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Green Synthesis and Characterization of Silver Nanoparticles Synthesized Using Leaf Extract of *Passiflora Foetida* Linn

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Abstract: The silver nanoparticles (SNPs) are applied in nanomedicine, health, food safety and other applications. The topical methods are based on chemicals that are toxic in nature, thus to minimize the hazardous effects the natural sources need to be explored for synthesizing SNPs. The contemporary work reports the greener way of silver nanoparticles (SNPs) fabrication using phytochemical, functional groups and secondary metabolites from *Passiflora* aqueous leaf extract. The optimized condition for SNPs synthesis was 1 mM $AgNO_3$ 95ml + leaf extract 5 ml at alkaline pH 11 yields stable NPs. Various parameters such as temperature, pH, sunlight, dark, different concentration of $AgNO_3$ were evaluated. The UV-spectroscopic analysis confirms SNPs synthesis at 420 nm for sun light based, 428 nm at room temperature and 425 nm for microwave-based conditions. The FTIR determines alkanes, alkyne, amines, aliphatic amine, carboxylic acid; nitro-compound from the leaf extract have been used for reducing silver nitrate for SNPs synthesis. The SEM and TEM analysis confirms the 14.96 nm size SNPs under sun light condition shows antimicrobial activity against *E. coli* and *S. aureous* pathogens at 50 ug/ml concentration, which may have applicability to raised SNPs based green sustainable products in treating pathogens.

Keywords: Silver nano particle, *passiflora foetida*, UV spectroscopic analysis, FTIR, SEM, TEM, antimicrobial

1. Introduction

The silver nitrate is a solid compound called as "Lunar caustic," in English [1,2], which complexes as reducing agents for fabrication of metallic nanoparticles. The terminology nano refers by means of dwarf as manipulative synthesis of particles in nano scale dimensions (100 nm) such as coarse nano particles (10,000 - 2,500 nm), fine nano particles (2,500 - 100 nm), nano particles (100-1 nm) [3,4]. There are several sources for synthesis of nanoparticles such as, noble metals, i.e. silver, titanium dioxide, zinc oxide, platinum, gold and palladium. However, silver (Ag) is rare, basic and natural element possessing high electrical, thermal conduction, ductility and malleability [5-7]. It is used as nitrate to induce the antimicrobial effects, which makes it suitable for development into novel applications in curative, pharmaceutical, textile, paint, food and agriculture sectors [8-10]. Usually, the silver nanoparticles are printed or complex with other materials such as polysaccharides, proteins, biopolymer, hydrogel, to form nano emulsion, composite, dendrimer, liposome and warps for medical, food and agricultural applications [2].

The basic mechanism of action of the SNPs against the aerobic and anaerobic bacteria is still being explored [11]. However, the SNPs could be transported to the cytoplasm by endocytosis, accumulated with plasma membrane and release of free metals within surface of the bacteria. The Ag+, interact with phosphorus-containing compounds like DNA and inhibit their function. They aggregate on semi-permeable cell walls of bacteria and get accumulated through channels and carriers at membrane [12-15]. The silver nanoparticles (SNPs) are largely useful in various activities including anti-bacterial, anti-fungal, larvicidal, anti-inflammatory, antiplatelet, anti-angiogenesis and anti-viral activity [16-19].

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The silver nanoparticles formulation is being the most important aspect which uses sophisticated techniques and the type or form of SNPs formulated depends on the kinetics of reaction that relays on the adopted methodology. The NPs fabrication is the very critical process were environmental factor's temperature, pressure; light intensity, dark conditions play an important role. The action of silver on microbes is not fully understood [20] and no reported evidence to suggest humans are affected by using products containing SNPs. The nanoparticles toxicity on living organism was performed on some of the species, including bacteria, algae, invertebrate, for example, nematodes and crustaceans, vertebrates such as fish and rats [21,22]. Large numbers of available specialized methods that are used in the biosynthesis of SNPs are ultrasound, microwave, UV radiation, aerosol technologies, lithography, laser ablation, ultrasonic fields and photochemical reduction. All these methods require consumption of energy form of heat, electricity and high-priced chemicals, which are expensive and use hazardous chemicals. In addition, during NPs synthesis, the chemical and physical reduction of silver salts yields noxious chemicals affecting environment, plant, animals and humans. Therefore, the greener source used for the SNPs formulation have advantage of simple, ecofriendly, cost effective, less time consuming, reproducible, and produce zero contamination for environment [5-7].

Passiflora species have reported with significant medicinal properties and are used in the treatment of several diseases, such as insomnia, anxiety, and hysteria. It is also used in the treatment of tuberculosis, worms, coughs and colds [36]. The possible starting natural green source from *Passiflora foetida* (P. foetida), could be seed, leaves, flower bud, flower, fruit, tendril, stem, bracts and roots that possess antioxidant and antibacterial compounds are considered as the biological source for nanoparticle synthesis. The leaves of this plant have the potential anti-inflammatory, antioxidant, anti-helminthic (intestinal nematodes and flatworms), antidiabetic, analgesic and antibacterial potential. These activities are due to the phenolic, polyphenolic and flavonoids compounds. Therefor the aim of this study was to investigate an ecofriendly method that uses minimum energy, electricity, less time for synthesis of silver nanoparticles using the aqueous leaf extract of *P. foetida* to determine their activity against *E. coli* and *S.aureous* pathogens. Therefore, in this work, we show the potential of using Passiflora leaf extract as a green method to synthesize SNPs. This study uses a natural plant extract for nanoparticles synthesis and were characterized and their antibacterial susceptibility and efficacy was estimated against *E. coli* and *S.aureous.* The methods for SNPs synthesis have been explored that uses less energy for SNPs synthesis thus will likely contribute to the development of green, nontoxic formulation supporting greener sustainability.

2. Materials and Methods

The material required for silver nanoparticles formulation were 250 ml flask, 100 ml measuring cylinder, $AgNO_3$ from (Sigma-Aldrich). The instruments used were UV spectrophotometry (Shimadzu Dual Beam spectrometer, ModelUV-1650 PC), FTIR (Shimadzu, Japan) [23], Scanning electron Microscopic (SEM) and Transmission electron microscopic examination (TEM). Bacterial strains for study of antimicrobial activity were *Escherichia coli* (ATCC no.1105) and *S. aureus* (ATCC no. 6538). Sample collection the plant of *Passiflora foetida* has been collected from green house and garden of Department of Biotechnology, Sant Gadge Baba Amravati University, Amravati 444602, Maharashtra, India.

2.1 Silver Nanoparticle Synthesis Using Leaf Extract of *P. foetida*

The 10 gm leaf of *P. foetida* washed with tap water, dried at room temperature and chopped them into small pieces and boiled into 75 ml autoclaved ddH_2O for five min. The aqueous extract allowed to cool and filtered using cotton muslin cloth. The filtrate is set for centrifuge at 4000 rpm for 10 min. The filter supernatant is again filter by syringe filters and used for formulation of silver nanoparticles (SNPs). The 5 ml leaf extract was added dropwise into the 95 ml silver nitrate (1 mM), and pH adjusted to 11 using 0. 1 mM NaOH solution. The solution is observed for change in color from light green to dark brown.

2.2 Optimization of Parameters

There are several important parameters that are considered for optimization of SNPs fabrication, such as volume of extract, concentration of $AgNO_3$, temperature of reaction, pH of reaction and steering time effect. Varying extract compositions, the biosynthesis of the SNPs was carried out for different compositions of the extract and $AgNO_3$ solution in ratio of 1:99, 2:98, 3:97, 4:96, 5:95, 10:90 and 15:85 (v/v). Varying $AgNO_3$ concentration, several studies indicated that the 1 mM $AgNO_3$ is standard and produces SNP, thus in the current study, we have used 1 mM as standard for SNP synthesis [25]. Effect of pH, Once the silver nitrate 95 ml (1mM) and 5 ml leaf extract were mixed in a selected ratio at room temperature (27°C), and synthesis of SNPs performed at specified pH 5, 6, 7, 8, 9, 10, 11. Effect of temperatures, The effect of temperature at the rate of formation of SNPs was studied at- 20°C , 4°C , RT (27°C), 20°C , 40°C, 60°C , 80°C and 100°C. Effect of reaction stirring time, The reaction stirring time was studied for 0 min, 1 hr, 24 hr, 48 hr, 72 hr and 96 hr. Effect of light, In order to study the effect of light on synthesis of SNPs, the SNPs synthesis performed under sun light.

2.3 SNPs Synthesis

Microwave oven (MIC) assisted SNPs, the silver nanoparticle biosynthesis was carried out under microwave condition maintained at 100 W power. The reaction mixture was given quick burst of microwave for 5 min. The color change as well as absorbance of the reaction mixture was monitored spectrophotometrically for every 30 sec. Sonication (SON) assisted SNPs, The SNPs biosynthesis was carried out in sonicator 130 Watts; 20 kHz until the resultant solution changes from light green to dark black-red color and the UV-visible spectrum were recorded. Autoclave (AUT) assisted SNPs, The SNPs formation was carried out under autoclave condition maintained at 125°C and 5-7 lbs pressure. The optimized reaction mixture of $AgNO_3$ and leaf extracts was kept in the autoclave for 30 sec. Light source assisted SNPs, the silver nanoparticle biosynthesis was carried out under sun light (SUN).

2.4 Stability, Visual Examination and Separation of SNPs

The stability of SNPs was determined at room temperature using the UV spectrophotometer at intervals of days. The reaction mixture monitored for visual change in colour by naked eyes. The reaction mixture completes in 96 hr and SNPs are separated by using centrifuging at 13,000 rpm for 15 min. The dark-brown color SNPs forms colloidal pellets at bottom. The same process repeated by dispersion of SNPs pellets in water, to obtain colored supernatant solutions, that are discarded. The pellets were dried and powdered was used for characterization.

2.5 Characterization of Silver Nanoparticles

The synthesized SNPs were used for characterization by various available techniques. The FTIR is used to detect the functional group that has taken part in reducing and capping a surface of SNPs. The shape and size of synthesized SNPs was determined by using SEM and TEM. The samples for SEM and TEM were prepared by depositing a drop of colloidal solution on an aluminium grid sample holder and allowed to dry at RT.

(a) UV-Visible Spectroscopy

UV spectroscopy is the primary instrument used for characterization of SNPs. The formation of SNPs by reduction of silver ions to nano particle is monitored by measuring UV-Vis spectra of mixed $AgNO_3$ extract solution diluted with 1000x autoclaved ddH_2O. Autoclaved ddH_2O was used as blank for auto-zero and baseline correction and spectrophotometer was operated at a resolution of 190-1100 nm [23].

(b) FTIR Instrument

The FTIR was operated in ranged from 4000 to 450 cm^{-1} at a resolution of 4 cm^{-1} by making a KBr pellet with AgNPs. Latest FTIR spectroscopy instruments use a pinch of material to place on sample holder and arm needle is placed over it and readings are noted for functional group that is identified by standard IR absorption frequencies [24].

(c) Scanning Electron Microscopy (SEM)

The liquid colloidal SNPs solutions were dried in powder and send to SAIF Indian Institute of Technology, Bombay for SEM analysis. SEM analysis was done using Carl Zeiss SEM-EVO18 with accelerated voltage of 130 kV. The morphology and size of silver nanoparticles were investigated using SEM specific software.

(d) Transmission Electron Microscopy (TEM)

The liquid colloidal SNPs solutions were dried in powder and send to SAIF IIT Bombay for TEM analysis. The sample was dried on a copper grid covered with a conductive polymer. The 25 µl of sample was applied to check size and shape of SNP synthesized using *P. foetida* leaf extracts was visualized using 200 kV ultra-high resolution TEM (JEOL-2010).

2.6 Antimicrobial Analysis of SNPs

The antibacterial activity of silver nanoparticles synthesized using fresh aqueous *P. foetida* extracts was determined using well diffusion method [25]. The 100 ml nutrient broth (NB) medium was prepared and poured in six test tubes, in order to inoculate two test bacteria. The tubes were sterilized by autoclaving at 121°C for 15 minutes at 15 psi pressure. Further, bacterial strains were inoculated and incubated at 37°C for 24 hours. The Mueller-Hinton Agar (MHA) 40 ml was poured aseptically into each aseptic Petri dish and allowed to solidify at room temperature. The test bacteria were spread on the MHA agar petri plates using sterile swabs. Sterile wells 6 mm diameter were made with the help of an antiseptic corn borer. Then 20 µl of control Dimethyl Sulfoxide (DMSO) and sample (SNPs+ DMSO) in various dilutions of SNPs (12.5, 25, 50, 100 µg/ml) were incubated at 37°C for 24 hours. Next day, zone of inhibition in mm was measured by using a scale.

3. Results and Discussion

The green synthesis of silver nanoparticles using leaf extracts of *P. foetida* was carried out in the present study. The procedures adapted for SNPs synthesis was optimized, used for its characterization and to study its antimicrobial property.

3.1 Phytochemicals Present in *P. foetida*

The phytochemical and secondary metabolites that are found in *P. foetida* extract are alkaloids, phenolic compounds and tannin's carbohydrates, glycosides, flavonoids, proteins, steroids and tannins. Polyphenol and flavonoids could be responsible for capping and reduction of silver ions [26].

3.2 Visual Conformation of SNPs

The mixing of 95 ml silver nitrate (1 mM) and 5 ml leaf extract of *P. foetida* results in change in color of solution. A color changes from light green to black can be visible by eye. The color intensity of synthesized SNPs increased with duration of incubation (Fig. 1). The extract color became deep white after 1 min, light-brown color appeared after 2 min of incubation and deep reddish brown after 5 min. After 1 hr there is appearance of dark brown-black color, which indicates the formation of silver nanoparticles. It was observed that the reduction of silver ions reaches saturation at 1 h of incubation. The complete process of green synthesized SNPs under SUN is shown in a diagrammatic form in Fig 2. The silver nanoparticles synthesized with 5 ml of leaf extract of *P. foetida* and 95 ml of 1 mM silver nitrate with pH 11 under various experimental conditions were used for further optimization.

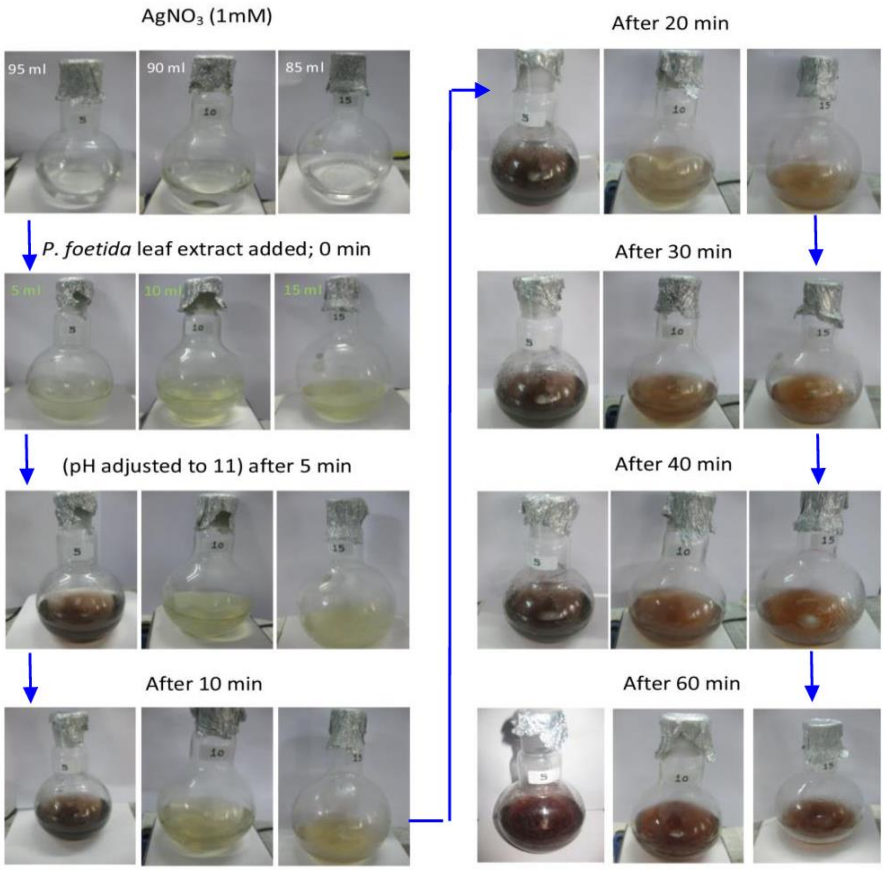

Fig. 1 - Visual examination of silver nanoparticles synthesis observed by color change at a different time intervals from 0 min- 60 min. The leaf extract of *P. foetida* (5, 10, 15 ml) and 1 mM $AgNO_3$ (95, 90, 85 ml)

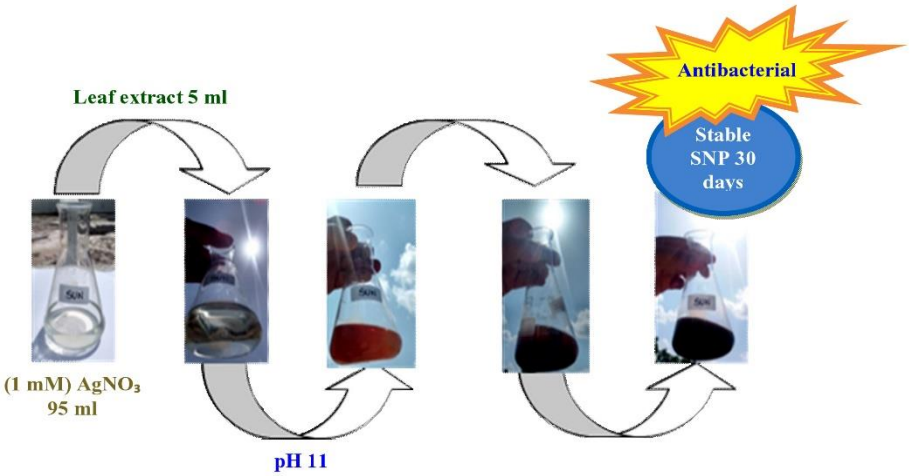

Fig. 2 - The complete process of green synthesis of silver nanoparticles under sun light condition. From left to right: The leaf extract (*P. foetida*) light green color used for reduction of silver salt. The desired pH of mixture is adjusted to 11 and synthesized stable silver nanoparticles

3.3 Ratio of Volume of Extracted $AgNO_3$

The time taken in the formation of silver nanoparticles was found to be less for 5 ml of extract and 95 ml of 1 mM $AgNO_3$ solution, thus it was considered as standard.

3.4 Effect of Leaf Extract Quantity

The concentration of leaf extracts influenced the size of SNPs. Here, different volume 1, 2, 3, 4, 5 ml of leaf extracts were studied and found that 5 ml is efficient for reduction of silver nitrate to zero valent silver ions.

3.5 Effect of $AgNO_3$ Concentration

The $AgNO_3$ concentration was considered for SNPs fabrication. Generally, a minimum amount of silver salt concentration is used to avoid the issue of toxicity. The experimental phases use $AgNO_3$ in range of (0.1 mM – 1 mM) and 1 mM $AgNO_3$ was found standard to produced SNPs.

3.6 Effect of Stirring Time

In the present study, boiling method prepared extract found to reduce silver to nanoparticles speedy as compared to the extract prepared without boiling this may be due to greater kinetics in case of boiling method. Heating the reaction mixture increases the kinetics of components and produces SNPs. Thus, some plants quickly produced SNPs in 1 min and other may take 24 hrs, 48 hrs, 72 hrs and 96 hrs to complete a reduction process. The stirring time is dependent on reaction mixture acidity, basicity, temperature, reducing power of extract, light intensity, enzyme and secondary metabolites of extract [30]. In present study silver nanoparticles are produces at 1 hr stirring time.

3.7 Effect of pH on SNPs Synthesis

Once the silver nitrate and leaf extract were mixed in an optimized ratio, out of tested pH after 5 minutes, pH 11 was found to produced SNP in no time. The SNPs formation was observed by taking UV-spectra in range of 400 nm-450 nm range. The alkaline pH environment enhanced the reducing and stabilizing capability of the antioxidants presents in the *P. foetida* leaf extract. The preliminary UV spectroscopic confirmation for SNPs synthesis using 5 ml leaf extract + 95 ml of 1 mM $AgNO_3$ was done at various time intervals of 72 hrs are shown in Fig. 3(A).

3.8 Effect of Temperature on a Biosynthesis of SNPs

Fig. 3(B) shows spectroscopic analysis at - 20°C, 4°C, RT (27°C), 20 °C, 40°C, 60°C, 80°C and 100°C on synthesis of SNP at time interval of 72 hr. The synthesis of nanoparticles was increases while elevating the reaction temperature by using the leaf extract of *P. foetida*.

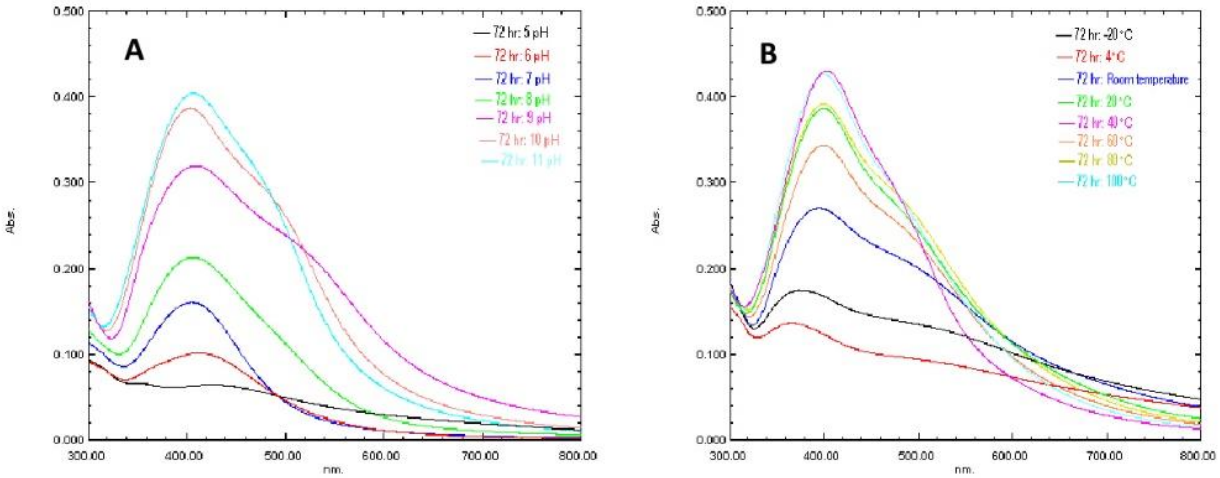

Fig. 3 - Spectroscopic analysis of synthesized silver nanoparticles; (a) at pH range (5-11); (b) at different temperature - 20°C, 4°C, RT (27°C), 20 °C, 40°C, 60°C, 80°C and 100°C

3.9 Stability of SNPs

The UV-visible spectrum for the biosynthesized SNPs was recorded over a period of time for 15 and 30 days. There was a little decrease in the UV visible spectrum of sun light. However, the biosynthesized nanoparticles from sun light conditions were found to be lasting for 60 days at pH 11 without any sign of precipitation and without any change in λmax value. The stable SNPs was colloidal in nature for use in its various applications such as hydrogel, earthen pots, clothing and medical utensils.

3.10 UV-Vis Analysis of SNPs

The UV-visible spectra show an absorption band at 421 nm which corresponds to the absorbance of silver nanoparticles. The synthesis of SNPs revealed that the sun light method of production of the AgNP's was efficient and complete in 1 minute.

3.11 FTIR Analysis of SNPs

The powder of SNPs was used for FTIR analysis which confirm alkanes, alkenes, alkyne, amines, aliphatic amine, aromatic amines, 1° amines, α β unsaturated esters, carboxylic acid from the aqueous leaf extract to reduce silver nitrate to produce zero valent silver NPs. Fig. 4 shows the FTIR analysis of; (A): *P. foetida* fresh leaf extracts, and (B): synthesized SNPs. The conformational states and chemical changes between silver nitrate and leaf extract is studied through the modifications in the vibrational spectra in FTIR.

3.12 SEM Analysis of SNPs

Experimental SEM image results showed that the diameter of the prepared nanoparticle is about 7-26 nm and the shape is not definite, relatively looks like mountain rocks particles. SEM micrographs of the synthesized silver nanoparticles using the extract of leaves of *P. foetida* fabricated under microwave oven, sun as 2 µm and room temperature 10 µm is shown in Fig. 5. The microwave oven SNPs looks like coral reef, sun SNPs are like small rocks and RT SNPs are large rock mountains type. The particle size was found to less than 50 nm in all three experimental conditions.

3.13 TEM Analysis of SNPs

TEM images of SNPs recorded after 60 days of reaction time. Fig. 6 shows transmission electron micrographs of silver nanoparticles synthesized by *P. foetida* leaf extract under microwave oven, SUN and RT conditions with their respective electron diffraction pattern. The TEM results show that the SNPs is smaller than 36 nm in most of the cases. It can be seen that the average size of the SNPs was around 13.60 nm for microwave, 14.96 nm for Sun, 31 nm at RT with indefinite spherical shape. The silver nanoparticles seen to be surrounded by a faint layer of capping organic material from *P. foetida* extract.

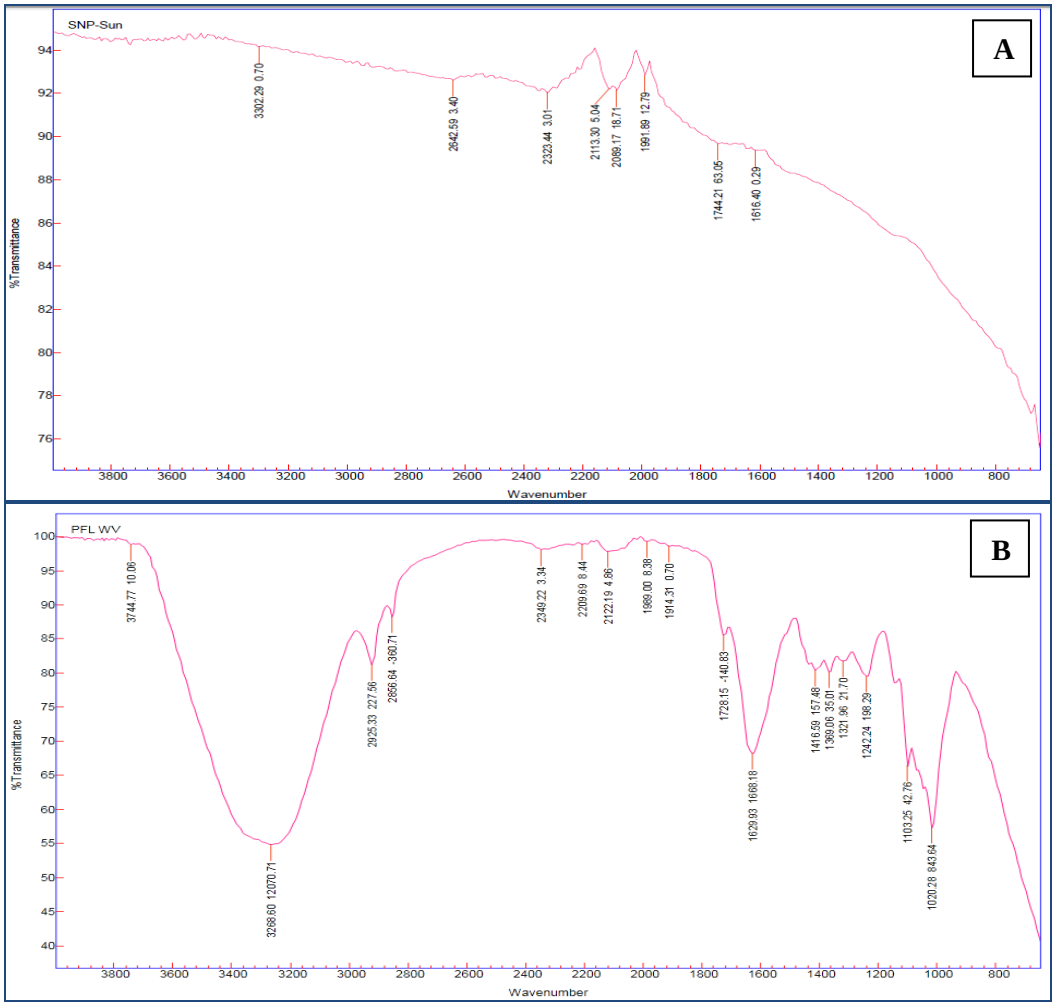

Fig. 4 - FTIR analysis of; (a) *P. foetida* fresh leaf extract (before reduction), and (b) synthesized silver nanoparticles (after reduction)

3.14 Antimicrobial Activity of SNPs

Antimicrobial activity of biosynthesized silver nanoparticles was examined on, *E. coli* and *S.aureous*. In the agar well diffusion method was Amikacine sulfate 10µg/ml was used as +ve control and DMSO as negative control. Fig. 7 shows bar diagram of antimicrobic activity of SNPs fabricated from *P. foetida* leaf extract against *E. coli* and *S.aureous*. We have confirmed the water-soluble functional groups such as alkanes, alkenes, alkyne, amines, aliphatic amine, aromatic amines, 1° amines, α β unsaturated esters, carboxylic acid, alkaloids, phenolic, carbohydrates, glycosides, flavonoids, proteins, steroids and tannins from the aqueous leaf extract reduces silver nitrate to produce zero valent silver NPs.

In literature, few reports suggest the use of potential *P. foetida* for nanoparticles synthesis with few publications for silver nanoparticles using aqueous leaf extract of *P. foetida*. However, recently [26] used aqueous leaf extracts of *P. foetida* for green synthesis of iron oxide nanoparticles. They found phenolic, alkaloids, and tannins to be very effective reducing and stabilizing agents for NPs based on phytochemical results. Similar to our work [27] performed SNPs synthesis using carob leaf extracts 5 ml to reduce 1 mM silver nitrate solution 100 ml for 10 min. They followed boiling of leaf extract, same followed as leaves cut into small pieces and boiled in 200 ml water for 10 min, but we have used 10 gm in 75 ml water. The optimization parameters such as volume of extract, concentration of $AgNO_3$, temperature of reaction, reactions stirring time and pH are very crucial parameters for optimization. The size of nanoparticles differs upon change in variables, increasing concentration of plant extracts leads to decrease in size of particles. The volume ratio of extract to $AgNO_3$ effects the SNP formation, we have tried leaf extracts (1, 2, 3, 4, 5, 10, 15 ml) and found 5 ml as best rather than 10 or 15 ml. In contrast, [28] used 10 ml of leaf extracts from *Ocimim Basillicum* for reduction of $AgNO_3$.

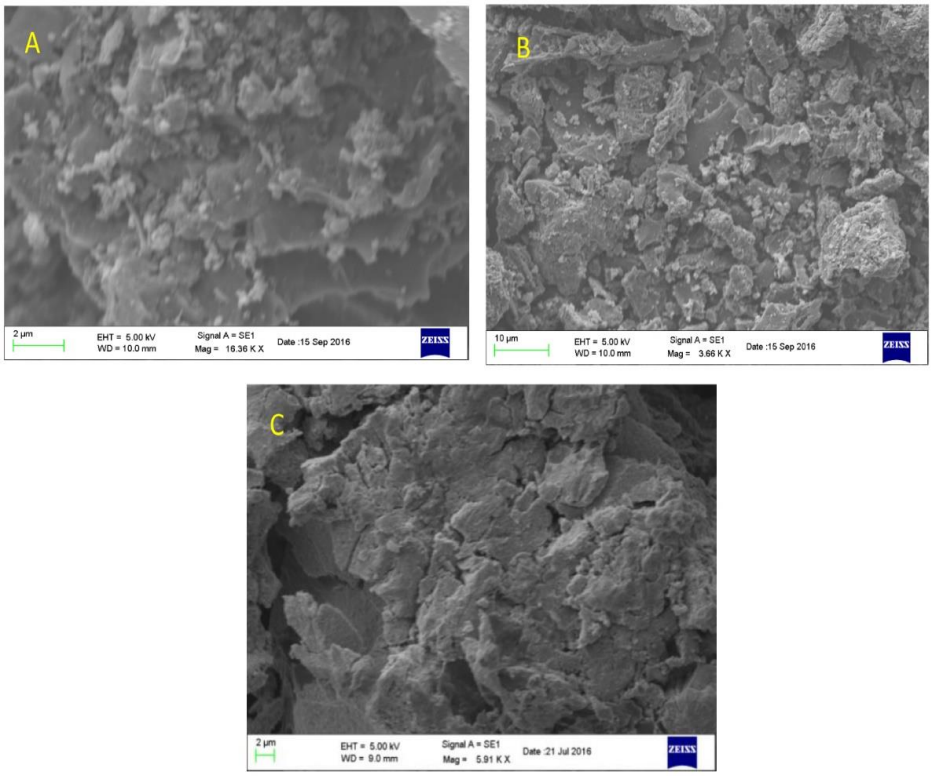

Fig. 5 - SEM micrograph showing surface morphology of the silver nanoparticles synthesized using leaf extract of *P foetida* under; (a) microwave oven; (b) sun light; (c) room temperature

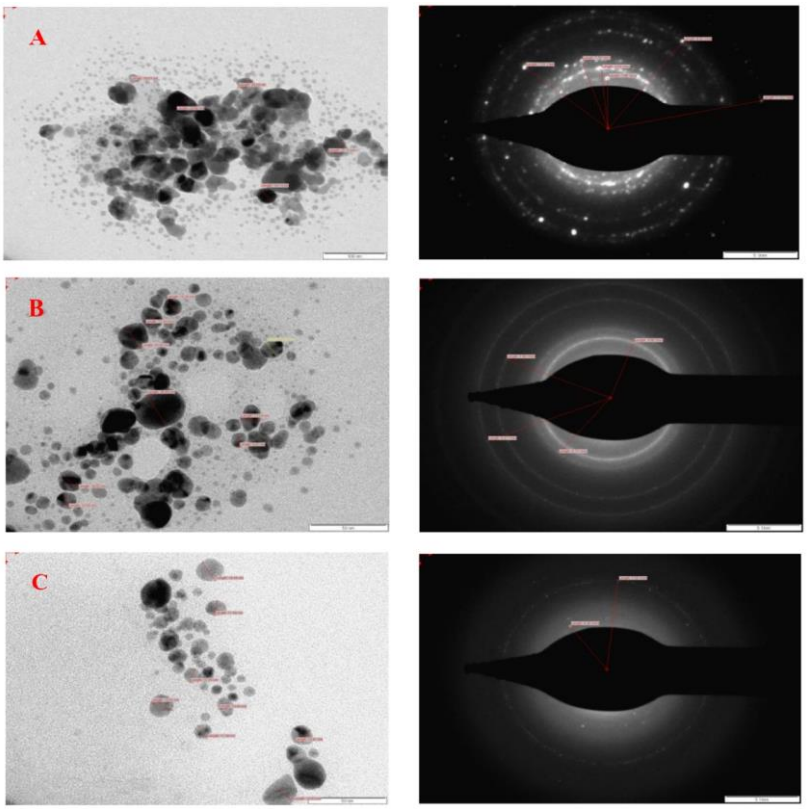

Fig. 6 - Transmission electron micrographs and selected area electron diffraction pattern (SAED) of silver nanoparticles synthesized by *P. foetida* leaf extract under; (a) room temperature; (b) sunlight; (c) microwave oven conditions

The [29] synthesis SNPs of 2-6 nm with the surface plasma resonance at 449 nm using 5 gm of Cycus leaf in 200 ml of 50 % ethanol in boiling steam bath. In contrast to this, our optimized sunlight induced SNPs synthesis is devoid

of any such chemical solvent eg. ethanol and steam bath or consumption of energy/ electricity thus very simple and cost effective, which produces SNPs of 31 nm. The 1mM $AgNO_3$ was optimized and used in experiments similar to report of [28]. However, the reports by [3] indicate the use of 100 mM, 50 mM for SNPs production. We observed that in low pH (5, 6, 7 pH), small with broadening SPR band was formed that's mean formation of large size of nanoparticles. The particle size is expected to be greater in an acidic medium than in the basic mediums [25]. However, alkaline 11 pH show a narrow peak at 425 nm which indicates a formation of spherical shape of silver nanoparticles.

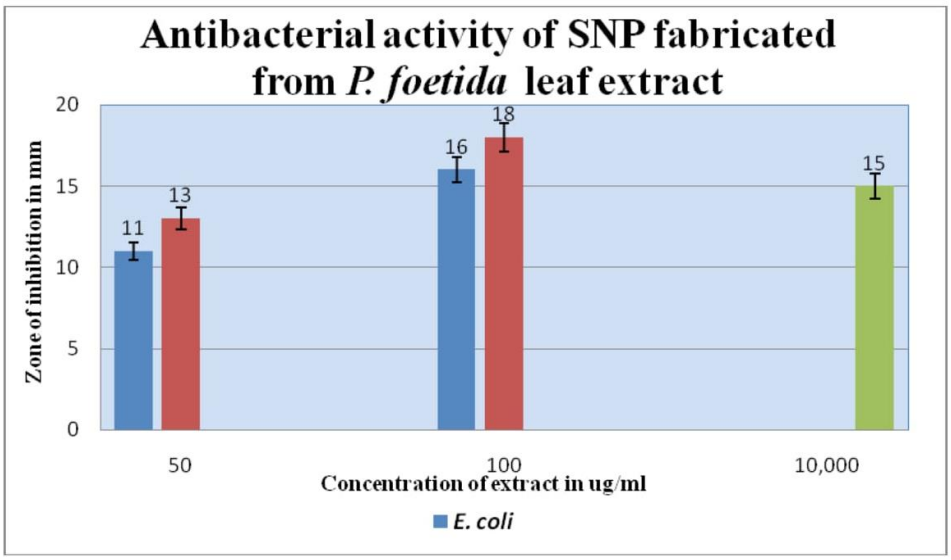

Fig. 7 - Antimicrobial activity of SNPs fabricated from *P. foetida* leaf extract against *E. coli* and *S.aureous*

We have studied temperature effect and the absorbance at the absorption maximum was measured spectrophotometrically [25]. At high temperature 100°C narrow peak was obtained which indicates small size SNPs with the higher rate of reduction of silver ions. In comparison of all, the SNPs was formed in 30 minutes at room temperature without adjusting any pH. In contrast, the SNPs were formed within 1 minute of pH adjusted at 11 at 27°C however, at 40°C the SNPs are formed at 1 minutes and under 60°C and 100°C conditions it was formed within seconds. Hence higher temperature favors the formation of SNPs. The absorbance band was broadened and positioned at 550 nm and 350 nm at the temperature of 20°C, 40°C, 60°C and 100°C. It was observed that the absorbance peak become sharper and band narrow with an increase in temperature.

If the leaf extracts carry abundance of phytochemicals or secondary metabolites, SNPs formation takes in the faster way. We have optimized steering time for RT ie. 60 min, for microwave is 30 sec and sunlight within 30 sec. However, [30] studies the effect of stirring time on SNPs prepared to use glutathione as reducing agent, it produces SNPs of stability at 72 hr stirring time [25]. The SNP synthesized at room temperature takes more time than sunlight. The microwave method also produced SNP in less than 30 sec confirmed by UV- spectroscopic analysis, SEM and TEM. The [31] results support our work that got same results while performing green synthesis using 25 ml leaf extracts 100 ml of 0.1 mM $AgNO_3$ solution in a microwave oven at 180 watts for 30 minutes. It was observed that, the rate of formation of SNPs was higher under microwave condition, which is found to be 30 sec with a sharp absorption peak and is the most stable at 72 hr. The sun assisted SNPs are found to produce quick and are of smaller size. This may be due to the light induced enzyme that may be present in extract, which get activated upon receiving sun light elevating kinetics.

The [16] uses Kiwifruit juice and stability of SNPs was determined at room temperature using the UV spectrophotometer at an interval of 24 hr, 48 hr, 72 hr, 96 hr and 30 days. The same absorbance of UV at a longer time means firmer NP. The SNP synthesized by RT, sunlight and microwave, autoclaves are found most stable. However, in order to select method that uses less or no energy input and a simple process; we used sunlight induced SNPs for final selection. It was observed that the initial color of silver nitrate treated *P. foetida* extract turned from light to dark brown of the reaction after 5 min at RT, 30 secs at microwave and within 30 secs in sunlight, which indicates the formation of silver nanoparticles. The instant color change could be due to reaction between Ag ions and secondary metabolite solution that resulted in faint white color of solution. Placing the reaction solution in bright sunlight reduced the Ag ions. Therefore, the color of the solution changed into dark brown, which indicated the formation of SNPs as reported in our previous study of [28] were they added extract of *Ocimum basillicum* to silver nitrate solution results in dark-brown color from yellow.

If the SNPs not stabilized, become heavy and will be seen at bottom of flask or beaker. The aggregation problems can be sorted by sonicating the SNPs solution. Usually, NaCl Salt hides the charges allowing the particles to clump

together to form aggregates. A new broad peak may appear around 350-525 nm along with a decrease in the intensity of the plasmon absorbance. A well dispersed SNPs solution shows an absorption peak at 425 nm with no evidence for aggregation [3], which was observed in case of *P. foetida* extract at pH 11, Sun, sonication, RT and microwave oven conditions derived SNPs. Similarly, observed in [33] with maximum absorption in range of 425 to 475 nm. The SNPs from *P. foetida* secondary metabolites show characteristic absorbance between 320 and 550 nm, were as [28] show absorption in 300 nm-380 nm. The [16] observed weak peak at 415 nm at RT due to prolonged reaction time and lead to wider SNPs size than of boiling water that we used in extract preparation.

The FTIR spectra of *P. foetida* extracts, and their corresponding synthesized silver nanoparticle showed shifting in peaks along with appearance and disappearance of absorption bands in the FTIR Spectrum. The major shifts in band intensities were observed at 3268–2856, 2349–2122, 1989–1728, 1629 cm-1, when exposure to IR leads to change to 3302, 2642, 2323, 2113, 1991, 1744, 1616. The [25] found FTIR spectrum of Broccoli extracts and synthesis of SNPs spectrum shows peaks at 3424, 2924, 2856cm, 2333, 3433, 1625, and 1383cm^{-1}. The plain extract of *P. foetida* shows a strong peak at ~3268, 2925, 1629, 1242 and 1103 cm^{-1} indicating the presence of alcohols and phenols group, alkanes, aliphatic amines, 1° amines groups respectively. The peaks at 2209.69 cm^{-1} and 2122.19 $^{cm-1}$ indicate the presence of alkynes group (C≡C) compounds. The peak 2122.19 $^{cm-1}$ in extract is displaced in formed SNP to 2113.30 thus alkynes could be a strong contented. The peak 2856.64, 2349.22 and 1728.15 $^{cm-1}$ from extract is disappeared and formed SNP show peaks at 2642.59, 2323.44 and1744.21 $^{cm-1}$ respectively. The corresponding groups are alkanes, carboxylic acids and α β unsaturated esters. This indicates water soluble C–H stretch, O–H stretch, C=O stretch groups present in the *P. foetida* extract gets adsorbed on the surface of Ag+ and performs catalytic reduction of Ag+ to Ag0 nanoparticles. The other groups that are located are aromatics, alkenes, aromatic amines and alkanes. The [26] also confirmed the involvement of alcoholic or polyphenolic stretching, carboxyl, conjugated carboxyl, aliphatic, aromatic, methyl, methylene and methoxy groups for SNP synthesis using FTIR in related Passiflora species, i.e. P. tripartita whose pigments are composed of glucose, C-glycosides and O-glycoside derivatives that involved in reducing the Ag^{+} to Ag^{0}.

TEM images reveal that the *P. foetida* silver nanoparticles were indefinite and spherical in morphology, which correlated to the results of [25]. Interestingly, the size of *P. foetida* reduced silver nanoparticles were found to range from 6-36 nm under SEM and TEM observation. Similar to the observation of [26]. The antibacterial property of silver nanoparticles has been investigated against *S. aureus*, and *E. coli*. The [32] uses agar well diffusion method for evaluation of antimicrobial activity at 12.5, 20, 50, 100 ug/ml concentration, similarly we have used same concentration of sunlight induced SNPs against *E. coli* and *S. aureus* and detected that 50 ug/ml as MIC with 11 mm and 13 mm of zone of inhibition respectively. [33] studied green synthesis, antimicrobial activity, minimum inhibitory concentration against *S.aureus* 23 mm and *E. coli* 20 mm. The TEM and SEM analysis show dominance of spherical size of 200 nm particles with inhibition property against *E. coli* culture [34]. Banana SNPs 14 mm, Neem SNPs 12 mm and Tulsi SNPs shows 14 mm MIC against *E. coli*. Therefore, These SNP could be complexed with the other products such as film or composite to have specific application [35].

4. Conclusion

The water-soluble compounds present in leaf extract of *P. foetida* are efficient for fabrication of silver nanoparticles. Among several tried methods, the sunlight induced silver nanoparticles are more efficient. UV-spectroscopic analysis confirms at 420 nm for sunlight-based SNPs, 428 nm for room temperature and 425 nm for microwave-based SNPs. FTIR determines alkanes, alkyne, amines, aliphatic amine, carboxylic acid; nitro-compound from the leaf extract have been used for reducing silver nitrate to produce zero valent silver NPs. SNPs was successfully fabricated at alkaline pH 11, and acidic pH was unsuitable for stable Ag-NP synthesis. The parameters such as the leaf extract volume [5ml], the [pH 11], effect of temperature [100°C] were successfully optimized for production of stable NPs. The SEM and TEM analysis confirms the size SNPs as 13.60 nm for microwave, 14.96 nm for sunlight, 31 nm at room temperature. The sunlight-based SNPs was selected and used for antimicrobial test against *E. coli* and *S.aureous* pathogens and MIC was found to be 50 ug/ml concentration. SEM analysis clearly figured coral reef, small rocks and big rock mountain like morphology of microwave oven, sunlight and room temperature and the particle size was found to less than 50 nm in all three experimental conditions. This sunlight induced SNPs synthesized using plant extract could be integrated in pharmaceutical applications.

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