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ORIGINAL ARTICLE

Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities

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Abstract There is an increasing commercial demand for nanoparticles due to their wide applicability in various areas such as electronics, catalysis, chemistry, energy and medicine. Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable. In this research article we present a simple and eco-friendly biosynthesis of silver nanoparticles using *Garcinia mangostana* leaf extract as reducing agent. The aqueous silver ions when exposed to leaf extract were reduced and resulted in silver nanoparticles whose average size was 35 nm. The silver nanoparticles were characterized by UV–Visible, Fourier transform infra-red spectroscopy (FT-IR) and transmission electron microscopy (TEM) techniques. Furthermore these biologically synthesized nanoparticles were found to be highly effective against different multi-drug resistant human pathogens.

1. Introduction

The field of nanotechnology is one of the most active areas of research in modern material science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. New applications of nanoparticles and nanomaterials are emerging rapidly (Jahn, 1999; Naiwa, 2000; Murphy, 2008). Nanocrystalline silver particles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnostics (Schultz et al., 2000), antimicrobials and therapeutics (Rai et al., 2009; Elechiguerra et al., 2005), catalysis (Crooks et al., 2001) and micro-electronics (Gittins et al., 2000). However, there is still a need for economic, commercially viable as

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well environmentally clean route to synthesize silver nanoparticles.

A number of approaches are available for the synthesis of silver nanoparticles for example, reduction in solutions (Goia and Matijevic, 1998), chemical and photochemical reactions in reverse micelles (Taleb et al., 1997), thermal decomposition of silver compounds (Esumi et al., 1990), radiation assisted (Henglein, 2001), electrochemical (Rodriguez-Sanchez et al., 2000), sonochemical (Zhu et al., 2000), microwave assisted process (Pastoriza and Liz-Marzan, 2002) and recently via green chemistry route (Begum et al., 2009; Bar et al., 2009; Song and Kim, 2009).

Unfortunately, many of the nanoparticle synthesis or production methods involve use of hazardous chemicals, low material conversions, high energy requirements, difficult and wasteful purifications. Biosynthetic methods employing either biological microorganisms or plant extracts have emerged as a simple and viable alternative to chemical synthetic procedures and physical methods. Most of the methods are still in the developmental stage and various problems are often experienced with the stability of nanoparticle preparations, control of crystal growth and aggregation of particles.

Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large-scale synthesis and further there is no need to use high pressure, energy, temperature and toxic chemicals. Using plants for nanoparticle synthesis can be advantageous over other biological processes because it eliminates the elaborate process of maintaining cell cultures and can also be suitably scaled up for large-scale synthesis of nanoparticles under non- aseptic environment.

Silver nanoparticles play a profound role in the field of biology and medicine due to their attractive physiochemical properties. Silver products have long been known to have strong inhibitory and bactericidal effects, as well as a broad spectrum of antimicrobial activities, which has been used for centuries to prevent and treat various diseases, most notably infections (Shankar et al., 2004). Silver nanoparticles are reported to possess anti-fungal (Wiley et al., 2006; Ramirez et al., 2009), anti-inflammatory (Panacek et al., 2009), anti-viral (Nadworny et al., 2008), anti-angiogenesis (Rogers et al., 2008) and anti-platelet activity (Gurunathan et al., 2009).

Here in, we report for the synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the cell free aqueous extract of *Garcinia mangostana* leaf. Furthermore these biologically synthesized nanoparticles were found to produce a high bactericidal activity.

2. Material and method

2.1. Plant material and preparation of the extract

Fresh and healthy mangosteen leaves were collected, washed thoroughly with distilled water, incised into small pieces and air-dried. About 25 g of thus finely cut mangosteen leaves were weighed and transferred into 500-ml beaker containing 100 ml distilled water, mixed well and boiled for 25 min. The extract obtained was filtered through Whatman No.1 filter paper and the filtrate was collected in a 250-ml Erlenmeyer flask and stored in refrigerator for further use.

2.2. Synthesis of silver nanoparticles

Aqueous solution (1 mM) of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 5 ml of mangosteen leaf extract was added into 95 ml of aqueous solution of 1 mM silver nitrate for reduction into Ag^+ ions.

In a typical synthesis of silver (Ag) nanoparticles the leaf extract (1.5 ml) was added to 30 ml of 10^{-3} M $AgNO_3$ aqueous solution in a 250-ml Erlenmeyer flask and heated on water bath at 75 °C for 60 min. Reduction of silver nitrate to silver ions was confirmed by the color change from colorless to brown. The formation of silver nanoparticles was also confirmed by spectrophotometric determination. The fully reduced solution was centrifuged at 5000 rpm for 30 min. The supernatant liquid was discarded and the pellet obtained was redispersed in deionized water. The centrifugation process was repeated two to three times to wash off any absorbed substances on the surface of the silver nanoparticles.

2.2.1. UV–Vis spectra analysis

UV–Vis spectral analysis was done by using GBC UV–Visible Cintra 101/202/303/404 spectrophotometer. UV–visible absorption spectrophotometer with a resolution of 1 nm between 300 and 700 nm possessing a scanning speed of 300 nm/min was used. The reduction of pure Ag^+ ions was monitored by measuring the UV–Vis spectrum of the reaction medium after diluting a small aliquot of the sample into deionized water. One milliliter of the sample was pipetted into a test tube and diluted with 4 ml of deionized water and subsequently analyzed at room temperature. The nanoparticle solution showed maximum absorbance at 438 nm.

2.3. Fixation of different parameters

2.3.1. Temperature

The above mentioned procedure was repeated for optimization of temperature, where the reaction temperature was maintained at 37, 45, 50, 65, 75, 80 and 90 °C, respectively, using water bath. The absorbance of the resulting solutions was measured spectrophotometrically.

2.3.2. pH

The above mentioned procedure was repeated for optimization of pH where the reaction pH was maintained at 4, 7 and 8, respectively. The pH was adjusted by using 0.1 N HCl and 0.1 N NaOH. The absorbance of the resulting solutions was measured spectrophotometrically.

2.3.3. Time

The above mentioned procedure was repeated to optimize the time required for the completion of reaction, where the reaction was monitored from 0 to 70 min at 10 min time interval. The absorbance of the resulting solutions was measured spectrophotometrically.

2.3.4. Concentration of silver nitrate solution

The above mentioned procedure was repeated for optimization of silver nitrate concentration, where the reaction was monitored using different concentration of silver nitrate (0.25, 0.5, 0.75, 1, 2 and 5 mM). The absorbance of the resulting solutions was measured spectrophotometrically.

2.3.5. Concentration ratio of silver nitrate and leaf extract
The above mentioned procedure was also repeated for optimization of silver nitrate and leaf extract concentration required for the maximum production of silver nanoparticles, where the reaction was monitored by using different ratio of silver nitrate and leaf extract solution (0.5:19.5, 1.5:20, 1:19, 2:20 and 2:25). The absorbance of the resulting solutions was measured spectrophotometrically.

2.3.6. Stability study
The stability of the resultant solution was determined at room temperature, at interval of 12 h, for 30 days.

2.4. Transmission electron microscopy (TEM)

TEM technique was employed to visualize the size and shape of Ag nanoparticles. The 200 kV Ultra High Resolution Transmission Electron Microscope (JEOL-2010) was used. TEM grids were prepared by placing a drop of the particle solution on a carbon-coated copper grid and drying under lamp.

2.5. FTIR analysis of dried biomass after bio-reduction

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 5000 rpm for 10 min the supernatant liquid was decanted. The resulting suspension was redispersed in 10 ml sterile distilled water and centrifugation process was repeated for three times. Thereafter, the purified suspension was freeze dried to obtain dry powder. Finally, the dried nanoparticles were analyzed by FTIR-JASCO 4100 spectrophotometer.

2.6. Antibacterial assays

The antibacterial assays were done on human pathogenic *Escherichia coli* and *Staphylococcus aureus* by standard disc diffusion method. Mueller Hinton (MH) agar medium was used to cultivate bacteria. Fresh overnight cultures were taken and 100 µl of inoculum were spread on the MH agar plates. Sterile paper discs of 5 mm diameter (containing 20 µg/ml silver nanoparticles) along with four standard antibiotic containing discs (30 µg/ml) were placed in each plate.

3. Results and discussion

It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Krishnaraj et al., 2010). As the mangosteen leaf extract was mixed with aqueous solution of the silver nitrate, it started to change the color from watery to brown due to reduction of silver ion; which indicated the formation of silver nanoparticles. It is generally recognized that UV–Vis spectroscopy could be used to examine size and shape-controlled nanoparticles in aqueous suspensions (Shrivastava and Dash, 2009). Fig. 1 shows the UV–Vis spectra recorded from the reaction medium after heating the solution at 75 °C for 60 min. Absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak at 438 nm and broadening of peak indicated that the particles are polydispersed.

Different parameters were optimized including temperature, pH, concentration of silver nitrate, concentration ratio of silver nitrate and mangosteen leaf extract, and time which had been identified as factors affecting the yields of silver

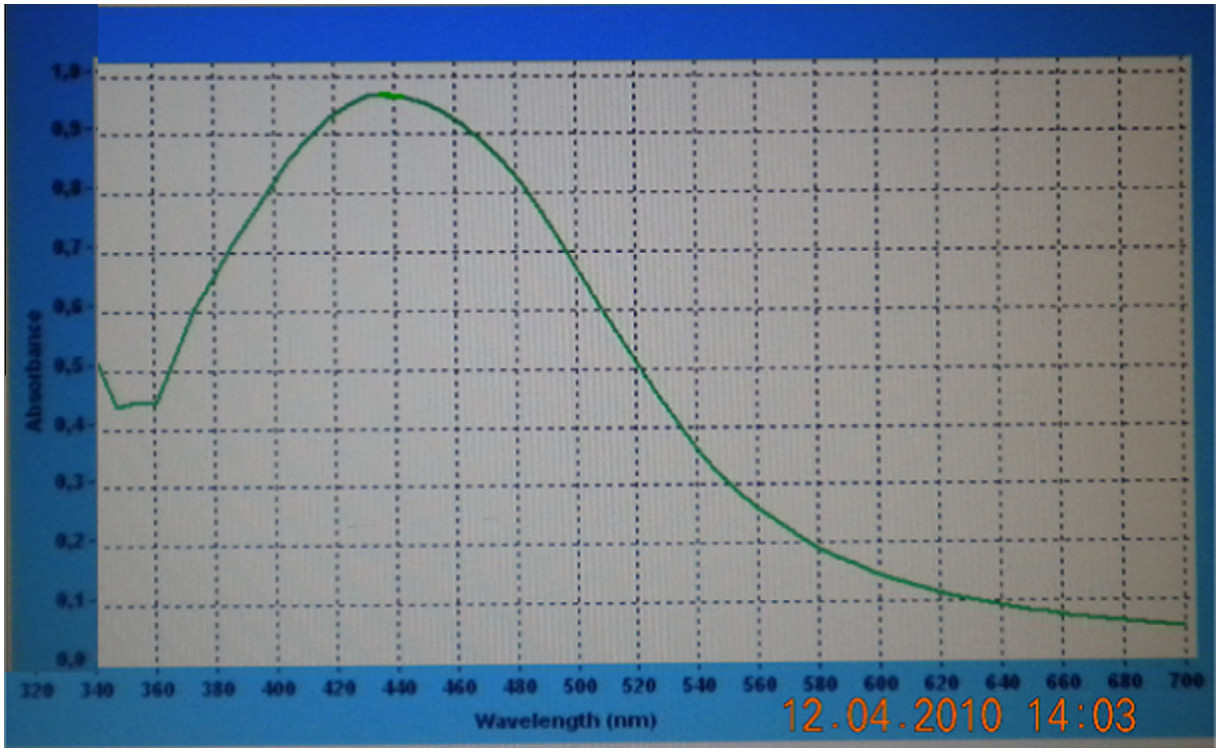

Figure 1 Absorbtion spectrum of silver nanoparticles.

nanoparticles. The first factor considered was temperature, as the temperature increased, the rate of silver nanoparticles formation also increased. The size is reduced initially due to the reduction in aggregation of the growing nanoparticles. Increasing the temperature beyond a point (75 °C) aids the growth of the crystal around the nucleus (Fig. 2) which leads to decrease in absorption.

The second factor considered was pH of the reaction medium. Acidic condition suppresses the formation of silver nanoparticles but the basic condition enhances the formation of silver nanoparticles. Large nanoparticles were formed at lower pH (pH 4), where as small and highly dispersed nanoparticles were formed at high pH (pH 8). At low pH, the aggregation of silver nanoparticles to form larger nanoparticles was believed to be favored over the nucleation (Fig. 3). At higher pH, however, the large number of functional groups available for silver binding facilitated a higher number of silver nanoparticles to bind and subsequently form a large number of nanoparticles with smaller diameters. But at higher pH agglomeration of nanoparticles took place (Fig. 4).

The third factor was the time required for the completion of reaction. As the duration of reaction increases, more silver nanoparticles are formed. Due to the instability of the silver nanoparticles formed, an optimum duration is required, as silver nanoparticles agglomeration after the optimum duration resulting in larger particle sizes. The optimum time required for the completion of reaction from our study was 60 min (Fig. 5). The next factor was concentration of silver nitrate solution. Different concentration of silver nitrate solution was used to get maximum silver nanoparticles. We got a maximum yield with 1 mM silver nitrate solution.

Besides that, the ratio of silver nitrate solution (1 mM) and the leaves extract was altered to investigate the optimum composition to maximize the yield of silver nanoparticles. Enough leaf extract must be added to reduce the silver nitrate present in solution. It was found that the optimum ratio for the reaction is 1:19 based on the number of trials and the optimum yield.

The overall optimized reaction condition was: temperature = 75 °C, time = 60 min, concentration of silver nitrate = 1 mM, pH – neutral, and the concentration ratio of silver nitrate and mangosteen leaf extract = 1:19. The prepared silver nanoparticles are stable up to 30 days at room temperature (Fig. 6).

Fig. 7 shows the TEM micrograph of the synthesized Ag nanoparticles. It is observed that most of the Ag nanoparticles were spherical in shape. A few agglomerated Ag nanoparticles were also observed in some places, thereby indicating possible sedimentation at a later time. Fig. 8 shows the particle size frequency histogram taken from a large number of micrographs. It is evident that there is variation in particle sizes and the average size estimated was 35 nm and the particle size ranged from 6 to 57 nm.

FTIR analysis was used for the characterization of the extract and the resulting nanoparticles. Absorbance bands were observed at 1619, 1522, 1340, 1160 cm^{-1}. These absorbance bands are known to be associated with the stretching vibrations for –C–C– [(in-ring) aromatic], C–O–C (ethers) and C–O (–C–OH). In particular, the 1160 cm^{-1} band arises most probably from the C–O of aromatic-OH group (such as hydroxyflavones and hydroxyxanthones). The total disappearance of this band after the bio-reduction may be due to the fact

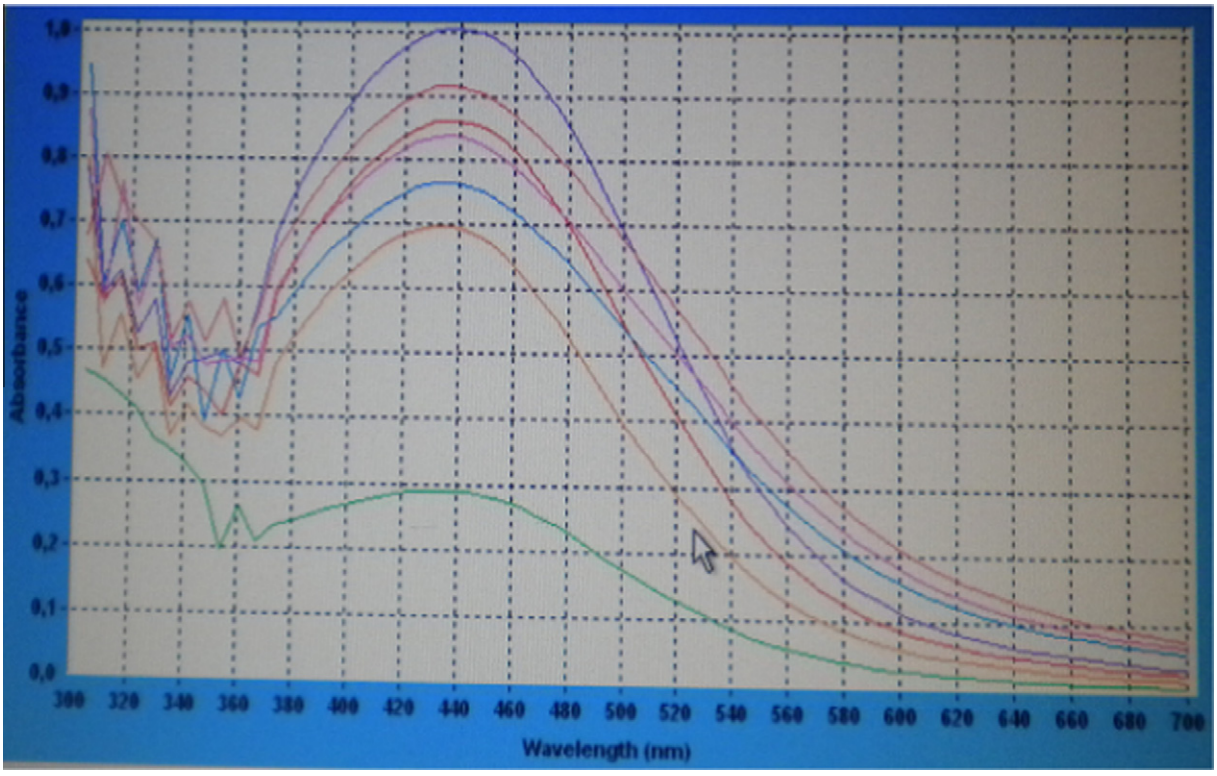

Figure 2 Effect of reaction temperature on production of silver nanoparticles. —— = 37 °C, —— = 45 °C, —— = 50 °C, —— = 65 °C, —— = 75 °C, —— = 80 °C, —— = 90 °C.

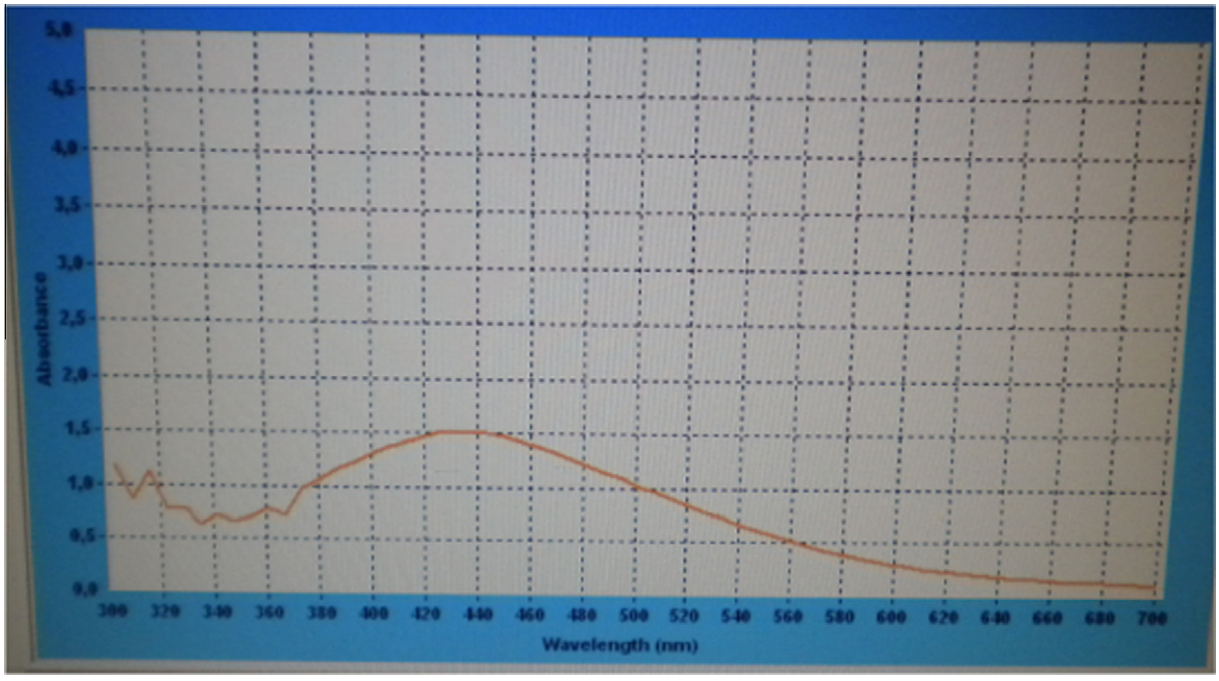

Figure 3 Effect of reaction pH (acidic) on production of silver nanoparticles.

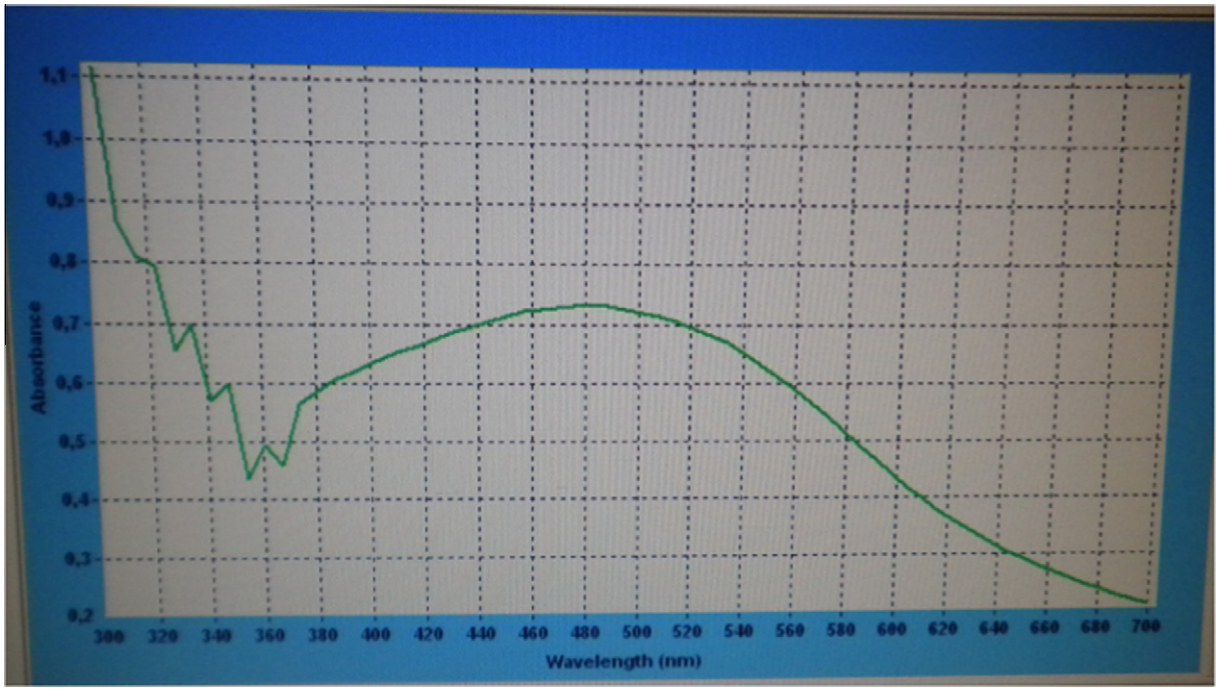

Figure 4 Effect of reaction pH (basic) on production of silver nanoparticles.

that the polyols are mainly responsible for the reduction of Ag ions, whereby they themselves get oxidized to unsaturated carbonyl groups leading to a broad peak at 1660 cm^{-1} (for reduction of Ag).

Furthermore the nanoparticle syntheses by green route are found to be highly effective against multi-drug resistant human pathogenic bacteria. Antibacterial activity of silver nanoparticles against *E. coli* and *S. aureus* were investigated and compared with the standard drug. For *E. coli*, the zone of inhibition of amikacin (30 μg/ml) and cefalotaxin (30 μg/ml) were 20 and 36 mm, respectively while the zone of inhibition of silver nanoparticles (20 μg/ml) was 15 mm (Fig. 9a). For *S. aureus*, the zone of inhibition of penicillin (30 μg/ml) and sulpha methoxazole (30 μg/ml) were 40 and 25 mm, respectively while the zone of inhibition of silver nanoparticles (20 μg/ml) was 20 mm (Fig. 9b). Antibacterial effects of Ag

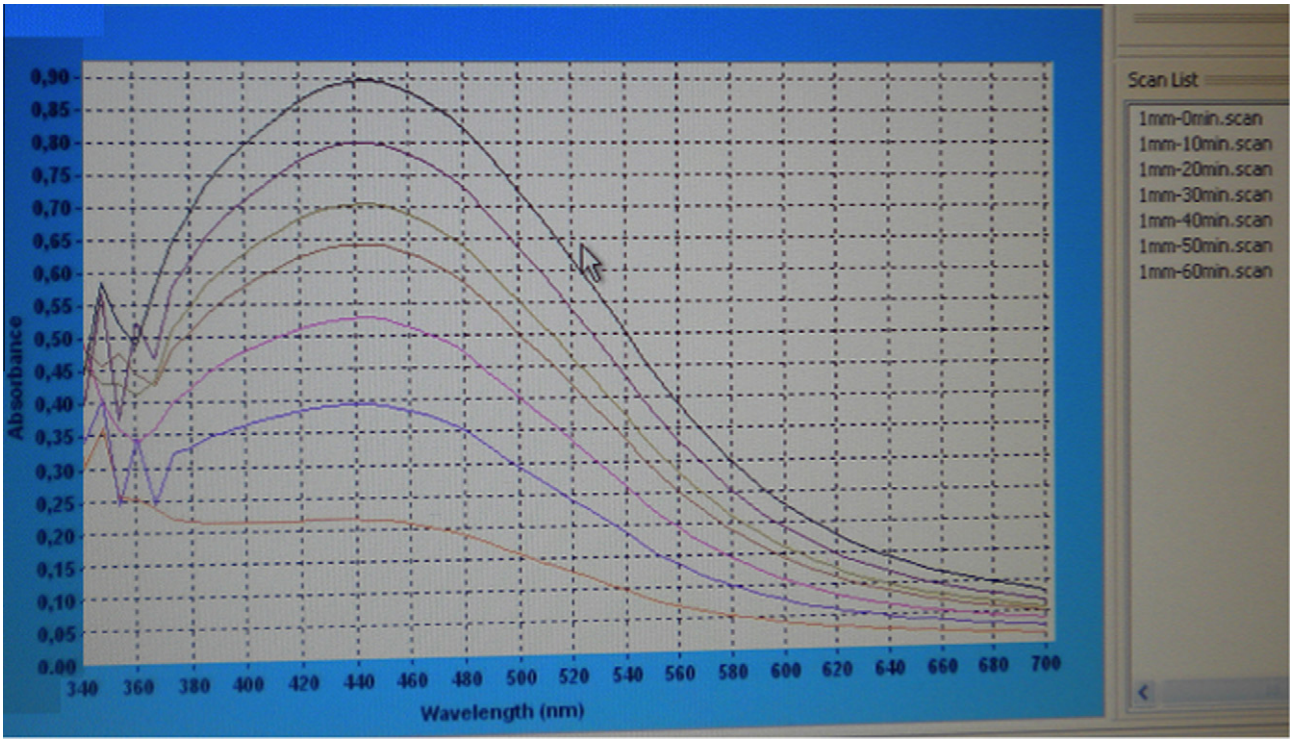

Figure 5 Effect of reaction time (in min) on production of silver nanoparticles. —— = 0 min, —— = 10 min, —— = 20 min, —— = 30 min, —— = 40 min, —— = 50 min, —— = 60 min.

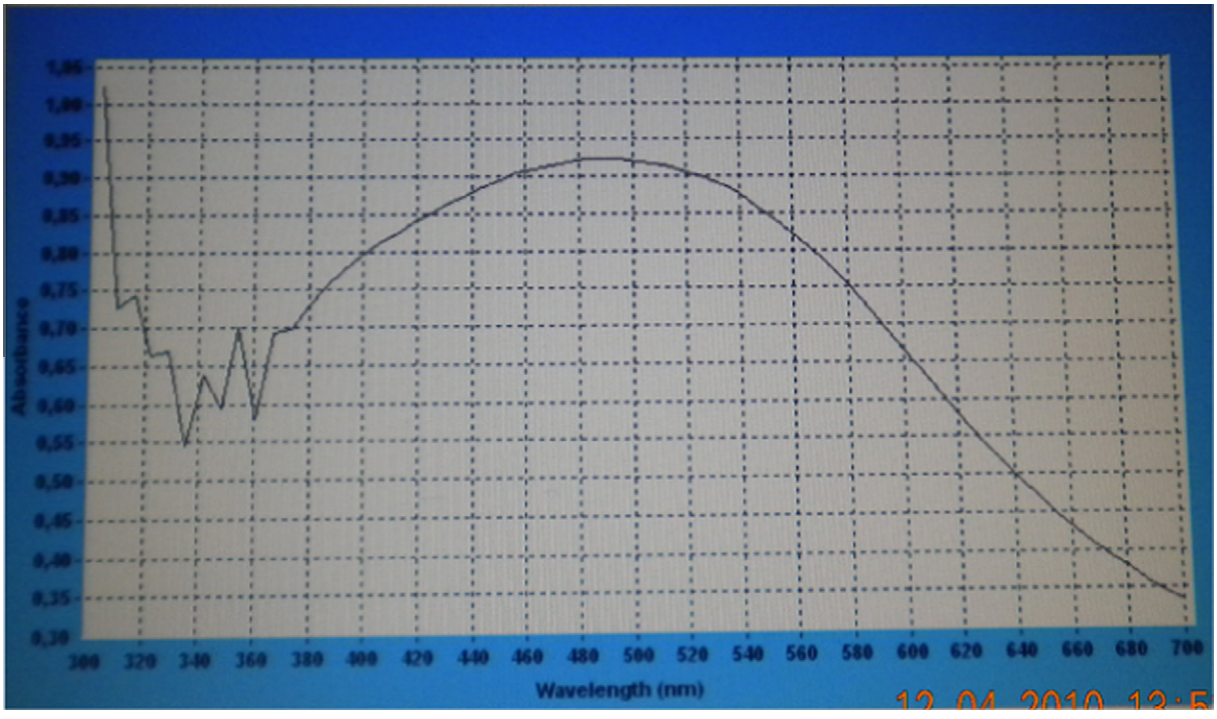

Figure 6 Absorption spectrum of prepared silver nanoparticles on 31st day.

nanoparticles obeyed a dual action mechanism of antibacterial activity, i.e., the bactericidal effect of Ag^{+} and membrane-disrupting effect of the polymer subunits. Antibacterial activity produced by the silver nanoparticles prepared by using mangosteen leaf extracts are not better than the antibacterial activity exhibited by silver nanoparticles prepared by using *Acalypha indica* leaf extracts (Krishnaraj et al., 2010) and papaya fruit extracts (Jain et al., 2009).

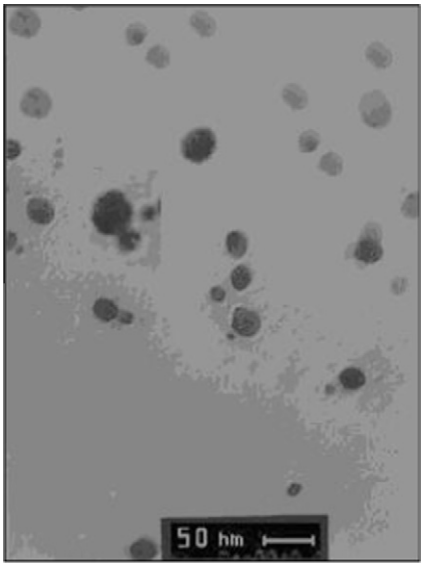

Figure 7 TEM image of the synthesized silver nanoparticles at a visible-light density of 1000 μmol/m^2 s (scale bars correspond to 50 nm).

4. Conclusion

A critical need in the field of nanotechnology is the development of a reliable and eco-friendly process for synthesis of metallic nanoparticles. We have demonstrated that use of a natural, low cost biological reducing agent, *G. Mangostana* leave extracts (aqueous) can produce metal nanostructures, through efficient green nanochemistry methodology, avoiding the presence of hazardous and toxic solvents and waste. The biosynthesized silver nanoparticles using mangosteen leaves extract proved excellent antimicrobial activity. The antimicrobial activity is well demonstrated by considerable zone of inhibition against *E. coli* and *S. Aureus*. The present study showed a simple, rapid and economical route to synthesize silver nanoparticles.

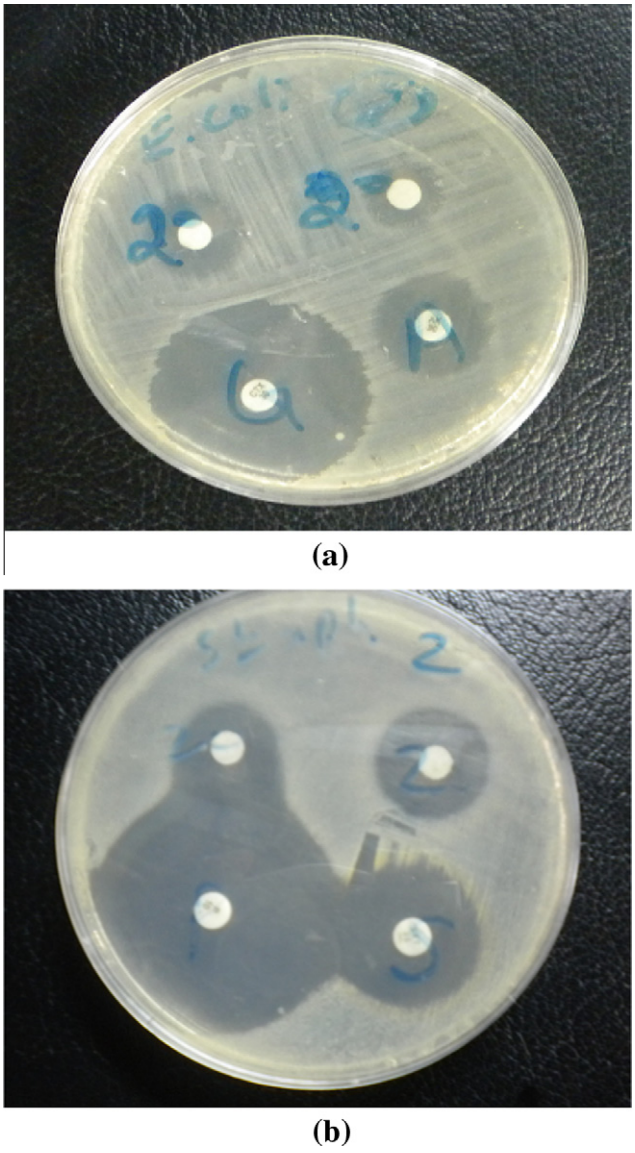

Figure 9 Activity of silver nanoparticles against (a) *E. coli* (b) *S. aureus*.

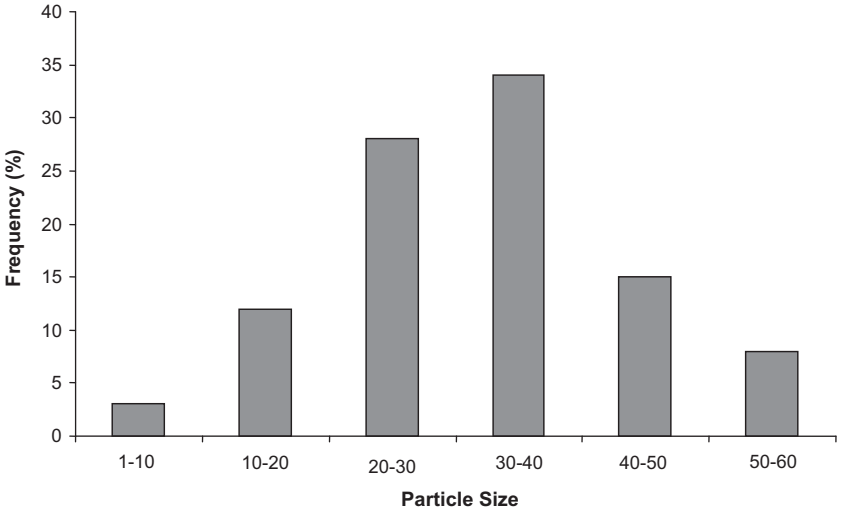

Figure 8 Particle size distribution of silver nanoparticles.

Applications of such eco-friendly nanoparticles with bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of nanoparticles.

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