires developing a green synthesis method for nanomaterial synthesis. A wide range of metal NPs such as Ag, Au, ZnO, TiO$_2$, CuO, In$_2$O$_3$, etc, have been synthesized using the help of herbal plant extracts. Various, plants have been used for the synthesis of metal nanoparticles including medicinal plants. Ag has long been reputed for its goods inhibitory effect on many bacterial strains and catalytic activity. So, normally used in industrial and medical products so, in this research work, we have synthesized AgNPs with the help of *Phyllanthus emblica* plant extract for the conversion of silver ion to silver nanoparticles by silver nitrate solution.

**Fig. 1: Phyllanthus emblica fruit**

Present investigation focused on the effects of the different optical parameters on the silver nanoparticles and investigates of the antibacterial effect of the synthesized AgNPs. *Phyllanthus emblica* is a common plant, which is more amount of exceedingly in a nearby part of Indian subcontinents.
as shown in figure 1. Some organic phytochemicals compounds are present in plant extract so *Phyllanthus emblica* fruit extract can be used in the synthesis of another different type of nanoparticles like Au, Ag, Pd, Pt, ZnO, and TiO$_2$, etc. Present phytochemicals in plant extract such as terpenoids and flavanones which act as stabilizing and reducing agents for the nanoparticle synthesis process. Synthesized AgNPs show the no harmful effects on the human body as well as environmental health, natural antibacterial activity in the direction of pathogens like as viruses, fungi and bacteria etc.

**Experimental**

*Phyllanthus Emblica* Extract

Sigma-Aldrich grade AgNO$_3$ (99%) solutions was prepared by distilled water and all glassware was purified by distilled water and dry and glassware was autoclaved before the experiment. Extraction of plant fruit and ready the aqueous plant extract by the dry fruit. Firstly washed carefully by distilled water to make free from dust particles and dried 24 h on room temperature. After that 2 gm dry fruit was soaked in 50 mL of pure distilled water for 24 h. Then synthesized plant extract was filtered and stored for nanoparticle synthesis.

**Nanoparticle Synthesis**

Synthesis of AgNPs by green synthesis process. *Phyllanthus emblica* fruit extract (0.5% (w/v)) was mixed drop by drop in aqueous 0.001 M AgNO$_3$ solution and kept in boiling tube at around 25°C temperature till 12 h aging. Then the solution of a mixture of silver nitrate turns brown with increasing time interval; its optical property shows the confirmation of AgNPs. Synthesized silver nanoparticles are established without any chemical reagent and their stability for a long time due to antimicrobial properties of this plant extract and now synthesized silver nanoparticles was separated by centrifugation technique at 5000 rpm for 20 minutes. For the further settlement of particles, the supernatant material was transferred to a beaker and frequent centrifugation process was carried out to clean AgNPs. The obtained nanoparticle pellet was dry in an oven and stored for further study.

**Results and Discussion**

Synthesis of AgNPs was noted by optical observation with reaction time, initially a silver salt solution is mixed to *Phyllanthus emblica* plant extract, it observed a color change from light yellow to brown color within 12 h of incubation as shown figure 2, after that the color of the solution not be changed and stable therefore observed change of color is strong indication formation of nanoparticles and the stability of color shown that means it's have been completely done the conversion of silver ions to silver nanoparticle due to SPR.

![Fig. 2: *Phyllanthus emblica* fruit extract after the addition of silver salt shows the coloration due to the synthesis of silver nanoparticles](image)

It’s indicated these plants extract reducing agents such as terpenoids and flavanones are responsible for the reduction of Ag$^+$ to AgNPs. It is suggested, the time duration is an important role during the nanoparticle synthesis process, and present analysis indicates that’s 12 h time duration is more appropriate for the complete synthesis of silver nanoparticles.

**UV-Visible Analysis**

Synthesized AgNPs were characterized and confirmed by respective UV-visible absorption
band at appearing around 400-450 nm at 12 h shown in figure 3, due to the strong surface plasmon resonance. This wavelength corresponds to the AgNPs and a previous study revealed that the SPR value for the AgNPs was in the range 400-450 nm.\textsuperscript{34-36} After 12 h of reaction time UV-visible absorption band did not change peak position and highly stable which means 12 h reaction period is more reliable for synthesis and stability of silver nanoparticles.

Fig. 3: UV-Visible spectrum of synthesized silver nanoparticles

Surface plasmon resonance (SPR) is the excitation of free electrons within the conduction band. Hence the broad absorbance was observed. This broad absorbance, indicating the amount of synthesized AgNPs. The broad spectra could be generated due to some reasons (1) organic elements that are produced by plant extract (2) homogeneity,\textsuperscript{37-38} and extra-fine nature of AgNPs,\textsuperscript{39} (3) Moreover many other factors such as the size and shape of nanoparticles.\textsuperscript{40} The TEM image from this study confirms the spherical shape of synthesized silver nanoparticles.

Fig. 4: X-ray diffraction patterns of synthesized silver nanoparticles

\[\text{Ag NPs}\]
XRD Analysis
Figure 4 shows the XRD patterns of synthesized AgNPs by Phyllanthus emblica extract. Some intense characteristic diffraction peaks at 2θ angles of 38.4, 44.5, 64.8, 77.6 are observed, which crystallographic planes of (111), (200), (220), and (311). According to the TEM, synthesized AgNPs are evidently showed the most AgNPs were highly dispersed in spherical shapes. Synthesized silver nanoparticle directly corresponds with JCPDS 00-004-0783. No, any other peaks were obtained, only cubic shape AgNPs appeared. Usually, XRD peaks width directly related to the crystallite size of nanomaterial. Which are useful in the debye scherrer equation for the analysis of average particle size.\(^1\) That means Diffraction peak \(D = \frac{(k\lambda)}{(β \cos θ)}\), here \(λ\) wavelength of Cuku, \(D\) is the crystalline size of the sample powder, \(β\) is the full width at half maxima, \(θ\) is the bragg diffraction angle and the \(K\) is the constant. The highest peak (111) was picked to determine the crystalline size of nanomaterial. According to this equation, the average size of AgNPs is found 25 nm and mostly spherical. That's the result that was found consistent with the TEM analysis.

![TEM image of synthesized Ag nanoparticles](image1)

![HRTEM image of spherical shape Ag nanoparticles](image2)

TEM Analysis
Synthesized silver nanoparticles shape and size are calculated by with the help of HRTEM image shows in figure 5. Aliquots silver nanoparticle solutions were placed on a copper grid and dry under ambient conditions than after the HRTEM image was recorded. These results shows the particles are still in the range of nm scale with a spherical shape. These spherical shape synthesized AgNPs with a size range of 20-25 nm.

![ATR spectrum of synthesized silver nanoparticles after 12 h](image3)
ATR Analysis

ATR measurement was used for identifying the responsible biomolecules for capping and stabilizing agent in AgNPs. Two types of infrared bands are observed at a position of 3321 cm\(^{-1}\) and 1635 cm\(^{-1}\) figure 6. Present intense peak around at 3321 cm\(^{-1}\) responsible for OH stretching of alcoholic and phenolic groups\(^{42}\) and another intense peak around at 1635 cm\(^{-1}\) appear from the C=O stretching mode of amine group these groups are found in proteins\(^{43}\) and these groups of proteins as a capping and stabilizing agent for synthesized AgNPs, which are responsible for increasing the stability of the synthesized nanoparticles.\(^{44}\) It is observed the structures of proteins were not affected by the silver ions before and after binding with AgNPs.\(^{45}\) Previous studies by\(^{46-48}\) have also identified the plant extracts in the samples and proposed that these alcoholic and phenolic groups could serve as organic reducing as well as capping agents.

Photoluminescence Analysis

Figure 7 represents the photoluminescence spectra of the synthesized silver nanoparticles by \textit{Phyllanthus emblica} plant extract that are studied through emission spectroscopy. This is one of the technical methods for observation of the optical properties of synthesized AgNPs. Synthesized AgNPs are mixed in distilled water then emission spectra are obtained within a range from 200 to 800 and for the wavelength of excitation at 289 nm. It exhibits the emission peaks around 400 to 600 nm which belong to the green-yellow region. The intensity of emission peak continues increased up to 469 nm, then after it’s gradually decreased up to 650 nm. The luminescence spectrum of AgNPs is generated by the excitation of an electron from occupied d bands into states above the Fermi levels its responsible for luminescence spectra of AgNPs\(^{49}\) and broad peak in green-yellow emission is produced by radial recombination of photoexcited holes with the electrons in oxygen vacancies and indicates that AgNPs have a good crystal structure.

Antibacterial Activities

Antibacterial activity of synthesized silver nanoparticles analysed against \textit{E.Coli} bacterial samples, shown in figure 8. During this process in which synthesized silver nanoparticles are used in different concentrations (2, 4, 8, and 10 mg) are further agar dishes containing colony. The dishes are visible every concentration with a bacterial colony and experimentally observed after 24 h incubating time at 37\(^{\circ}\)C. The zone of approval is experiential maximum at 10 mg of AgNPs. The antibacterial activity results show due to modifying bacteria cell permeability as well as enzyme degradation by synthesized AgNPs. The resultant zone of inhibition increased as well as increases the concentration of AgNPs.
Conclusions
We have reported a reasonable, eco-friendly, and easy approach for the synthesis of AgNPs. *Phyllanthus emblica* plant extracts which act as a reducing, stabilizing, and capping agent for nanoparticle synthesis. This is a simple, green efficient method for the synthesis of silver nanoparticles at room temperature without using any harmful reducing and capping agent. The green synthesized silver nanoparticles were collected of spherical shape which was highly stable and crystalline. These AgNPs were characterized carefully by XRD, TEM, FTIR, PL, and UV-visible spectroscopy. The UV-visible spectra showed a characteristic range of 410-425 nm for nanoparticle. XRD results showed the crystalline nature of AgNPs, and size with the help of TEM (20-25 nm) and XRD (25 nm). ATR analysis found numerous phytochemicals and functional groups these groups are responsible for stabilizing and creation nanoparticle. Antibacterial activity of AgNPs analyzed against *E.Coli* bacteria and the result shows that a higher concentration of AgNPs is increasing as well as the zone of inhibition increased. This synthesizes process is cheaper, a single step, and faster as compared to chemical and biological methods as well as eco friendly, of low cost, and simple therefore can promote the application of the green method for silver nanoparticle synthesis.

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