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# A Study On Reaction Parameter Optimization For Synthesis Of Silver Nanoparticles By *Parthenium Sp.* Aqueous Leaf Extracts And Their Antimicrobial Potential

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**Abstract**:
Present study reveals an appropriate and extracellular process using aqueous leaf extract of *Parthenium sp* for reducing silver nitrate resulting in fabrication of nanoparticles. Plant leaves were subjected to extraction of metabolites by decoction, cold percolation and soxhlet procedure. The metabolites were then tested for the presence of various metabolites including alkaloids, protein, phenol, reducing sugar, steroid, saponin and tannins. Owing to the maximum extractive yield in case of the different aqueous extracts, these were then used to reduce silver ions by adding 5ml plant leaf extract to 95ml of $10^{-3}$M silver nitrate solution. The quick reduction of silver ions was observed as a change in leaf extract colour from green to dark redish brown on treating with silver nitrate. The bioreduction was also monitored spectrophotometrically that shows a broad surface plasmon resonance absorption peak for silver nanoparticles at wavelength 422 nm. Effect of several factors, including the quantity of plant extract, the incubation period, the temperature, the amount of silver nitrate, and the pH were investigated and silver nanoparticles synthesis was followed under optimum parameters. X-ray diffraction, Fourier transforms infrared spectroscopy and transmission electron microscopy was used to examine and characterize biosynthesized silver nanoparticles. Due to the existence of the highly intense peak for Face-centred cubic (FCC) material (1 1 1) reflection, the XRD examination assured the fabricated particles to be Face-centred cubic (FCC). By using FTIR measurements, potential biological capping molecules were predicted as well as effective stability of synthesized nanoparticles was ensured. Numerous absorption bands in the spectrum show that the produced silver nanoparticles contain active functional groups. Using TEM examination, the silver nanoparticles' size and form were identified. Mean diameter of spherical silver nanoparticles as exhibited in TEM image was 23.01 nm. The antimicrobial potential of the aqueous leaf extract and the fabricated nanoparticles was estimated using agar well diffusion and growth curve analysis, against various bacteria and fungi. The aqueous leaf extract and the synthesized silver nanoparticles showed pronounced activity against the microbes used in the study, with high zone of inhibition with silver nanoparticles as compared to the plant extracts. Current study concluded that aqueous extract of the weed under investigation provides the reducing power for fabrication of silver nanoparticles with an effective biocidal activity against the test microbes that makes them an efficient and candidate for inclusion in pharmaceutical goods that could aid in halting the spread of drug-resistant pathogens.

**Keywords:** Green synthesis**,** Silver Nanoparticles, Antimicrobial activity, XRD, *Parthenium sp.*

## INTRODUCTION

In the era of recent scientific development, nanoparticles fabricated from metals have attracted the attention of research community owing to their recognizable chemical, electrical optical, mechanical and magnetic proficiency. Their small sizes, large surface to volume ratios and crystallographic surface structure are

responsible for their unique properties. Nanomedicine, which results from the merger of nanotechnology with medicine, is a rapidly developing new science that employs nanoparticles. Silver nanoparticles can be synthesized by various techniques, such as chemical reduction of silver ions in aqueous solutions with or without stabilizing agents, thermal decomposition in organic solvents, chemical reduction and photoreduction in reverse micelles, and radiation chemical reduction. The majority of these techniques use dangerous, poisonous chemicals, which could pose threats to the environment and human health. They are also quite expensive

Noble metals like lead, silver, gold, and platinum are the metals of choice for synthesizing nanoparticles by chemical methods. Among the noble metals, silver (Ag) is the metal of choice in the field of biological systems, living organisms and medicine. Since noble metal nanoparticles are widely applied to areas of human contact, there is a growing need to develop environmentally friendly processes for nanoparticle synthesis. Due to slower kinetics, biological techniques of synthesis have provided an option for the "greener synthesis" of nanoparticles, which appears as superior method since it allows for improved control and manipulation of the crystal development and stabilization. As a result, research on synthesis techniques that enable more precise form and size control for a variety of nanotechnological applications has increased. The use of environmentally benign materials like plant extract (Singh *et al*., 2017), bacteria, fungi and enzymes for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Weeds, considered undesirable flora competing with cultivars for nutrients and space, may be the plant material of choice for the task. The strategy will offer a useful substitute for using this undesirable flora.

## MATERIALS AND METHODS

### A. *Plant material collection and aqueous extract preparation*:

*Parthenium sp.* leaves were collected from the fields and thoroughly washed with running fresh water and two times with sterilized distilled water before being air dried for duration of 7 days. The leaves were crushed into powder which was in turn used for extraction purpose using one of three methods: soxhlet extraction process (SPaq), cold percolation process (CPPaq) and decoction process (DPaq). The weight of the extract obtained was measured after being dried over anhydrous sodium sulphate to eliminate any remaining water.

### B. *Calculation of crude yield*:

The dish was weighed empty and its weight was recorded in order to calculate the crude yield of the extract. After any remaining water had evaporated, the dish weight and its contents were measured. Crude yield was quantified by formula:

$$\textbf{Crude yield} = \frac{\textbf{Weight of the sample extract obtained (g) X 100}}{\textbf{Weight of the powdered sample used (g)}}$$

### C. *Phytochemical analysis*:

The produced aqueous leaf extract was tested chemically using a variety of methods (Santhi & Sengottuvel, 2016) to screen for and identify bioactive chemical ingredients.

### D. *Bio inspired synthesis of silver nanoparticles*:

Brown vials were used to prepare and store the silver nitrate solution (1 mM). A 95 ml solution of $AgNO_3$ was poured to 5 ml of aqueos lead extract that was placed in a BOD bottle. The commencement of the reaction was defined as the moment the extract was introduced to the aqueous $AgNO_3$ solution. After being exposed to the plant extract, silver ions were transformed into silver nanoparticles, and their colour changed from yellow (pale) to dark yellow brown. Spectrophotometric analysis was performed for confirming production of silver-nanoparticles.

**E. Reaction parameters optimization:**

Change in the different parameters may lead to a variable change in reduction process thereby leading to the change in the shape and appearance of the final product. Different parameters were altered to study their effect on synthesis of silver nanoparticles.

**1. *Plant leaf extract and silver nitrate ratio*:**

For examining the impact of reducing agent and metal ion ratio on process of silver nanoparticle formation, four distinct ratios of aqueous extract and silver nitrate were taken into consideration. To achieve this, various ratios of leaf extract and silver nitrate were introduced to four separate test tubes (7:93, 5:95, 3:97 and 1:99). The mixture was left to sit at room temperature while a colour change was monitored and a UV-visible spectral analysis was performed.

**2. *Reaction time*:**

*Parthenium sp* leaf extract (2.5 ml) was added with continuous stirring into 1 mM (47.5 ml) silver nitrate solution that was taken in various containers. At various times, the change in colour of reaction mixture was monitored using UV-visible spectrophotometric examination at different time intervals.

**3. *Reaction temperature*:**

**E**ach plant extract **(**2.5 ml) were added along with steady stirring to 1 mM silver nitrate (47.5 ml) solution collected in four different containers. The reaction was then allowed to happen for up to 24 hours while the tubes were subjected to various temperatures such as 10$^{o}$ C, 30$^{o}$ C, 50$^{o}$ C, and 70$^{o}$ C. Periodically, the solution colour was checked, and Spectrophotometric study (UV-Visible) was also performed.

**4. *Reaction pH*:**

Plant extract (2.5 ml) was added to 1 mM silver nitrate solution (47.5 ml), and the pH was adjusted in different conicals (pH 5, 7, and 9). The reaction mixture pH was adjusted using standard NaOH (0.1N) and HCl (0.1N) solutions. The colour of solution was checked on a regular basis, and the absorbance of solution was measured with spectrophotometer (UV-Visible).

**F. *Silver nanoparticles synthesis under optimized parameters:***

The bio reduction of silver ions was carried out under ideal circumstances following the adjustment of several parameters for the creation of nanoparticles. 1 mM silver nitrate solution (45 ml) was taken in various containers, and plant leaf extract (5 ml) was added. The reaction mixture's pH was brought down to 7.0, and it was incubated for up to 24 hours at 70$^{o}$C. The colour change of the solution was observed after 24 hours and the absorbance of resulting solution was monitored by UV-Vis spectrophotometer (Perkin-Elmer lamda-25) with the wavelengths of 300 – 700 nm to analyse the Surface Plasmon Resonance band.

**G. *Silver nanoparticles recovery*:**

To obtain pellet, the mixture was centrifuged for 30 minutes at 10,000 rpm (REMI, Model C-24BL). The resulting pellet was centrifuged after being re-suspended in deionized water. To remove any biological impurities, the pellets were washed three times carefully with deionized water. The dust was dried and put away for later use.

***H. Phyto silver nanoparticles characterization***

**1. *X -ray diffraction (XRD) study*:**

X-Ray diffraction was used to observe the crystallinity and phase of silver nanoparticles. X-Ray Diffraction grid was coated with air dried silver nanoparticles and examined using an X-Ray Diffractometer with an X-Ray generated operator at a voltage of 40 KV and current of 30 mA with Cu-K radiation (= 1.54120A).

**2. *Fourier transforms infra red spectroscopy (FTIR):***

By using FTIR analysis (Shimadzu), the surface of silver nanoparticles were analysed for the existence of various functional groups and the spectra were scanned in the region of 4500-500 $cm^{-1}$ with a resolution of 4

$cm^{-1}$. Silver nanoparticles were uniformly scattered in a KBr matrix for sample preparation, and was compacted into a transparent disc.

**3. *Transmission electron microscopy*:**

Transmission electron microscopy (TEM) examination was done to look at the silver nanoparticles that were created and determine their size and shape. The solvent was evaporated for 30 minutes under infrared light, after a drop of this solution was put onto copper grids that had been coated with carbon. Utilizing TEM, the sample was examined. The software Image J 1.50i was then used to analyse the image. To ascertain the frequency of nanoparticles of various sizes, the information was shaped as a histogram created in Excel 2007.

***I.Antimicrobial activity of fabricated silver nanoparticles:***

Cultures of *Salmonella sp*. (MTCC No. 3215), *Escherichia coli* (MTCC No. 1722), *Klebsiella pneumonia* (MTCC No. 3384), *Proteus vulgaris* (MTCC No. 1771), *Staphylococcus aureus* (MTCC No. 1430) and *Streptococcus sp.* (MTCC No. 655) in lyophilized form were procured from MTCC IMTECH Chandigarh in lyophilized form. For determining the antibacterial activity of the silver nanoparticles two methods were used that includes agar well diffusion method and growth curve analysis. For these methods, 5 mg of extract and silver nanoparticles were taken in two separate tubes 10 ml sterile distilled was mixed to them so as to obtain 500 µg ml-1 of stock solution for each. Aqueous extract was filtered through 0.22 µm membrane filter.

**1. *Agar well diffusion method*:**

In a test tube, 20 ml nutrient agar was melted at 100°C and stabilized at 45°C for about 15 minutes. About 0.1 ml inoculum was added from culture tube to the agar in the test tubes by use of a loop. The test tube containing the inoculum and agar was then gently rolled between the palms to completely combine the inoculum and agar. Each time the loop was used, it was flamed. The test tube's contents were transferred to a petri plate and given time to set. The petri dishes were then labeled with the respective organism (inoculums) and date. By means of a 6 mm cork borer, four cups were bored, well separated, and equidistant from each other in the agar. The cups were labeled with the four crude extracts. Each cup was filled with its corresponding 500 µg $ml^{-1}$ SNP (silver nanoparticle) to about the three-quarters full. They were kept on a bench at room temperature for about 60 minutes (for the extracts to diffuse into the agar). After a 24-hour aerobic incubation period at 37°C, the plates were checked for any zones of inhibition. The same procedure was repeated with the control using the pure solvent *i.e*. water. Zones were read with reflected light on a dark background.. The diameters of the zones of growth of inhibition were measured with the help a scale from the underside of the covered plates. The average of the diameters was taken. By deducting the diameter of the cups (6 mm) from the overall zone of growth, the actual zones were determined. The test was performed in triplicate. Antimicrobial activity was expressed as the mean inhibition zone (mm) ± S.D produced by the silver nanoparticles.

**2. *Analysis of antibacterial activity by Growth curve analysis*:**

The antibacterial activity of nanoparticles, against various microbial cultures was analyzed also by their growth curve. 10ml of nutrient broth media was injected with fresh colonies from agar media. The media supplemented with 500 µg $ml^{-1}$silver nanoparticles and bacterial cultures was incubated at 37°C with continuous shaking. The microbial growth in broth media was indexed by measuring the optical density (at λ = 600nm) at regular intervals using UV-Vis spectrometer. To ascertain the effect of nanoparticles different controls were also prepared that includes one tube tube with aqueous plant extract, one with the reference antibiotic (ampicillin) and another with no other inhibitory agent in it

**RESULTS AND DISCUSSION**

**A. *Determination of crude yield*:**

The effectiveness of extraction procedure is crucial process in the extraction of bioactive components from plant material. This effectiveness can be expressed in terms of crude yield values which help in estimation of

specific constituents soluble in particular solvent (**Llorent-Martínez, *et al.* 2020**) as well as to determine the chemical composition of the extract. In present demonstration the crude yield was seen to differ depending on the techniques used. The soxhlet extraction technique was found to have the highest crude yield of all the processes used as can be seen in Figure 1. This may be due to the extensive heating of material in this process.

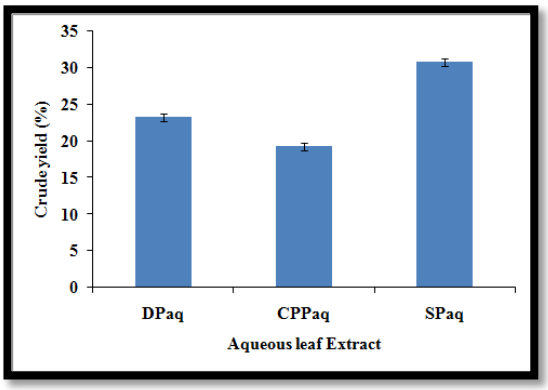



**Fig 1.** Crude yield of aqueous extract using different extraction methods

**B. *Phytochemical analysis:***

Plants have always been a source of various pharmacogonostic and therapeutic agents (Sharma and Tomar 2022) owing to the presence of phytochemicals present in them. The phytochemical analysis of *Parthenium sp.* aqueous leaf extracts (DPaq, CPPaq and SPaq) showed the presence of Protein, Alkaloid, Steroid and flavonoids in all the fractions (**Table 1**) that confer various pharmacological properties to the plant (Marimuthu *et al.*, 2015). The results are in line with the reports documenting *Parthenium sp* as a rich source of phytochemicals.

**Table 1:** Phytochemical analysis of *Parthenium sp*. aqueous leaf extracts **obtained using different extraction techniques**

| S. No. | Phytochemical | Method used | Leaf extracts | | |
|---|---|---|---|---|---|
| | | | DPaq | CPPaq | SPaq |
| 1. | Reducing sugar | Benedict's test | - | - | - |
| 2. | Protein | Biuret test | + | + | + |
| 3. | Phenol | Ferric chloride test | - | - | - |
| 4. | Alkaloid | Hager's test | + | + | + |
| 5. | Steroid | Liebermann Burchard test | + | + | + |
| 6. | Flavonoids | Alkaline Reagent Test | + | + | + |
| 7. | Saponin | Foam test | - | - | - |
| 8. | Anthraquinone | Modified Borntrager's Test | - | - | - |
| 9. | Tannin | Braemer's test | - | - | - |

**C. Bio inspired synthesis of silver nanoparticles:**

It is widely known that the activation of surface plasmon vibrations in silver nanoparticles causes them to appear brown in aqueous solution. For this the aqueous extract obtained by soxhlet extraction was used (as the crude yield was observed to be maximum by this process). Biofabrication of silver nanoparticles using *Parthenium sp*. leaves aqueous extract was validated by monitoring the aqueous extract's colour change with the addition of silver nitrate solution. Within two hours of incubation, the colour change was observed and was found to be stable after gradual alteration upto six hours. A slight change in colour of the reaction mixture was seen after 24 hours that became stable going dark brown from pale yellow, due to the silver ions reduction process, demonstrating the synthesis of silver nanoparticles (Velusamy *et al.*, 2016). Figure 2A

displays the change in colour of the plant extract. The findings are supported by similar findings as reported with various other plant extracts used by the scientific community.

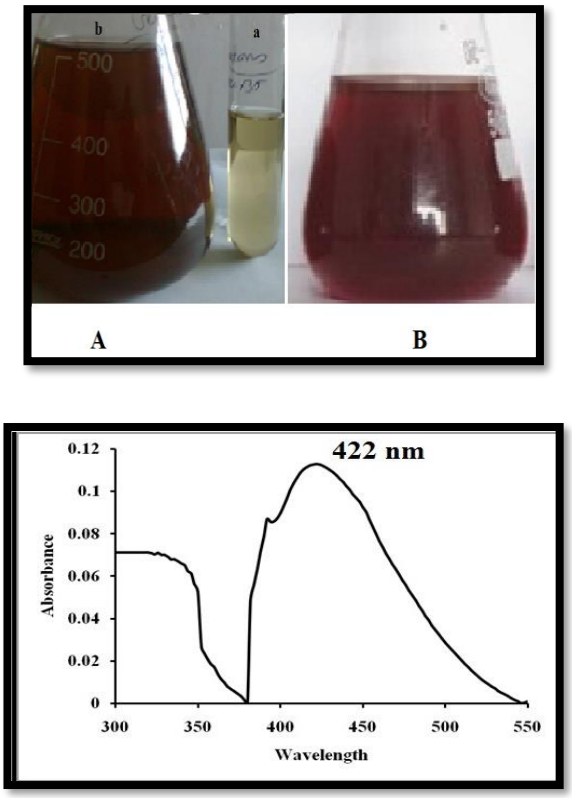



**Fig 2.** Reaction mixture showing change in colour after mixing plant extract to silver nitrate solution (B: under optimized condition) and UV visible spectrum analysis of silver nanoparticles synthesized under optimized condition

***D. Optimization of various parameters for nanoparticles synthesis***

**1. *Ratio of plant leaf extract and silver nitrate*:**

The ratio of metal ions to reducing agent affects the rate of synthesis as well as the shape of the nanoparticles. The UV-Visible spectra created for silver nanoparticles by altering leaf extract amount are shown in Figure No. 3A. With an increase in peak intensity, it was observed that the plasmon absorbance maximum moved somewhat towards longer wavelengths as the amount of aqueous extract in the combination increased. The peak shift was observed to be from 410 nm to 430 nm that indicates increase in the particle size. The increase in intensity of the peak may be due to an increased rate of silver nanoparticles synthesis as when the concentration of the biological material is increased, the higher content of biomolecules get involved in the reduction process resulting in more intense colour (Salunke *et al*., 2017). The optimal extract to metal ion ratio for the creation of silver nanoparticles was determined to be 5:95 since our goal was to create silver nanoparticles with strong antibacterial activity.

**2. *Reaction time*:**

With an increase in reaction time, an increase in the SPR band's intensity was seen without any change in the peak wavelength (Figure 3B). Initial observations of the colour shift were made an hour after adding the salt solution to the leaf extract. The rate of silver ion reduction was found to be gradual during the reaction period with an appreciable increase in the intensity. After 24 hours, the colour of the solution became practically consistent, suggesting that there was no longer any silver salt available for reaction (Sumitha *et al.*, 2018). The findings completely accord with earlier reports. Due to the fact that the intensity peaked at 24 hours, this time frame was chosen as the best one for the production of silver nanoparticles.

**3. *Reaction temperature:***

On incubating the silver nitrate and plant extract mixture at different temperatures there was a considerable difference in colour of the mixture after 24 hours. However the change in colour was observed to be delayed at 10$^{o}$ C and occurs after 6 hours. At other temperatures, the colour change was observed earlier. As can be seen in the results, with the increase in temperature, the reduction of silver salt is enhanced, as indicated by rapid change in the colour of the solution. The peak absorption wavelength shifted from high to low, as temperature varies from 10 to 70$^{o}$C. The shift of wavelength in *Parthenium* sp. was observed from 426 nm to 422 nm. The shift in the band maximum may be due to localization of surface plasmon resonance of the silver nanoparticles. Along with the change in the band maximum, the peaks were observed to be getting narrow with increase in the incubation temperature; this indicates that the size of the synthesized nanoparticles decreases with increasing temperature, which may be due to the faster reaction rate at higher temperature. At high temperature, the kinetic energy of the molecules increases and silver ions gets consumed faster, thereby leaving less possibility for particle size growth (Verma *et al*., 2016). Thus, smaller particles of uniform size are supposed to be formed at higher temperature. Therefore the optimum temperature for Silver nanoparticles synthesis was considered to be 70$^{o}$ C

**4. *Reaction pH*:**

Another important parameter which affects the formation of nanoparticles is the pH of the solution. It has been documented that change in pH affects the shape and size of the particles, as pH has the ability to alter the charge of biomolecules, which might affect their capping as well as stabilizing abilities. Fig 3D shows change in peak absorption wavelength and intensity on varying the pH of the solution. As the pH increases from 5 to 9, the absorption maximum shifts from low to high wavelength. In addition to the spectral shift, the absorption intensity increases with increasing pH. This indicates that pH 9 is the most favourable pH for the synthesis of silver nanoparticles using aqueous leaf extract. Further, it was observed that the pH enhances the rate of reduction reaction, the colour change was observed very fast when AgNO3 mixed with aqueous leaf extract, i.e. within few minutes the colour of the sample changed to dark brown. The shift in the peak wavelength indicates that the size of the particles increases with increasing pH of the solution. As the diameter of the particles get larger, the energy required for excitation of surface plasmon electrons decreases, as a result the absorption maximum shifted towards the longer wavelength region. Moreover, it was observed that at acidic pH i.e. pH < 7, the formation of nanoparticles is suppressed. At pH > 7, the bioavailability of functional groups in leaf extract promoted the synthesis of nanoparticles. It has been mentioned that the absorbance of the silver nanoparticles obtained from olive leave extract increases with increasing pH of the solution from 2 to 8. Upon further increasing the pH of the solution, the absorbance decreases. However, in the present study upon increasing the pH from 5 to 9 the absorbance increases monotonically along with a shift of peak from lower to higher wavelength, indicating that the alkaline pH is more favourable for the synthesis of silver nanoparticles with large size (Aslam *et al*., 2021). But since the particles tailored were required for antimicrobial activity the pH considered was 7.

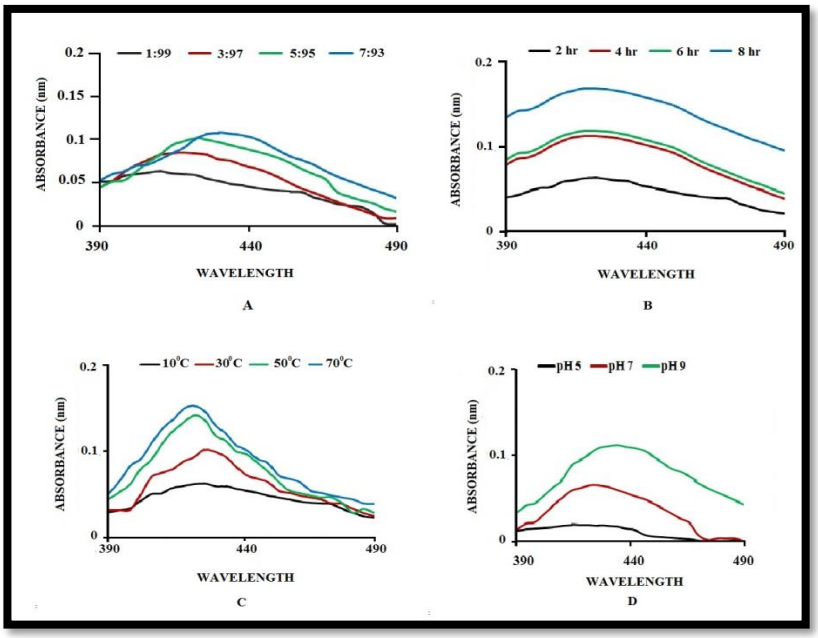



**Fig 3:** UV-Vis spectrum analysis of silver nanoparticles synthesized under different reaction parameters (A- Ratio of plant leaf extract and silver nitrate, B- Reaction time, C- Reaction temperature and D- Reaction pH)

***E. Bio-inspired synthesis of silver nanoparticles under optimized parameters***

Under the optimized conditions the colour of the reaction mixture was found to be more intense (Fig 2B) and the silver nanoparticles fabricated were characterized further.

***F. Characterization of phyto silver nanoparticles***

**1. *X -ray diffraction (XRD) study*:**

Figure 4 displays pattern of X-ray diffraction by fabricated nanoparticles. The XRD profile shows the crystalline nature and structural details of the produced silver nanoparticles. The miller indices were assigned following peak indexing. Standard powdered card on Powder Diffraction Standards (JCPDS), Silver file No. 04-0783, was then compared to three peaks at 2 theta values of 38.24º, 44.20º, 64.48º, and 81.72º, which correspond to the (111), (200), (220) and (222) Bragg reflection planes of silver detected. For the (111), (200), (220), and (222) planes, respectively, the computed interplanar spacing (d values) are 2.351, 2.047, 1.443 and 1.177, which are matched with conventional silver values. The sample shows that the high-intensity peak for FCC material is typically (111) reflection. The resultant silver nanoparticles are Face-centered cubic, according to XRD investigation. Our findings concur with those of other researchers who found peak values for the same type of silver nanoparticles generated using various plant extracts (Singh *et al*., 2019). Prior to XRD examination, nanoparticles were many times centrifuged and resuspended in sterilized distill water, eliminating the possibility of any free biological approach. Nevertheless, certain small peaks corresponding to impurities may be attributable to other organic compounds in plant extract. The erroneous diffractions show crystallographic impurities are present.

**2. *Fourier transforms infra red spectroscopy (FTIR):***

The FTIR spectra of *Parthenium* shows absorption bands at 3469.70-3204.51 $cm^{-1}$, 2939.31 $cm^{-1}$, 1641.31 $cm^{-1}$ and 1.333.68 $cm^{-1}$ that clearly indicates the presence of capping agent with nanoparticles (Figure 5). The band at 3469.70-3204.51 cm-1 in the spectra corresponds to N-H and O-H stretching vibrations indicating the presence of alcohol and phenol. Band at 2939.31 $cm^{-1}$ occurring as a result of the aromatic compound's C-H stretching. Stretching for C=C was allocated to the band at 1641.31 cm-1. The band at 1333.68 $cm^{-1}$ corresponds to the N=O symmetric stretching typical of nitro compound. C-N stretching of amines at 1333.68 $cm^{-1}$ is also attributed to C-F stretching, which is characteristic of alkyl halides (Elangovan *et al*., 2015).

**3. *Transmission electron microscopy*:**

By employing transmission electron microscopy (TEM) examination, the shape and size of the silver nanoparticles generated in ideal circumstances were analysed. Figure 6 displays TEM pictures of silver nanoparticles produced by aqueous *Parthenium sp*. leaf extracts. It is obvious that the nanoparticles have a form that is almost spherical (Shafaghat, & Shafaghatlonbar 2018). The histogram of the particle size distribution for *Parthenium sp.* demonstrates the size of the particles. The silver nanoparticles created by *Parthenium sp.* extract are spherical in shape and range in size from 1.13 nm to 34.17 nm, with an average size of 23.01 nm, according to TEM images of the particles. It's intriguing to observe that most of the particles in TEM pictures are not in direct physical touch with one another, but rather are separated by a largely consistent interparticle distance, which may be a sign that the particles are single crystals.

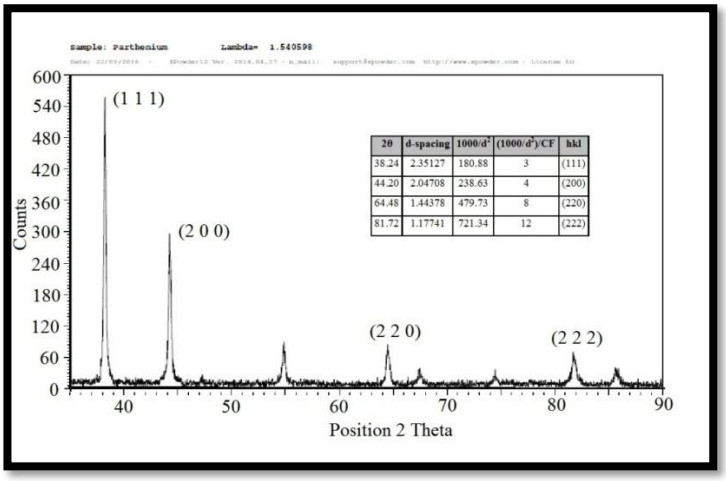
***G. Antimicrobial activity of fabricated silver nanoparticles***

1. ***Agar well diffusion method:***

It is clear from the graphs that in case of all the nanoparticles activity is maiximum against gram positive bacteria as compared to the gram negative bacteria. Silver nanoparticles are found to be more potent in comparision to the respective aqueous plant leaf extract. The phyto-nanoparticles are found to produce larger zone of inhibition than the control antibiotic in case of *Streptococcus sp., Staphylococcus sp., Klebsiella pneumoni.* . The antibacterial activity of Silver nanoparticles can be explained due to the change in the cell membrane permeability or degradation of enzymes in bacteria. It has been proposed that silver nanoparticles cause irreversible damage on bacterial cells by inhibition of bacterial DNA replication. Silver nanoparticles have been shown to be definitely an effective antibiotic against *Staphylococcus aureus* (Yekeen *et al.*, 2017)

*2.* ***Analysis of antibacterial activity by Growth curve analysis:***

The effect of silver nanoparticles when monitored by their growth curve analysis, it was observed that the presence of the silver nanoparticles decreases the slope of growth curve thereby affecting the generation time of the organism (Figure 7).




| 2θ | d-spacing | 1000/d² | (1000/d²)/CF | hkl |
|---|---|---|---|---|
| 38.24 | 2.35127 | 180.88 | 3 | (111) |
| 44.20 | 2.04708 | 238.63 | 4 | (200) |
| 64.48 | 1.44378 | 479.73 | 8 | (220) |
| 81.72 | 1.17741 | 721.34 | 12 | (222) |

**Fig 4:** XRD Pattern of silver nanoparticles synthesized using aqueous leaf extract of *Parthenium sp.* under optimized reaction conditions

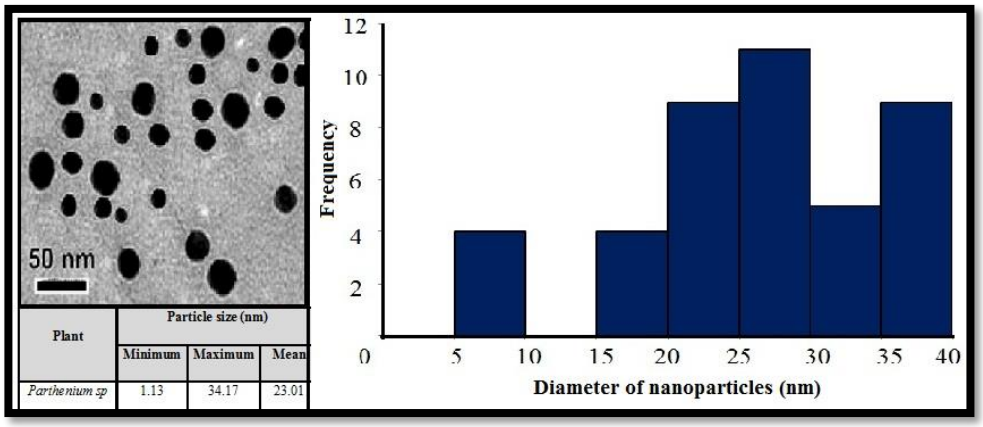



**Fig. 5:** TEM analysis image of silver nanoparticles synthesized using aqueous leaf extract of *Parthenium sp.* under optimized reaction conditions

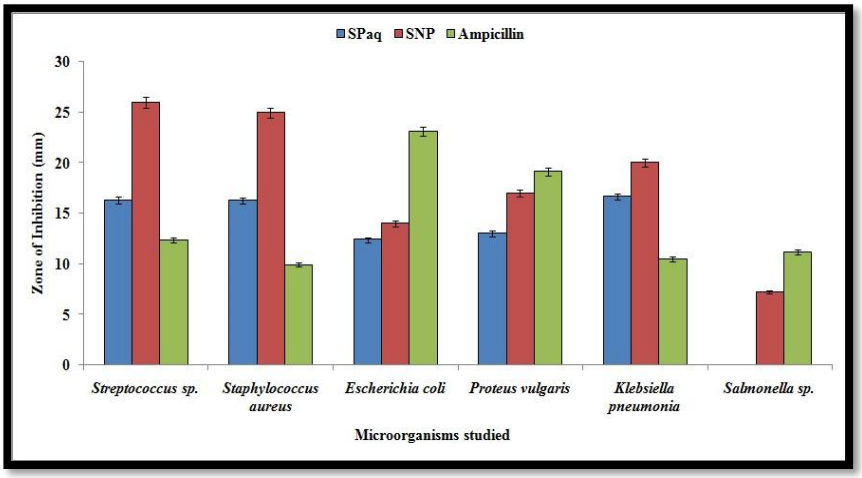



**Fig 6:** Antibacterial activity of silver nanoparticles synthesized using aqueous leaf extract of *Parthenium sp.* under optimized reaction conditions

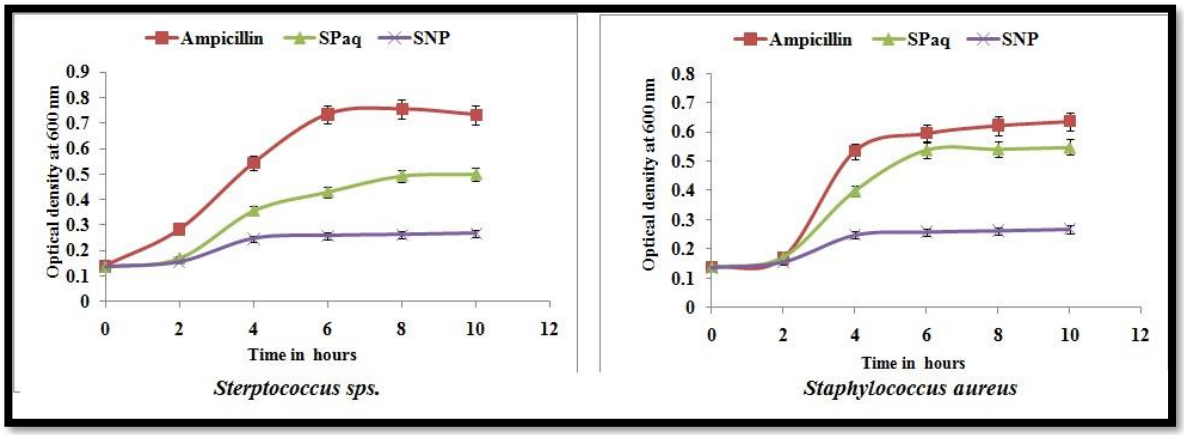



**Fig 7:** Growth curve analysis of silver nanoparticles synthesized using aqueous leaf extract of *Parthenium sp.* under optimized reaction conditions

## CONCLUSION

The present study concludes that *Parthenium sp* has capability to fabricate silver nanoparticles. The nanoparticles synthesized inhibited the growth of bacteria indicating that they can be potentially used as effective agents to control the growth of bacteria. The study also inferred that green synthesis is one of the simple fast, ecofriendly and low cost procedure and provides an alternative for treatment of bacterial infection and limitation of drug resistance too.

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