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MICROWAVE-ASSISTED GREEN SYNTHESIS OF SILVER NANOPARTICLES USING EXTRACT OF *SPONDIAS PINNATA* BARK

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ABSTRACT

Formation of silver nanoparticles with irradiation of microwaves using biological agents such as plant extracts is one of the eco-friendly green synthesis approaches which is economical in comparison with the physical and chemical methods. This study is focused at preparation of silver nanoparticles silver using *Spondias pinnata* bark extract by microwave irradiation and its comparison with aging method. Formation of silver nanoparticles was identified by observing the surface plasmon resonance absorption band peak at ~432 nm by UV-visible spectrophotometer. Optimization of the synthesis procedure was carried out using different amount of extract, concentration of substrate and microwave irradiation time and morphological features were characterised. A solution containing 8ml plant extract and 8 mM silver nitrate exposed to 90 sec microwave irradiation time was identified as optimised condition for the production of spherical shaped silver nanoparticles with a size of 34.52 nm which indicates that faster formation of silver nanoparticles compared to the aging method. Present research shows the importance of optimization of bio reduction conditions and the amount of reactants required for regulation of the morphology of silver nanoparticles as well as to obtain homogenous solution which is the main problem encountered in plant- mediated production of silver nanoparticles.

Keywords: Silver nanoparticle, microwave, optimization, *Spondias*, characterization, extract

INTRODUCTION

Nanoparticles have distinct property of size in nano range and large surface area, which change their chemical and physical properties making them better than their bulk counterparts with respect to their activity (**Iravani *et al*., 2014**). Among various nano formulations, silver nanoparticles (AgNPs) has gained more attention due to their prominent antimicrobial activity and finds its application in different areas such as cometic industry, pharmaceuticals, in refining of water, medical devices, clothing, agriculture, agriculture and in water treatment **(Loo *et al.*, 2012; Krishnakumar *et al*., 2014; Jaiswal *et al*., 2010; Awwad *et al*.,2013).** Silver nanoparticles are commonly prepared by other methods which include evaporation-condensation as their common technique and make use of various reducing agent for reduction of silver ions respectively, for the synthesis of stable silver nanoparticles. However, above-mentioned methods have drawback that it requires high energy and makes use of dangerous reagents. These problems can be avoided by using green synthesis method which involves synthesis of nanoparticles by making use of plant-based extracts. Plant intervened biosynthesis of silver nanoparticles is eco-friendly approach because plant extracts contain natural reducing/capping agents such as terpenoids, alkaloids, amino acids, vitamins, polysaccharides, proteins, tannins, saponins and phenolics, hence avoiding the usage of toxic reducing agents. In addition, plants are easily available and the process of preparation of extract is cheap, simple; making them cost-effective. For nanoparticle synthesis, use of phytoextracts is more beneficial than other biological agents because usage of radiation, maintenance of temperature and microbial strains, media for microbial growth are not necessary, avoids the chances of contamination during preparation and usage. Further, Plant mediated synthesis is carried out at biological pH and due to milder reaction conditions, it offers better handling and control, resulting in more stable nanoparticles (**Nath et al., 2013, Borase *et al*., 2014 , Iravani.,2011).**
In past years, microwave-assisted production of nanoparticles has set up itself as a cutting-edge method for producing distinctive nanomaterials. By utilizing microwave irradiation, it is possible to synthesize nanoparticles with accurate parameter control in less time and furthermore change in characteristics and size of nanoparticles as required. In comparison to conventional heating methods, microwave irradiation has several benefits in the synthesis of nanoparticles such as quick, with least usage of energy, with controlled rate of heating, even distribution of heat, economical and increased yield of synthesis, which reduces growth and avoids agglomeration of the nanoparticles (**Ahmadi *et al*., 2018**).
The plant *Spondias pinnata* (Family: Anacardiaceae) is a small, aromatic tree and fruits, leaves, bark of this plant are used as astringent, antidysenteric, antiseptic, antiscorbutic. The bark is employed for the treatment of dysentery, diarrhoea including its use as an antiseptic, tonic, astringent by different traditional system of medicine in India. Bark paste applied externally to articular and muscular rheumatism. The stem bark of the plant is reported to contain phytoconstituents like alkaloids, flavonoids, and polyphenols **(Bora *et al*., 2014**).
One of the problems encountered during the synthesis of silver nanoparticles is attaining the required dimension and size and this can be achieved by regulation of production variables. In addition, the quantity of reducing agents and reactants also influence the stability of nanoparticles.
Hence, present study is done to find out the potential of *Spondias pinnata* bark extract in reducing the silver ions to form nanoparticles.
Silver nanoparticle synthesis was done with the aid of microwave irradiation. In addition, for successful production of silver nanoparticles with required characteristics such as smaller size, spherical shape and higher stability, the reaction parameters such as amount of *Spondias pinnata* bark extract, amount of silver nitrate ($AgNO_3$) concentration and period of exposure to microwave time are optimised.

MATERIALS AND METHODS

Materials

Procurement of Silver nitrate (ACS reagent, ≥99.0% was from Sigma Aldrich chemicals, India and distilled water used for the study.

Preparation of *Spondias pinnata* bark Extract

Stem bark of *Spondias pinnata* were collected from local areas of Mangalore, authenticated at St. Aloysius College, Mangalore and a voucher specimen was kept at the Pharmacognosy Department, NGSM Institute of Pharmaceutical Sciences.

Plant material was cleaned with distilled water, dried and then powdered. 50g of powdered material was treated with 250 ml of double distilled water which was then boiled for 15mins which is called as hot percolation method (**Sahayaraj *et al*., 2011**). The bark decoction was cooled , filtered (**Maria, *et al*., 2015).**

Synthesis of Silver Nanoparticles (*Spondias*-AgNP) and its Optimisation

Production of silver nanoparticles using microwave was carried out in domestic microwave oven (CATA-R). 50 mL solution of silver nitrate (8mM) was taken in round bottom flask and *Spondias pinnata* bark extract (8 ml) was added and subjected to microwave irradiation of 90 seconds at 800W power and 2450MHz frequency. The resulting mixture was centrifuged for 10mins at 1000rpm, upper solution was removed and using distilled water nanoparticles were dispersed . The same procedure was adopted for optimization of nanoparticle synthesis process using different quantities of *Spondias pinnata* bark extract (1, 2,4,6 and 8mL), concentration of $AgNO_3$ (1, 2, 4, 6 and 8mM), and microwave irradiation time (30, 60, 90 and 240 sec) by keeping other parameters constant. The formation of silver nanoparticles takes place by bottom to top approach by assembly of atoms to form new nuclei which grows to form nanosized particle. After the addition of plant extract to the silver nitrate solution, phytoconstituents in the extract acts as reducing agent causing the reduction of silver ions which can be checked by subjecting to UV- Visible spectral analysis at different time intervals. UV- Visible spectroscopy will show a surface plasmon resonance (SPR) peak centred between 400-480nm for spherical silver nanoparticles based on the size and surrounding liquid medium and can be identified by changes in colour of solution to dark brown from light brown (**Sahayaraj *et al*., 2011)**
Producing silver nanoparticles by aging method involves synthesis at room temperature in which the reaction of silver nitrate and plant extract will be carried out for a duration of 24 hrs followed by UV-Visible spectrophotometric analysis at a time interval of 30 minutes.

Characterization of *Spondias*- AgNP

UV- Visible spectroscopy

Silver nanoparticles were analysed using UV- Visible spectrophotometer (UV 5704S from Electronic, India Ltd). The absorbance spectra were recorded at the wavelength region of 300-600nm for microwave assisted *Spondias*-AgNP solution obtained under various experimental conditions with varying amount of plant extract, concentration of $AgNO_3$, time of irradiation by keeping other parameters constant which is shown in Figure 1-3. Absorbance spectra was also recorded for silver nanoparticles solutions prepared by aging method which is given in Fig.4. The surface plasmon resonance (SPR) peak for different samples were observed and the nature of the peak indicated the morphological feature of nanoparticles.
Scanning Electron Microscopy: In order to observe the surface morphological features, the optimised silver nanoparticle formulation was subjected to Scanning electron microscopic analysis by using SEM (Jeol, JSM 6390LA).
Transmission Electron Microscopy (TEM): TEM was carried out specifically to determine the size of nanoparticles. TEM was done using JM 2100 (JEOL, Tokyo, Japan) transmission electron microscope.
Fourier Transform Infrared [FTIR] spectroscopy: FTIR spectrum of *Spondias*-AgNP obtained by microwave irradiation at optimised condition was recorded to find out the functional groups of plant extract responsible for reduction, stabilization of nanoparticles. Samples were prepared by KBr pellet method and spectrum was obtained using Bruker FTIR spectrophotometer which showed characteristic absorption bands corresponding to different functional groups.

RESULTS AND DISCUSSION

Visual observation and UV- Visible Spectroscopy

Formation of silver nanoparticles were observed by colour change, followed by UV–visible spectral analysis. Spectrum obtained by UV-Visible analysis is the primary method which indicates the bio reduction of silver to silver nanoparticles and in addition, gives the information about the structural changes of the nanoparticles **(Shankar *et al*., 2017).**
Production of nanoparticles was identified visible by change in colour of solution from light brown to either dark brown or black when the *Spondias pinnata* bark extract made to react to silver ions. SPR is caused by the excitation of free electrons which is indicated by the colour change of the solution. Metal nanoparticles shows electromagnetic absorption wave in the visible range due to SPR (**Barabadi *et al*., 2015**). SPR peaks play an important role in determining the dimension, morphology of silver nanoparticles **(Aromal *et al*., 2012)** and based on the parameters such as size of nanoparticles, shape, aggregation status, nature of medium, the SPR shows red or blue shift (**Shankar *et al*., 2017; Tripathy *et al*., 2010).**
In the present study, intensity of absorption peaks and intensity of colour was found to be enhanced with the rise in exposure time, silver nitrate concentration and quantity of extract in the reaction mixture which is also correlated with increase in the yield of the synthesized nanoparticles which was in line with previously reported studies **(Ahmed *et al*.,2016; Sasikala *et al*.,2014; Sinha *et al*., 2015).**

Synthesis of Spondias-AgNPs by microwave irradiation

Reaction mixture containing *Spondias pinnata* bark extract and aqueous silver nitrate solution when exposed to microwave irradiation, silver nanoparticle production were observed after 90 secs of microwave irradiation which is evident from the sharp SPR peak at about 432 nm in Figure 3. Beyond 90 seconds of irradiation, the SPR peak showed increase in intensity of absorption indicating that synthesis of nanoparticles increased with increase in irradiation time. Formation of silver nanoparticles containing *Spondias pinnata* extract with the use microwave irradiation occurred at faster rate in 90 seconds .This result is in alignment with the already reported studies, which suggest that application of microwave irradiation for synthesis of nanoparticle using extract of plant as reducing agents is found to be an efficient way of green synthesis with faster rate and it has useful advantages which includes less reaction time, less energy usage and increased yield of production. Faster and even heat distribution during exposure to microwaves results in uniform nucleation and growth condition of nanoparticles (**Nadagouda *et al*., 2011**; **Renugadevi *et al*., 2012**; **Abboud *et al*.,2013**). Microwave irradiation for 90 seconds is sufficient for the reduction of silver ions and production of stable nanoparticles which makes this method useful for the faster synthesis of silver nanoparticles.

Optimisation of microwave irradiated synthesis of Spondias-AgNP

Main problem involved in the generation of nanoparticles is acquiring the monodispersity in solution phase as well as obtaining desired morphological features of nanoparticles. Hence there is a need to optimize the nanoparticle synthesis by differing the few factors such as substrate concentration, reaction time etc. In this study, optimization of nanoparticle synthesis was done by changing the concentration of silver nitrate, quantity of extract, and microwave irradiation time. Nanoparticles production was determined by checking for the SPR peak by UV-Visible analysis. The peak absorption of colloidal silver solution will be ranging from 410nm due to SPR of metal nanoparticles **(Vaidyanathan *et al*., 2009).** SPR peak of *Spondias* silver nanoparticle was found at approximately 432 nm. In presence of visible light Combined oscillation of conductive electrons produces SPR peak and which gets affected by the morphological features of nanoparticles (**Link *et al*., 2003**).
The UV-visible spectral investigations suggest that the nanoparticles are having rounded shape and have uniform distribution. The primary fascination of microwave synthesis is that in short duration of reaction produces uniform particles with nano size range. The quick utilization of starting materials decreases the development of aggregates during synthesis of nanoparticles with the exposure to microwave producing particles with homogenous size distribution.

Effect of Substrate Concentration

Impact of silver nitrate concentration on silver nanoparticle production was observed by changing the amount from 1,,2,4,6 and 8mM and it was analysed by the pattern of SPR which is shown in the Figure 1. Lower sliver nitrate concentration of 1mM not produced SPR peak which may be due to the unavailability of adequate number of silver ions for reduction. Beyond 4mM concentration of silver nitrate, there was increase in intensity of SPR peak reflecting enhanced rate of reduction with the rise in concentration (**Zaki *et al*., 2011**) which occurred due to the longitudinal vibrations **(Prathna *et al*., 2011**). SPR peak showed shift from higher wavelength to shorter wavelength, above 4mM silver nitrate concentration. The reason being that as the particles size decreases, there is an increase in the sharpness of SPR peak, meanwhile peak shifts to the shorter wavelength (Heath 1989; DAS, R ***et al*.,2010**.,). Hence the silver nitrate concertation of 8mM is chosen as optimum quantity.

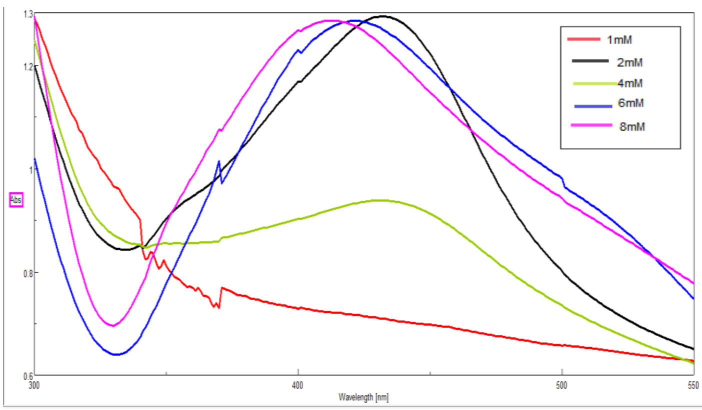

Figure 1 UV spectrum showing SPR peak of silver nanoparticles prepared using 1,2,4,6 and 8mM of silver nitrate.

Effect of Concentration Spondias pinnata Bark Extract:

Yield of nanoparticle synthesis depends on the amount of plant extract. Hence the amount of plant extract was changed from 1, 2, 4, 6 and 8mL in 50mL of silver nitrate solution in order to determine the suitable concentration required to form nanoparticles with required features. Figure 2 shows that the concentration of *Spondiosa pinnata* bark extract has significant effect in the synthesis of silver NPs, revealed by the red shift of SPR band from 432 nm with decreasing concentration of extract. Nanoparticles of bigger size at lesser concentration of extract are indicated by redshift **(Umadevi *et al.*, 2012).** Presence of large anisotropic particles results in broad SPR peak which was observed at lower concentration of *Spodias pinnata* extract. Extract at higher concentration (8ml) produced nanoparticles with smaller size and uniform shape.

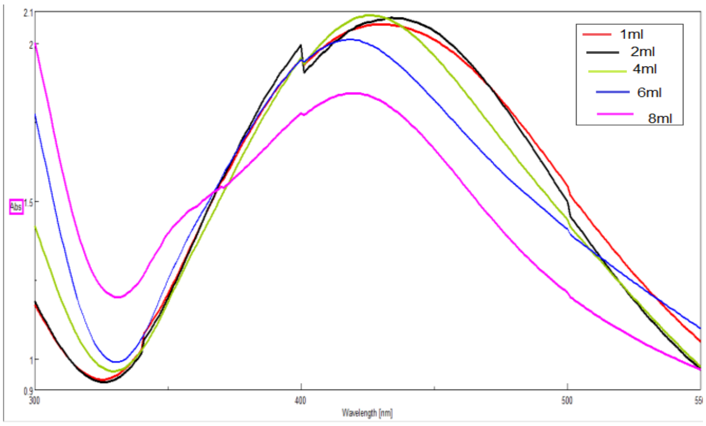

Figure 2 UV spectrum showing SPR peak of silver nanoparticles prepared using 1, 2, 4, 6 and 8mL of *Spondias pinnata* Bark Extract

Effect of irradiation time

Solution containing substrate and plant extract was exposed to microwaves for 30, 60, 90,120 and 240 seconds at constant temperature of 60°C and with irradiation power 850 w to observe the effect of microwave exposure period on synthesis of silver nanoparticles.Studies have reported that duration of exposure to microwave also affects the dimensions, production yield and stability of nanoparticles carried out with the use of plant based biomaterials **(Joseph *et al.*,2014; Kahrilas *et al*.,2014)**.At the initial stages of reaction time i.e. at 30 seconds, the SPR peak was absent and at 60 seconds SPR peak was broad as reflected in Figure 3. The strong SPR peak was seen at 432 nm with the irradiation of microwaves for 90 sec. Sharpness and SPR peak intensity increased with increase in microwave irradiation time from 90 to 240 seconds indicates that as the time period of microwave exposure increased there was increased production of AgNPs. Hence the ideal duration required for the production of nanoparticle was found to be 240 seconds.

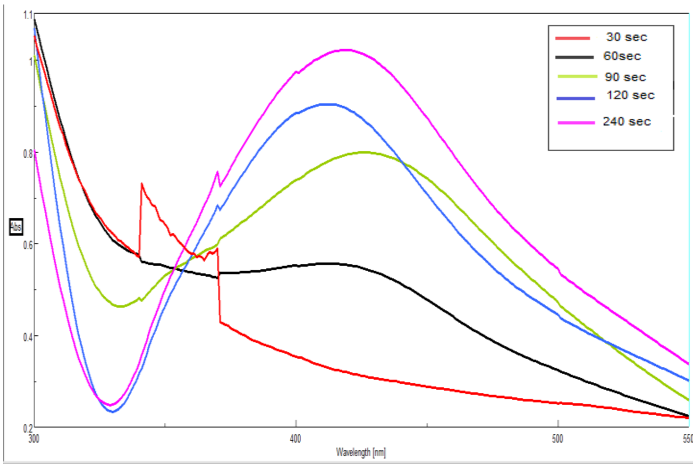

Figure 3 UV spectrum showing SPR peak of silver nanoparticles prepared by microwave irradiation time 30, 60, 90,120 and 240 seconds.

Based on the location ad shape of SPR peak, solution containing 8mM silver nitrate and 8ml of plant extract exposed to microwave for a duration of 240 sec concluded as the optimised condition appropriate for production of silver nanoparticles.

Production of Spondias-AgNP by Aging method

Production of silver nanoparticles by aging method resulted in reduced yield of nanoparticles but size of the nanoparticle increased with the rise in reaction time period as shown by the decrease in absorbance and sharpness of SPR peak respectively.

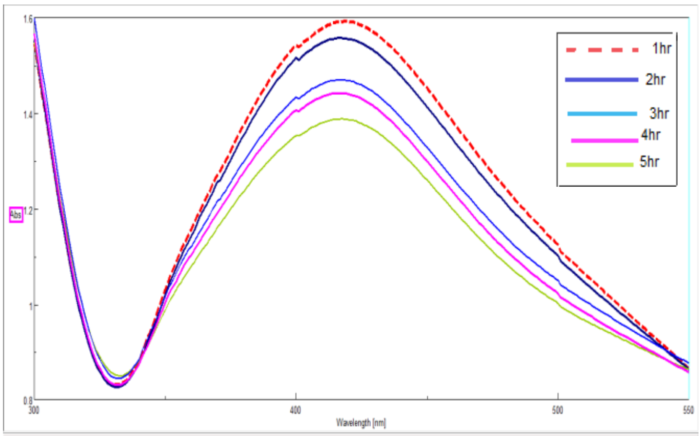

Figure 4 UV spectrum showing SPR peak of silver nanoparticles prepared at room temperature by aging method

Synthesis of silver nanoparticles with the exposure to microwaves produced smaller nanoparticles in faster rate compared with the aging method (without microwave irradiation).

SEM analysis

SEM provides data about the about the topography and morphology of the nanoparticles. Images of silver nanoparticles obtained by SEM indicate that nanoparticles synthesized by microwave irradiation and aging method are spherical to triangular in shape (Figure 5,6).

The nanoparticle shape is based on their interaction with stabilizing agents, surrounding medium and the method of preparation **(Haruta 2004).** It is also reported that rate of reaction affects the morphology of silver nanoparticles **(Xu *et al*., 2006).** Nucleation, seeding and growth are the stages involved in the growth of nanoparticles. At each stage, in order to control the morphological features of nanoparticles, kinetics and thermodynamics of the reaction can be changed **(Xia *et al*.,2009; Chang *et al*.,2011).** Silver nanoparticles shows antimicrobial actions by various routes. Reports shows that efficacy of silver nanoparticles as antimicrobial agents depends on morphological features such as high surface area and quantity of silver ions released **(Cheon *et al*., 2019)**. Therefore, penetrating ability of silver ions into the cell wall of microbes and silver ion release is based on the shape of the nanoparticles.

TEM Analysis

TEM give the precise information about the morphology of nanoparticles since TEM has more prominent magnification and resolution than SEM. Nanoparticles with an size of 34.52 nm with spherical shape were resulted with samples subjected to microwave irradiation when compared to room temperature synthesis which showed bigger particle size (Figure 7,8).

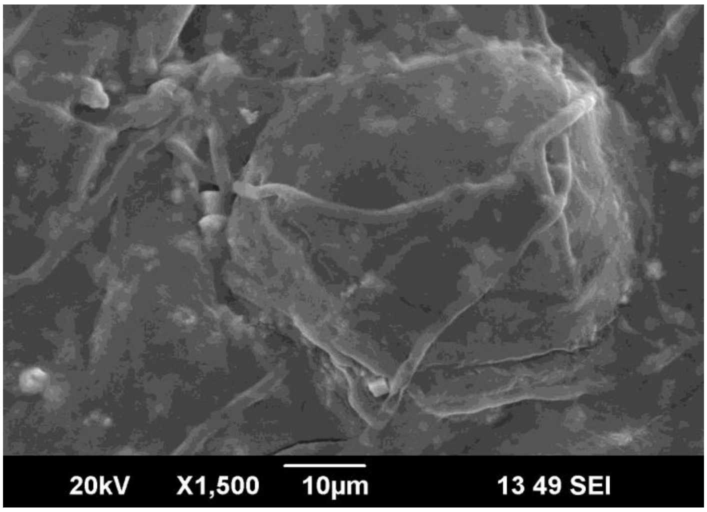

Figure 5 Images nanoparticles synthesised by aging method obtained by Scanning electron microscopy

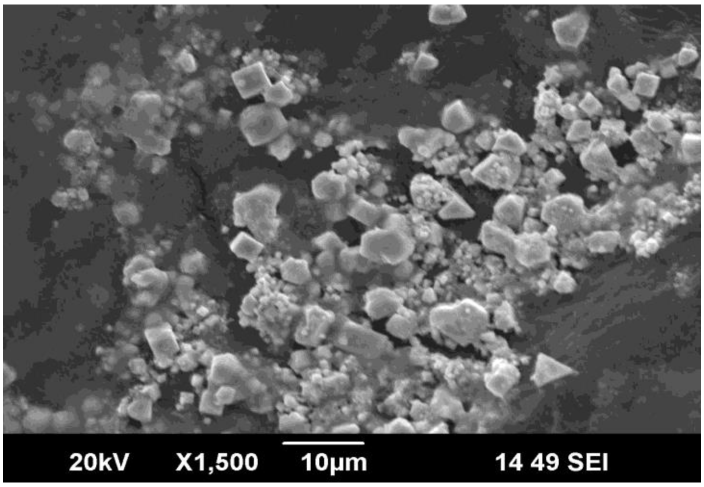

Figure 6. Images of nanoparticles synthesised by microwave irradiation obtained by scanning electron microscopy

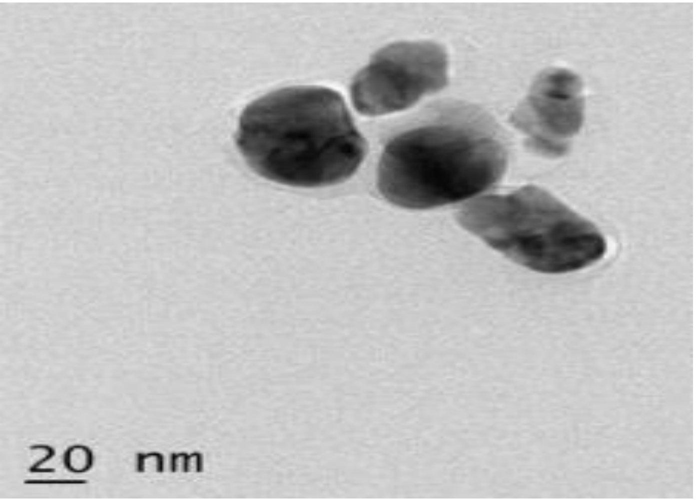

Figure 7. Images of nanoparticles synthesised by aging method obtained by TEM analysis

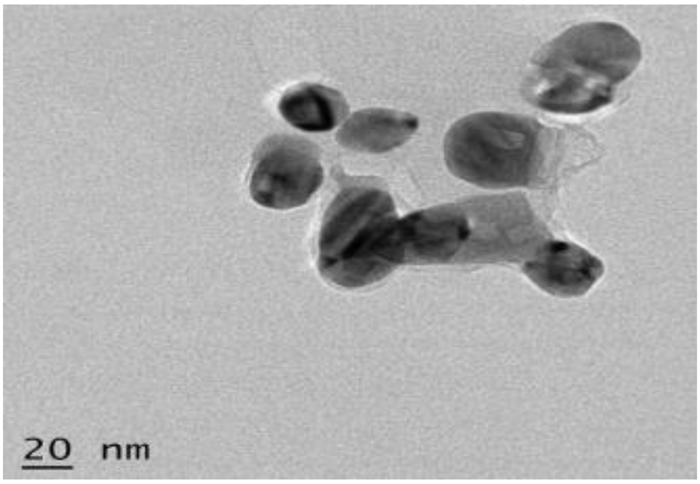

Figure 8 Images of nanoparticle synthesised by microwave irradiation obtained by TEM analysis

FTIR

Phytoconstituents with different functional groups present in the extract and in nanoparticle formulation were identified by FTIR, in addition it gives the indication of reducing agents involved in reduction of silver ions during the formation of silver nanoparticles.

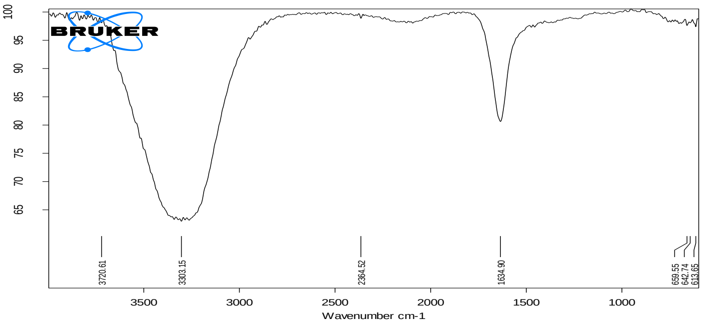

Figure 9 FTIR spectrum of silver nanoparticles

Results of FT-IR investigation of silver nanoparticles demonstrated sharp absorption peaks situated at around 3304 and 1635cm^{-1} (Figure 9). Carbonyl stretch in proteins due to the amide I bond produces peak at 1635cm^{-1} , while the N-H and O-H stretching in proteins produces broad peak at 3304 cm^{-1}(**Bindhani *et al*., 2015**). The peak at 1635 cm^{-1} indicates that proteins acts reducing agents for silver nanoparticles.

CONCLUSION

Present study highlighted the importance of use of microwave in producing silver nanoparticles in comparison with aging method. For formation of silver nanoparticles, the bark extract of *Spondias pinnata* has proven to be efficient reducing agent. Production of nanoparticle using the stem bark extract of *Spondias pinnata* by microwave irradiation is simple, fast, eco-friendly, economic and synthesizes smaller nanoparticles in very short duration of time. Use of optimised condition during microwave assisted synthesis of silver nanoparticle helps in achieving the desired size, shape and yield of nanoparticles. For large scale synthesis of silver nanoparticles, this green synthesis method can be applied which has many useful features. Future studies can include incorporation of silver nanoparticles into topical formulations and investigation of its ability as antimicrobial agents.

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