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Versatile application of silver nanoparticles from leaf extracts of *Arisaema leschenaultii* (Blume) *Cobra lily*: Microwave synthesis and characterization.

Jayanthi Kamalanathan, C. Sadhanandhan and *M. S. Suresh

PG and Research Department of Chemistry, Government Arts College, Udhagamandalam, Tamilnadu, India.

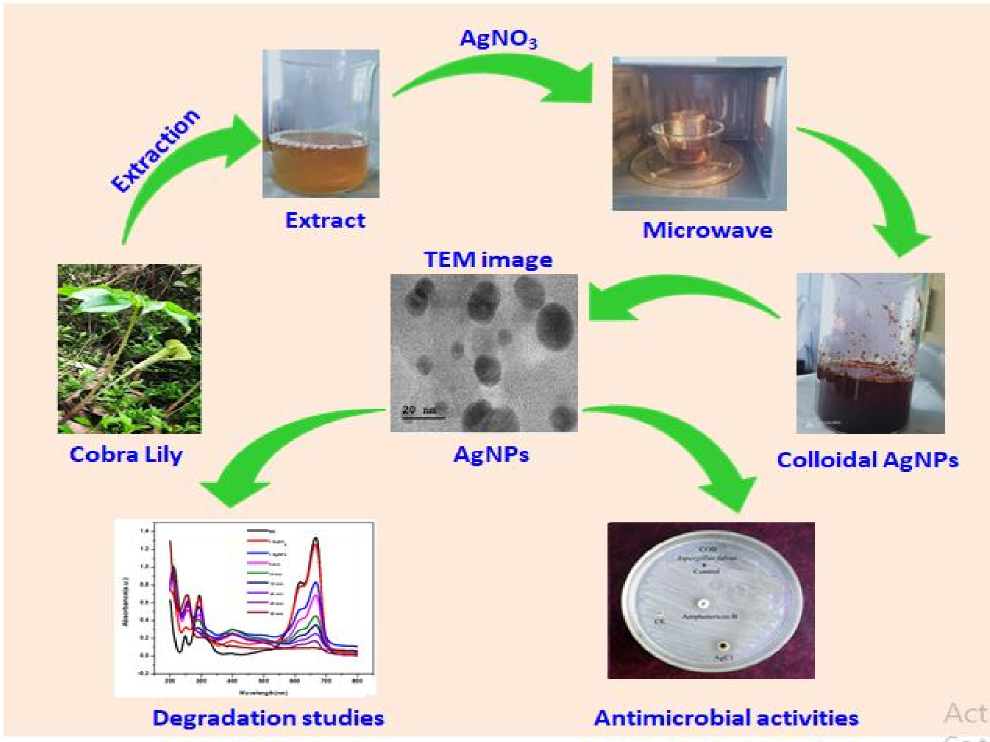
*Corresponding author: mssgacooty@gmail.com

Abstract

The present work describes the rapid synthesis of Silver nanoparticles (AgNPs) successfully produced within 2 minutes using an aqueous leaf extract of *Arisaema leschenaultii* (Blume) for the first time by microwave irradiation a green approach. Initially, it is confirmed by the colour change pale yellow to dark brown and further confirmed by Surface Plasmonic Resonance (SPR) band at 430 nm in absorbance spectra. The synthesized nanoparticles was characterised by UV-Vis, Fourier Transform Infrared (FT-IR), X-ray powder Diffraction (XRD), Field Emission Scanning Electron Microscope (FE-SEM), Energy Dispersive X-ray analysis (EDAX) and High Resolution Transmission Electron Microscopic (HRTEM) spectrum. From the HRTEM d spacing value and XRD, the average size of AgNPs measured was found to be 8.21 and 17.54 nm respectively. The antimicrobial assay was verified for the aqueous leaf extract and the synthesized AgNPs against some common human pathogens for biomedical applications. In addition, the catalytic activity of the AgNPs was gauged by observing the degradation of the organic dyes, Methylene blue (MB) and Congo red (CR) dyes with $NaBH_4$ that pose an environmental threat.

Keywords: AgNPs, *Arisaema leschenaultii* Blume, Antimicrobial, Catalytic activity, Microwave, Organic dyes.

Elementary graphical abstract



1. Introduction

Nanoscience and Technology has seen exponential growth in research in the past two decades traceable to its multidisciplinary approach. Nanoparticles have received global attention due to its ample applications and unique properties in various fields like catalysis, optics, bio medicals, technological and environmental challenges. Most of the Synthesized Nanoparticles by conventional methods use hard processing conditions, highly deleterious chemical reagents and solvents that cause huge environmental hazards. (Zhang et al. 2013 and Nawawi et al. 2016)As there is a growing need to develop eco-friendly methods in synthesizing nanoparticles, green approach using plants can serve as an alternative method.

Green synthesis of metal nanoparticles proves to be an imperative method because of its simplicity, economic, energy efficient, valuable alternative in large scale production, biocompatible, suitable alternative to toxic solvents and environmental friendly. (Fayezeh Samaria et al. 2019) Green synthesis by means of plants turns to be more advantageous than using microorganisms due to their ease of availability, natural capping and reducing agent, safe handling, reproducible, easy to scale up and highly stable. Plant extract of leaves, roots, latex, bark, stem and seeds are used for the synthesis of nanoparticles especially Silver (Bar et al. 2009 and Marambio Jones et al. 2010)as they have a wide range of active agents that can promote the Silver ions' reduction and stability. (Siby joseph et al. 2015 and Alsammarraie et al. 2018)Among various metal nanoparticles, AgNPs have gained considerable interest due to its fascinating characteristic (high stability, large surface area, low activation energy) and various applications in household and industries, such as cosmetics, food packaging and processing, tires, textiles, photonics, catalyst, wound care products in medical field, antimicrobial activities, therapeutic devicesand diagnostics and in delivering drug.(Abderrhmane Bouafia et al. 2021) Phytochemicals like reducing sugars, pigments, proteins and other bioactive molecules such as phenolics, terpenoids, flavones, alkaloids, enzymes, amino acids, and alcoholic compounds are found an inexpensive source in plants, that are able to lessen metal compounds into their corresponding stable nanoparticles.

On a large scale biosynthesis using plant extract isan overlong process (Hiral Vaghela et al. 2017) and hence this can be resolved by using microwave (MW) irradiation technique. MW assisted synthesis is a rapid and easy method that cause uniform heating, enhance reaction rates with immediate consumption of starting materials, prevents agglomeration, saves energy, promotes homogenous conditions of nucleation and growth, which results in monodispersed nanoparticles in short span of time.(Oluwatobi. S et al. 2016 and Nadagouda M N et al., 2011) Thus thisapproach stands as a greener, simpler, efficient, effective and economically viable technique for the synthesis of AgNPs which satisfies the benign of green chemistry and used to synthesise large quantities with defined shape and size.

In the present work *Arisaema leschenaultii* Blume (Ari-les) plant leaf is used in the synthesis of AgNPs as they show various therapeutic applications and also this plant has not been reported so far for the synthesis of AgNPs. *Arisaema leschenaultii* (Blume) (Family–Araceae) belongs to an Asian species universally known as Dhei or cobra Lilly. Flowering and fruiting of this plant fall in the months of June to September. It is widely distributed in Srilanka and in India in the hills of Assam, Karnataka, Kerala and Tamilnadu. [38] It is abundantly grown in the semi shaded, moist parts of hills' evergreen forest and specifically on shola floor at an altitude of 1200–2000m. From the earlier report, the phytochemically active compounds such as tannins, flavonoids, saponnin, steroids, terpenoids, carbohydrate, quinone, phenolic compounds and glycosides are present in the ethanoic extract of the leaves of Ari-les. (Fyson P.F. 1932)These secondary metabolites present in the Ari-les leaf might be responsible in the reduction of Ag+ to Ag^0, capping and stabilising the nanoparticles.

Thus, a green technique for the synthesis of AgNPs in aqueous medium using the leaf extract of *Arisaema leschenaultii* (Blume) is reported. The catalytic behaviourof synthesized AgNPs was examined in the degradation of heterocyclic organic dyes, Methylene blue and Congo red as they are considered to be common textile and tannery effluents which create impact on the organisms. As Silver flash sturdy toxicity to a variety of harmful microorganisms, antibacterial and antifungal studies of biosynthesized AgNPs were also investigated against some common human pathogens.

2. Medicinal use

In Nilgiri district, among tribes Ari-les plant is called as Amugidageddai. The entire plant was traditionally used in Ayurveda and folk medicines intended for the treatment of urinary diseases, snake bites, colitis, piles, haemorrhoids, fistula and sinus, indigestion, abdominal pain, dysentery and shows anti-nematode properties. (Krishnamurthy et al. 2017) Paste of the tuber and spadices of this species is used as an antidote or contraceptive in Nilgiris of Tamil Nadu. Natives of Munnar and Kodaikanal use this young shoots as vegetables. (Suresh et al. 2017 and Mathew KM, 1999)

Thus to the best of our knowledge this medicinally important plant extract is used in the green synthesis of AgNPs and it has not been stated earlier.

The Plant was authenticated by the department of Botany, Government Arts College, Udhagamandalam, Nilgiri district. Tamil Nadu, India,

3. Materials and Methods

3.1. Preparation of plant extract

Nilgiri Biosphere Reserve is one of the hot spots of the world with many more rare, endemic and threatened plants. (Nayar M P, 1996)For the present study, *Arisaema leschenaultii* (Blume) plant leaves were collected from sola grass lands of the Nilgris in the month of July. The leaves were properly washed several times with water, dried in shade for weeks and pulverised. To about 10 g of the powder leaves 100 ml of pure water is added and stirred continuously for 30 minutes at 45-50°C. It is then allowed to settle for two days and filtered using Whatmann No.1 filter paper and this freshly prepared extract was utilized for further studies.

Fyson, P.F. (1932). The Flora of the South Indian Hill

3.2. Microwave synthesis of Silver nanoparticles (AgNPs)

To 90 ml of 10^{-3}M solution of silver nitrate 10 ml of the extract was added and stirred well. It was then exposed to Microwave irradiation for 2 minutes in domestic microwave oven (whirlpool) operating at a power of 700 W. Consequently, the pale yellow solution changed into dark brown after irradiation, confirms the reduction of Ag^{+} to Ag^{0}. (Sastry et al. 1998) The colloidal solution was allowed to stand till the solid particles settles Down and thencentrifuged, (4000rpm, 15minutes) washed and dried overnight in oven.

3.3. Characterization of silver nanoparticles

The synthesized AgNPs using Ari-les plant extract was characterized by different spectrochemical techniques. The UV-Vis spectra were recorded on a Systronics spectrophotometer 2202; Fourier transform infrared (FTIR) spectra were measured using Perkin Elmer 1125000P spectrometer. The information about the particle size, crystalline nature and the purity of the nanoparticles was obtained from the Powder X-ray diffraction (XRD) data by employing the Rietveld refinement and recorded on a PAN analytical diffractometer utilising Cu-Kα radiation (λ= 0.1542 nm). Field emission Scanning Electron Microscope (FESEM) images and Energy Dispersive x-ray analysis (EDAX) measurement has been carried out using JEOL JSM-7610F.High Resolution Transmission Electron Microscopic (HRTEM) images were obtained from the spectrum model JEOL 200 operated at an accelerating voltage of 300 kV.

3.4. Catalytic studies

Catalytic efficiency of the AgNps was examined in organic dyes like Methylene blue (MB) and Congo red (CR) dyes at room temperature (14-16°C). About 2 ppm of dyes were prepared and used as a stock solution. To the 50 ml of the stock 1 ml of 10mM Sodium borohydride was added, magnetically stirred and spectrum was noted.

Same procedure followed with 1mg of AgNPs magnetically stirred for few minutes. Aliquots of 2-3 ml suspension was extracted using 0.2µm filter syringe and analysed for absorbance at regular interval of time using UV–visible absorption spectrophotometer.

The degradation of dyes is indicated by the decolourisation of the solution.

3.5. Antimicrobial assay (Antibacterial and Antifungal)

The antimicrobial potency of the leaf extract and the synthesized AgNPs was investigated following the standard agar well diffusion method. (Perez et al. 1990 and Erdemoglu et al., 2003) Each bacterial isolate was suspended in Brain Heart Infusion (BHI) broth and diluted to approximately 10^{5} colony forming unit (CFU) per mL. They were flood-inoculated onto the surface of Media (Mueller Hinton Agar for Bacteria and Sabouraud's Dextrose agar for fungi) and then dried. Using a sterile cork-borer, five-millimetre diameter wells were cut from the agar, into which, 30µL of the leaf extract and synthesised nanoparticle solutions were poured. The fresh cultures of four different bacteria in four media plates were incubated for 18h at 37°C. Similarly for 48h, four fungal plates were incubated at room temperature. Antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition in mm against the test microorganisms. Ciprofloxacin was used as reference antibacterial agent for positive control over antibacterial activity. A panel of four common human pathogenic organisms was used in this study, which includes two gram negative bacterial species (*Escherichia Coli*, *salmonella typhi*) and two gram positive bacterial species (*Staphylococcus aureus*, *Enterococcus faecalispos*). The pH was maintained 7.4. Similarly *Aspergillus niger*, *Candida albicans, Aspergillus flavus, Aspergillus fumigates* were used in to study antifungal activity and Amphotericin B was used as reference antifungal agent. The tests were carried out in triplicates. The synthesized AgNPs showed good stability and activity towards the microorganisms which reveals the capping efficiency of the plant extract.

All these work has been carried out in Micro Laboratory, Vellore, Tamil Nadu, India.

4. Results and Discussion

4.1. Synthesis of AgNPs

The formation of AgNPs using Ari-les leaf extract using microwave irradiation was easily confirmed by the colour change from pale yellow to dark brown in 2 minutes, due to the reduction of Ag^+ to Ag^0. It was also confirmed by surface plasmon resonance absorption (SPR) spectra. Agglomeration of nanoparticles was prevented by the microwave irradiation to a greater degree resulted in monodispersed AgNPs and hence it is an effective and economically liable heating technique in making of nanoparticles.

The absorption spectra of AgNPs displays a narrow absorbance peak at 430 nm in Figure 1, this value was in good pact with Mie's theory according to which, a single SPR band was displayed in small spherical or quasi-spherical nanocrystals, whereas two or three bands were shown in anisotropic particles, depending on their shape. (Vidhu V K et al. 2014)

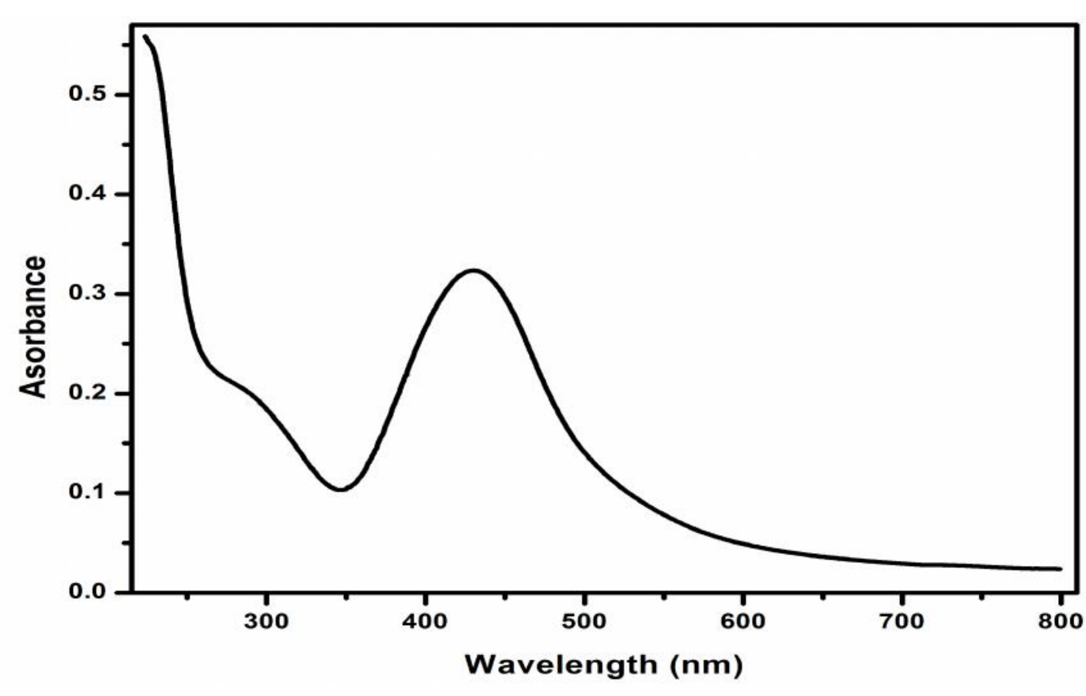


Figure 1. UV–Visible absorption spectra of AgNPs.

4.2. XRD method

X-ray diffraction studies further impart evidence ofthe crystalline nature of AgNPs. In Figure 2, four distinct peaks at two theta values of 38.30°, 44.26°, 64.71° and 77.55° were observed which are indexed the reflection of (111), (200), (220) and (311) crystallographic planes of face centred cubic (fcc) AgNPs. Each of the observed d-values correlates well with the standard values (JCPDS file no. 04-0783). (de Jesus et al. 2017) As compared to other reflection the intensity of the peak due to reflections of (111) plane is comparatively larger which denotes that the synthesized AgNPs were enriched in (111) facets and were crystalline in nature. Scherrer equation was used to gauge the particle size of the nanoparticles.

$$Dp = (0.94 \times \lambda) / (\beta \times \cos\theta)$$

Where, Dp = Average Crystallite size, β = full width at half maximum (FWHM), θ = Bragg's angle, λ = X-Ray wavelength.

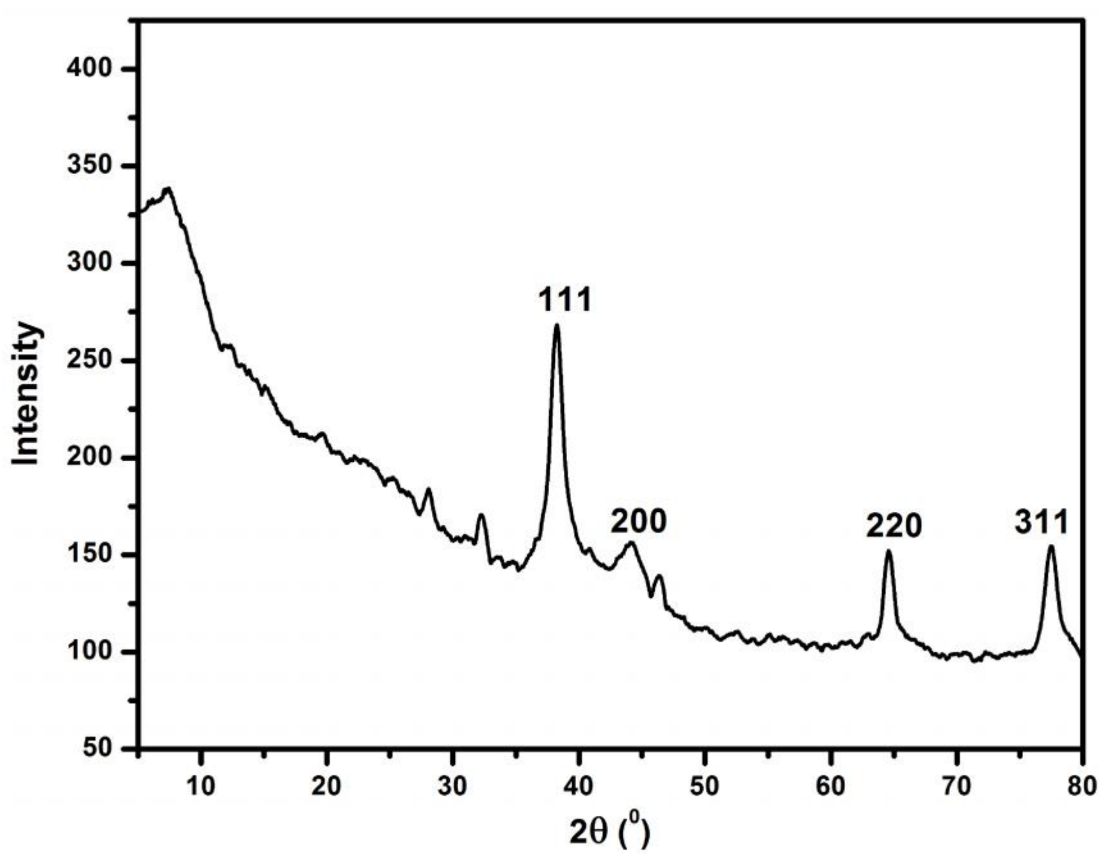


Figure 2. X-ray diffraction pattern of AgNPs.

The calculated average crystalline size of the AgNPs was found to be in the range of 17.54 nm.

4.3. FTIR

FTIR spectral analysis was a prominent tool used to identify possible biomolecules present in Ariles leaf extract that are likely to form and stabilize silver nanoparticles. Transmittance bands are observed at 1004, 1088, 1324, 1568, 1665, 2950 and 3316 cm^{-1} for green synthesised nanoparticles (Figure 3). The two peaks at 1665 and 1568 cm^{-1} were related to proteins, including a arising from amide I (mostly the C=O stretching vibrations of the peptide back bone) and to amide II (N-H bending coupled with C-N stretching) respectively. (Ji Zhang et al. 2017)Peak around 1324 cm^{-1}, involving amide III of proteins as well as COO^{-}vibration from amino acids and polypeptides. Peak at 1324 cm^{-1} resulted from COO^{-} vibrations of fatty acids, amino acids and polypeptides. The peak at 2950 $cm^{-1}$and 3316 cm^{-1} indicates C-H stretching due to alkanes and alkynes respectively. The peak at 3316 and 2950 cm^{-1} correspond to the OH stretching vibrations of alkanes, water soluble phenolic compound such as tannins and hydrogen bonded carboxylic acid which resembles the previously reported results of G.Sathish kumar et al. Peak at 1004 cm^{-1} indicates stretching due to alcohols, ethers, carboxylic acids, ethers and esters and C-N stretching due to amines. It can be concluded that the plant metabolites containing CO, OH and COO^{-} of proteins and phenolic compounds support the presence of Ari-les leaf extract on AgNPs and act as capping and stabilising agent as well as influence the process ofbio reduction of silver ions.

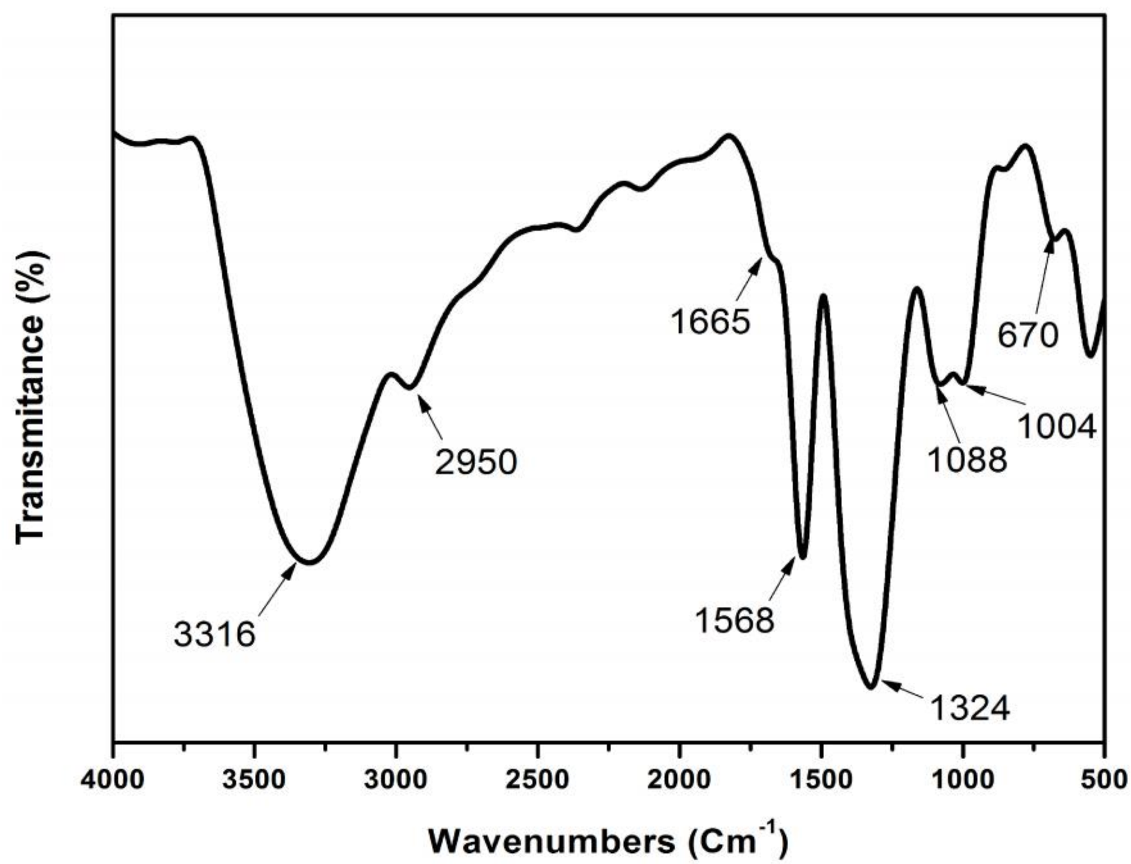


Figure 3. FTIR spectra of AgNPs.

4.4. FESEM and EDAX analysis

The morphology and elemental analysis of the newly synthesized AgNPs was performed using FESEM & EDAX techniques. FE-SEM image clearly reveals the spherical shape of AgNPs (Figure 4). The EDAX spectrum (Figure 5) recorded acknowledge the purity and the complete chemical composition of AgNPs. At the centre of the spectrum sharp peaks located between 2.6 and 3.6 KeV and those maxima are directly related to the K and L lines of metallic silver. (Puchalski M. et al. 2007) Due to surface plasmon resonance, silver show a strong optical absorption peak approximately at 3 KeV. The signals for carbon and oxygen may be originated from the substrate medium.

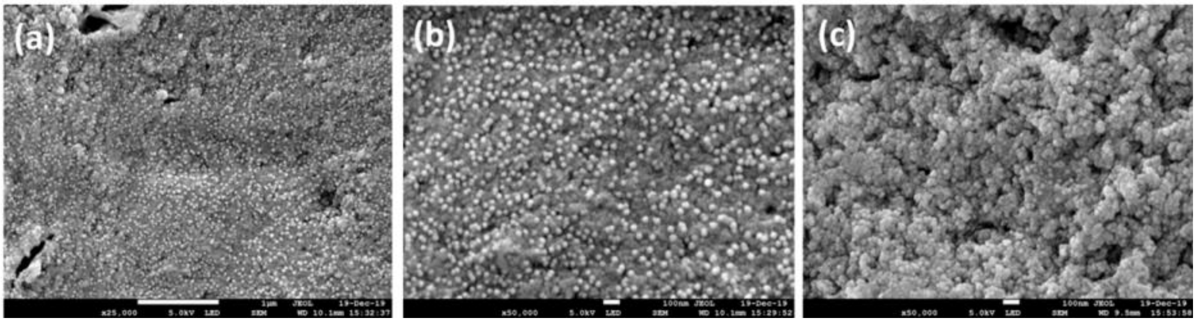


Figure 4. SEM images of AgNPs.

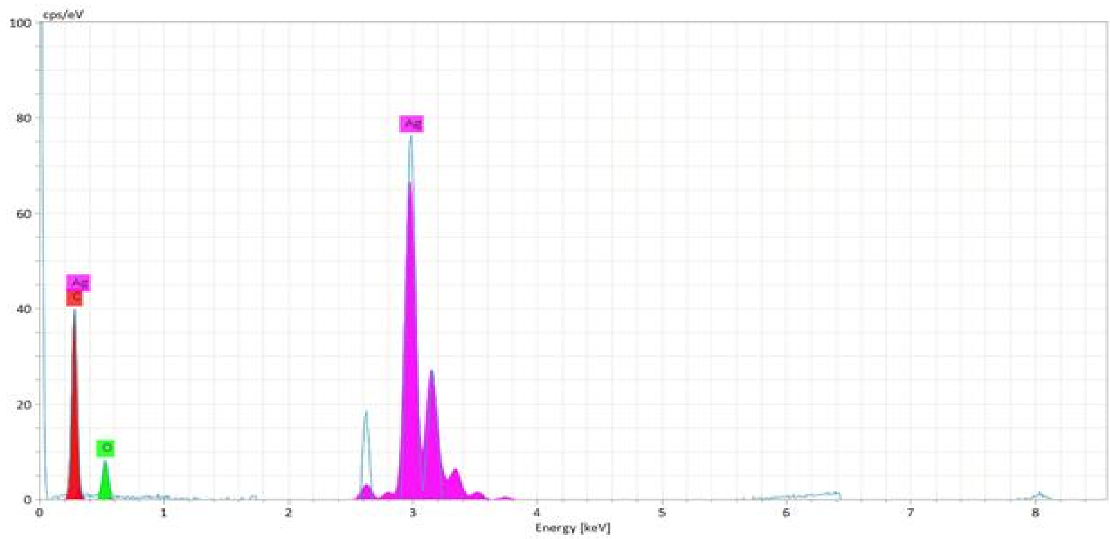


Figure 5. EDAX images of AgNPs.

4.5. HRTEM

HRTEM turns to be a main source in determining the shape, size and distribution of synthesized nanoparticles. The spherical and monodispersed morphology of AgNPs is confirmed from HRTEM at different magnification, indicating that the bioactive compounds present in the Ari-les leaf extract act as a reducing agent besides being a capping agent. The lattice spacing d measured from the high resolution images for AgNPs are shown in Figure6. This clearly confirm that the growth occurs preferentially in the direction of (111) plane. The SAED of a single spherical particle confirmed the crystalline nature of AgNPs. (Meena Kumari M et al. 2015)From HRTEM d spacing value the particle size calculated was found to be 8.21 nm. Thus rapid synthesize of AgNPs from Ari-les leaves by MW irradiation method results in smaller sized nanoparticles.

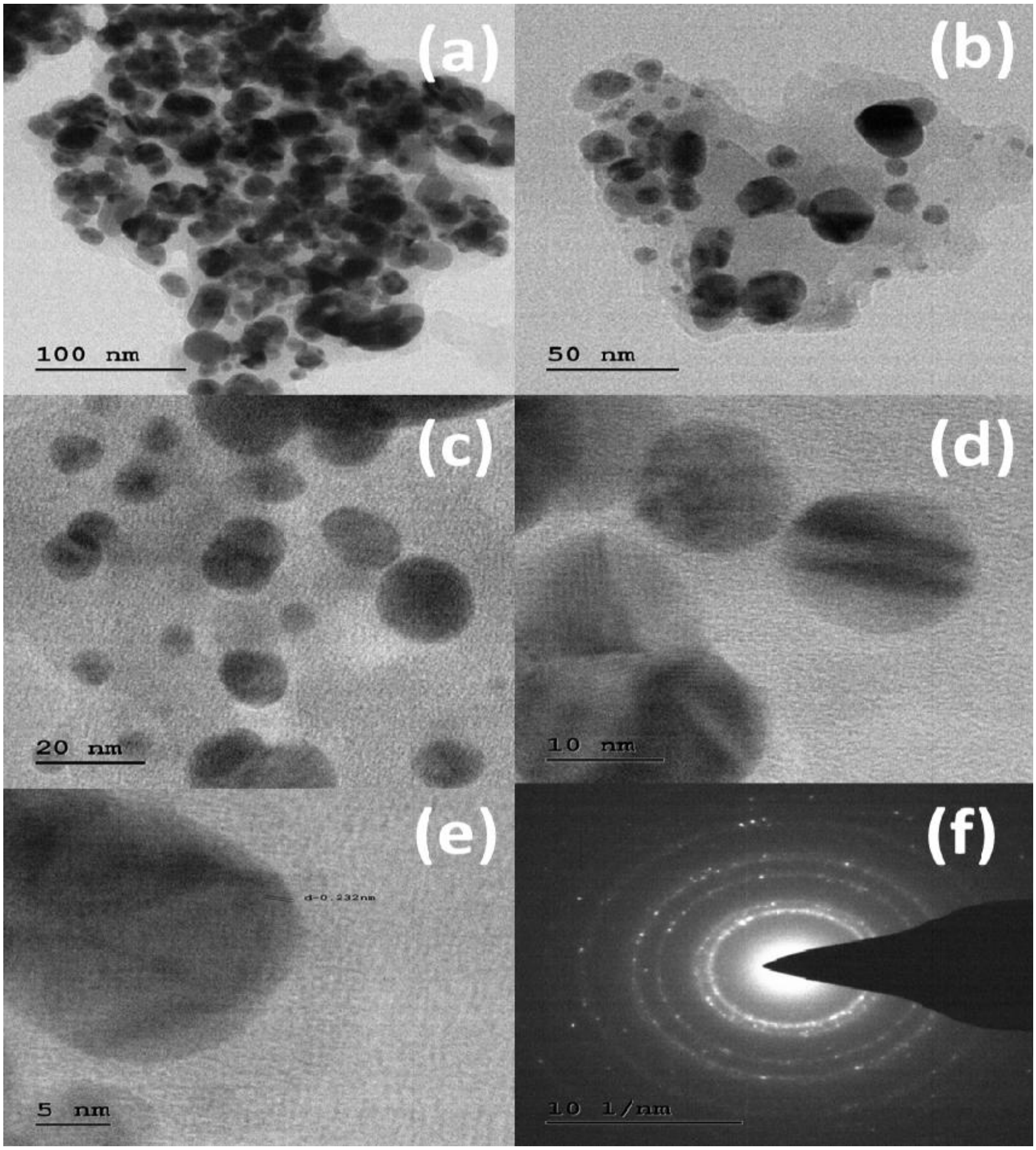


Figure 6. (a-d) HRTEM micrograph of MW synthesized AgNPs e) d spacing measurement f) SAED pattern showing the rings.

4.6. Catalytic Degradation of Dyes

The most significant application of metal nanoparticles is their catalytic role and that depends on their composition, shape and size. It is well known, that the nanoparticles have larger surface area and greater catalytic activity. Thus AgNPs was used as a catalyst for the degradation of Methylene blue (MB) and Congo red (CR) by $NaBH_4$.These two heterocyclic aromatic dyes are primarily considered as the most common organic pollutants released in large amounts from textile industries as effluent, which cause adverse effects on environment and organisms. Secondly their absorption peak does not overlap with that of SPR peaks of AgNPs and hence easily monitored.

Although $NaBH_4$is considered as a strong reducing agent, in aqueous solution its efficiency was found to be very less due to the difference in their redox potential, large kinetic barrier and low encounter probability ($<$ 3 %). (Komalam A. et al. 2012) Thus even after 30 minutes of the reaction between the dyes and $NaBH_4$, only meagre change was noted in the absorption peak of the respective dyes. At the same time, if the degradation of MB and CR by $NaBH_4$ carried out in the presence of AgNPs, rate of degradation is found to be greatly enhanced. This reaction kinetics was studied using spectrophotometric by monitoring the absorption peak that appears at 612 nm and 664 nm for MB andat 350nm and 450nm for CR. The intensity of these peaks decreased gradually indicating the photocatalytic degradation of the dyes. Decrease in the absorption peak of dyes and rise in the intensity of AgNPs peak point out the catalytic reduction and continuing evolution of the silver particles respectively (Figure 7). Gradual decrease of the absorbance of the dye and approaching the base line confirms the completion of the catalytic reaction. In this reduction reaction AgNPs act as an effective redox catalyst between the donor ($NaBH_4$) and the acceptor dyes. The degradation percentage of the dyes (MB and CR) was calculated by the following equation:

Degradation percentage = **$[A_0\text{-}A_t/A_0]$ x 100**

A_0 is the initial absorbance of the dye solution and A_t is the absorbance of dyes at time t.

From the slopes of the graph drawn between ln (A/A_0) and time, rate constants of the reaction and its corresponding correlation coefficient were estimated. (Table 1) The kinetic data obtained from methylene blue and Congo red is fitted to both first order and second order rate equations. After constant analysis, the linear plot between ln (A_t/A_0) and the reduction time (min) and its k value with correlation coefficient clearly reveals that the degradation of the dyes appears to follows the pseudo first-order kinetics.(Ravichandran et al. 2019)

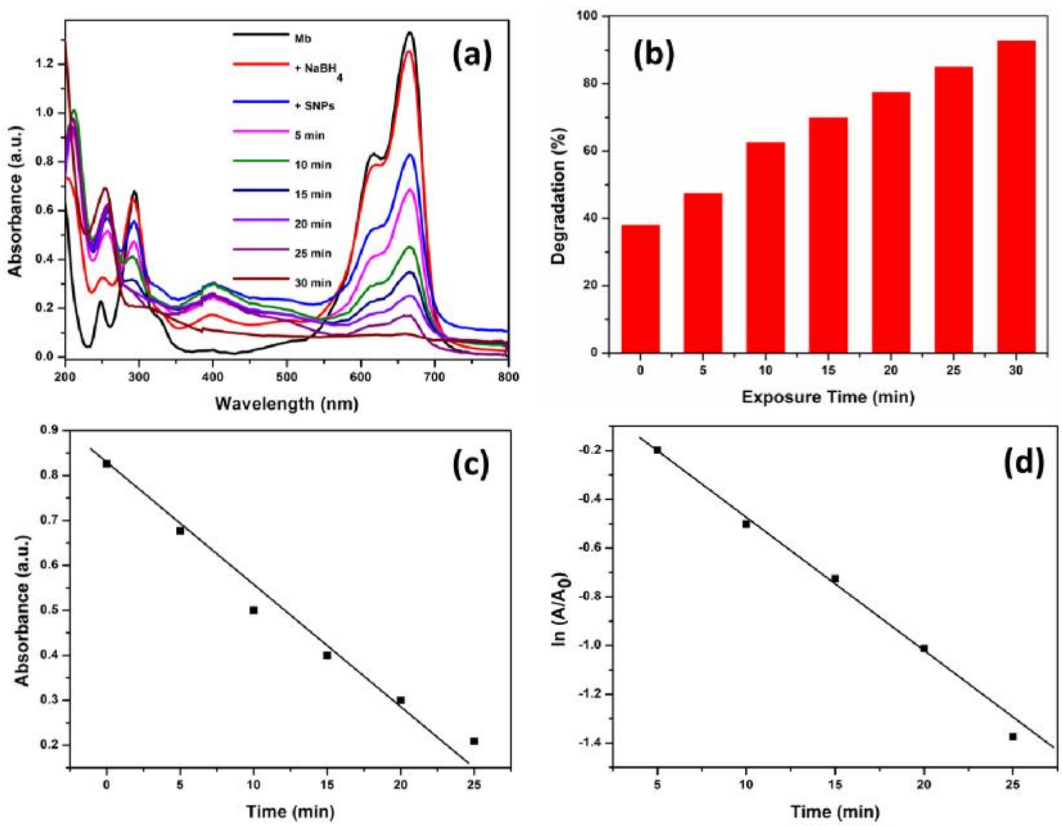


Figure 7. a) The absorption spectra of aqueous solution of methylene blue treated with 1 mg of synthesized AgNPs using Ari-les at different time intervals. b) Percentage of dye degradation by 1mg of synthesized AgNPs at different time intervals. c) & d) kinetic plot absorbance Vs time and ln A/A_0 Vs time respectively.

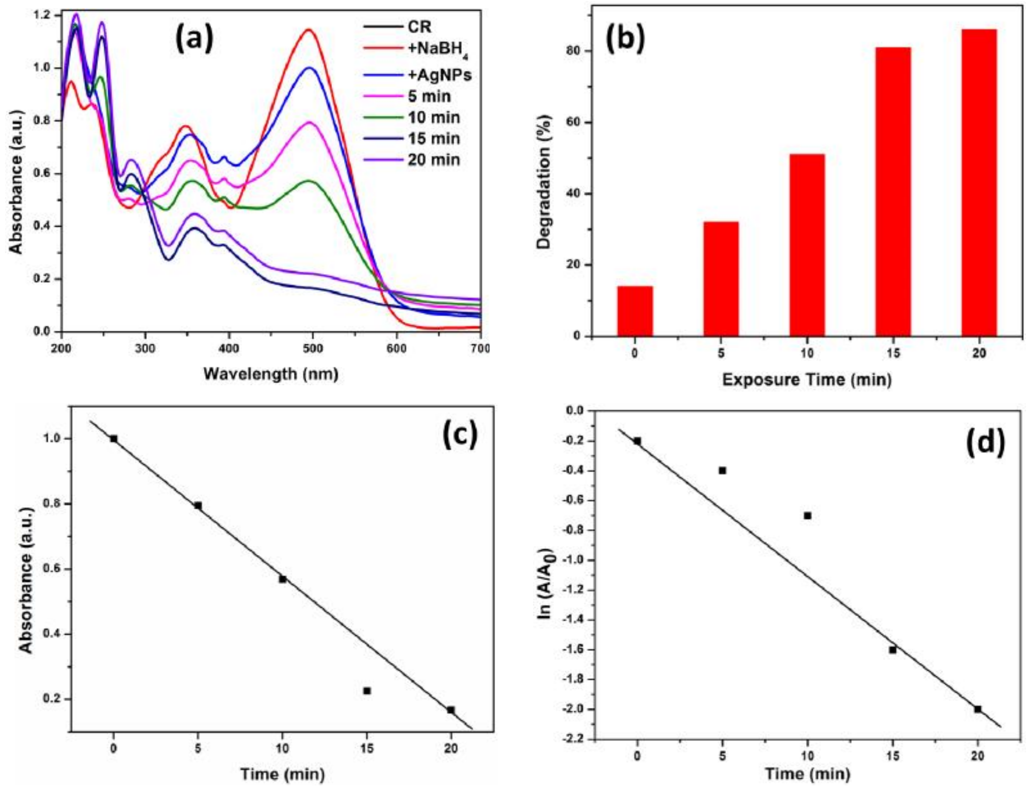


Figure 8. a) The absorption spectra of aqueous solution of methylene blue treated with 1 mg of synthesized AgNPs using Ari-les at different time intervals. b) Percentage of dye degradation by 1mg of synthesized AgNPs at different time intervals. c) & d) kinetic plot absorbance Vs time and ln A/A_0 Vs time respectively.

Table 1. Degradation results of MB and CR

Dye	Degradation Time (Min.)	Degradation (%)	R Square	Rate Constant
Methylene Blue	30	93	0.9922	0.070
Congo Red	20	86	0.9851	0.096

The calculated degradation efficiency (η) of AgNPs for MB was 93% at 30 min and for CR (Figure 8) its η was 86 % at 20 min. Thus the results obtained certify that the green synthesized AgNPs can turn into an efficient catalyst in the reduction of MB and CR by $NaBH_4$.

4.7. Antibacterial activity

The antimicrobial activity of aqueous extract and green synthesised AgNPs was tested against four bacterial species and compared to that of the antibiotic ciprofloxacin (Table 2). No zone of inhibition was observed for control. The zone of inhibition for the gram negative bacterial species for AgNPs are *Salmonella typhi* 20 mm and *Escherichia coli* 22 mm and for the gram positive bacterial species for *Enterococcus faecalis* 32 mm and *Staphylococcus aureus* 13 mm respectively.

The aqueous extracts show zone of inhibition only against *Salmonella typhi* the gram negative bacteria. Zone of inhibition for standard ciprofloxacin (30µg/disc) for the above gram negative bacteria are 32 and 15 mm. The aqueous extract does not show zone of inhibition against the gram positive bacteria. Zone of inhibition for standard ciprofloxacin (30µg/disc) for the above gram positive bacteria are 14 and 22 mm. The details given in the Table 2. reveals, that the aqueous extract of the plant doesn't show much activity against the selected pathogens, but the synthesised AgNPs show considerable activity against those pathogens. Comparatively *Escherichia coli* and *Enterococcus faecalis* showed high activity against the standard ciprofloxacin.

Figure 9. Antibacterial activity of the leaf extract of Ari-les (CE) and AgNPs (AgC1) against the pathogens, Ciprofloxacin is a common antibiotic used for comparative study

Table 2. Zone of inhibition in mm of Ciprofloxacin, Ari-les plant extract (CE) and AgNPs for four different bacteria.

S.No.	Microorganisms	Control	CE	AgNPs	Ciprofloxacin
		Zone of inhibition in mm			
1.	*Salmonella typhi*	-	15	20	32
2.	*Escherichia coli*	-	-	22	15
3.	*Enterococcus faecalis*	-	-	32	14
4.	*Staphylococcus aureus*	-	-	13	22

4.8. Antifungal activity

Antifungal screening was carried out against four fungi *Aspergillus niger, Candida albicans, Aspergillus flavus, Aspergillus fumigatus*. Amphotericin B has been used as reference antifungal agent for the above used fungus.

The antifungal activities of plant extract of Ari-les, AgNPs and standard Amphotericin B (50 µg/disc) were determined against four pathogenic fungi (Table 3). The highest activity of 25 mm of zone inhibition was found against *Aspergillus niger* followed by 15 mm of zone inhibition against *Candida albicans* and *Aspergillus fumigatus*. In contrast, the lowest activity of 10 mm of zone inhibition was identified against *Aspergillus flavus*. In all, the AgNPs showed significant activity against all the tested pathogenic fungi.

Table 3. Zone of inhibition in mm of Amphotericin-B, Ari-les plant extract (CE) and AgNPs for four different funguses.

S.No.	Microorganisms	Control	CE	AgNPs	Amphotericin-B
		Zone of inhibition in mm			
1.	*Aspergillus niger*	-	10	25	12
2.	*Candida albicans*	-	-	15	12
3.	*Aspergillus flavus*	-	-	10	12
4.	*Aspergillus fumigatus*	-	-	15	11

Figure 10. Antifungal activity of the leaf extract of Ari-les (CE) and AgNPs (AgC1) against the pathogens, Amphotericin-B is a standard used for comparative study.

5. Conclusion

Arisaema leschenaultii Blume leaf aqueous extract was used successfully to synthesize silver nanoparticles within two minutes by microwave irradiation of average particle size ranges from 8nm to 17nm, which substantiates the capability of the plant leaf in the formation of the AgNPs. This work emphasises simple, eco-friendly, economical, solvent free alternative method for the synthesis of AgNPs in large scale production. The plant mediated AgNPs so produced exhibits broad bacterial inhibitory functions comparable enough to Ciprofloxacin activities and better antifungal activity against human pathogens and thereby making the synthesized Silver nanoparticles a good antimicrobial agent. At the same time, AgNPs reveals the degradation efficiency of (in presence of $NaBH_4$) 92% for MB in 30 minutes and 86 % for CR in 20 minutes.

Thus the rapidly green synthesized AgNPs can serve as an efficient catalyst in waste water treatment as well as an effective therapeutic agent in biomedical field.

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