Resource-Efficient Technologies 3 (2017) 303–308

Contents lists available at ScienceDirect

Resource-Efficient Technologies

journal homepage: www.elsevier.com/locate/reffit

Research paper

Green synthesis of silver nanoparticles using *Capsicum frutescence* and its intensified activity against *E. coli* ☆

Thangaraj Shankar[a], Perumal Karthiga[a], Kalaiyar Swarnalatha[a,*], Kalaiyar Rajkumar[b]

[a] *Manonmaniam Sundaranar University, India*
[b] *Madras Veterinary College, India*

ARTICLE INFO

Article history:
Received 25 July 2016
Revised 7 November 2016
Accepted 17 January 2017
Available online 6 March 2017

Keywords:
Silver nanoparticles
Sweet pepper fruit extract
Biosynthesis
Bactericidal
Capsicum frutescence

ABSTRACT

The purpose of this study was to expand a trouble free biological method for the synthesis of silver nanoparticles using the fruit extract of *Capsicum frutescence* (Sweet pepper) to act as reducing and stabilizing agent. Water soluble organics played a vital role for the reduction silver ions into silver nanoparticles. The fruit extract was exposed to silver ions and the resultant biosynthesized silver nanoparticles characterized by UV–Vis spectrophotometry indicated the surface plasmon resonance band at 385–435 nm. X-ray diffraction spectrum showed crystalline structure while scanning electron microscope analyses exposed the monodispersed distribution and particle size of 20–25 nm. The elemental analysis displayed strong signal at 3 keV that agrees to silver ions and confirms the presence of metallic silver. The antibacterial activity of silver nanoparticles was determined by agar well diffusion method against gram positive and gram negative bacteria. Maximum and minimum zones of inhibition were renowned against *Escherichia coli* (11.5 mm) and *Bacillus subtilis* (10.5 mm), respectively. This study exposed that silver nanoparticles retained good bactericidal activity at 80 µg/ml concentration.

1. Introduction

The exploit of green chemistry for the synthesis of biocompatible silver nanoparticles has gained substantial awareness in the latest years for prospective appliance in biomedicine. Metal nanoparticles are of interest in both research and technology, due to their particular properties not offered in isolated molecules or bulk metals. Because of these properties nanoparticles have many imperative applications in catalysis, sensing and imaging etc. Among the gracious metals (e.g. Ag, Pt, Au and Pd), silver (Ag) is the metal of abundance for prospective applications in the field of biological systems, living organisms and medicine. Due to their elite properties, silver nanoparticles (AgNPs) may have quite a lot of applications, such as catalysts in chemical reactions [1,2] electrical batteries and in spectrally discriminative coatings for absorption of solar energy [3,4] as optical elements, pharmaceutical works and in chemical sensing and biosensing [5–7]. The pace of synthesis of nanoparticles through plant extract is as good as to those of chemical methods and more rapidly than green synthesis by microorganisms.

The plant used in this research, belongs to the genus *Capsicum frutescence* (Sweet pepper) in the family Solanaceae [8]. The sharp taste of *Capsicum* peppers is due to a fusion of seven allied alkaloids of which capsaicin is the most ubiquitous. The substances responsible for the pungency are the capsaicinoid alkaloids. They are characterized by means of a high biological activity and their pharmacological, neurological and dietetic activities are well known. When used at minimum levels in the usual diet, they extensively decrease serum, myocardial and aortic entire cholesterol levels [9]. The biological activity mainly predicts the bactericidal activity. *Capsicum frutescence* fruit can be used for the synthesis of silver nanoparticles, since this fruit extract contains many secondary metabolites which potentially act as a best reducing and stabilizing agent of the silver ions. In this paper we report the synthesis of silver nanoparticles by reduction of Ag^+ with *C. frutescence* fruit aqueous extract. The formation of the nanoparticles was recorded by UV-Vis spectroscopy, whereas the size and shape were determined by scanning electron microscope (SEM). The relation of nanoparticles with *C. frutescence* fruit extract was confirmed

☆ Peer review under responsibility of Tomsk Polytechnic University.

* Corresponding author. Manonmaniam Sundaranar University, Tirunelveli, India. Fax: 0462 2334363.
E-mail address: swarnalatha@msuniv.ac.in (K. Swarnalatha).

https://doi.org/10.1016/j.reffit.2017.01.004

using FT-IR spectroscopy. The crystallite size and elemental position were authenticated via XRD and EDAX. The bactericidal effect of fruit extract and silver nanoparticles were evaluated with the help of suitable clinical pathogens like *Escherichia coli* and *Bacillus subtilis*.

2. Experimental methods

2.1. Chemicals

All the chemicals used for synthesis [Silver nitrate ($AgNO_3$), Potassium Bromide (KBr)], were of analytical grade from Merck Limited, Mumbai, India. Nutrient Agar, Nutrient Broth, Agar Agar, Muller Hinton Agar (MHA) purchased from Himedia Laboratories, Mumbai, India. The aqueous solutions were prepared using triple distilled water.

2.2. Preparation of fruit extract

Fresh and healthy fruits of *Capsicum frutesence* (Fig. 1) were washed several times with deionized water to remove the filth particles and then air dried to remove the residual moisture and cut in to small pieces. Twenty-five grams of fruit in a round bottomed flask with 100 ml deionized water and refluxed for half an hour, cooled at room temperature and filtered with Whatman No.1 filter paper.

2.3. Biosynthesis of silver nanoparticles

The nanoparticles were synthesized by known concentration of *C. frutesences* broth was interacted with 1 mM silver nitrate. For the reduction of silver ions, 10 ml of fruit extract make up to 100 ml volume in 250 ml Erlenmeyer flask. The flask was incubated in a rotary shaker at 150 rpm speed for a desired time at room temperature for the development of silver nanoparticles.

2.3.1. Characterization of synthesized silver nanoparticles

The nanoparticle solution thus obtained was purified by repeated centrifugation at 20,000 rpm for 30 min followed by redispersion of the pellet in distilled water. UV-vis spectra were recorded as a function of reaction time on a Perkin Elmer UV-Vis spectrophotometer operated at resolution of 1 nm. After drying of the purified silver particles, the structure was predicted by scanning electron microscopy (SEM, Hitachi S-2500C), energy dispersive X-ray spectroscopy (EDS, Sigma). The crystalline character of silver nanoparticles was confirmed with the help of XRD. The X-ray patterns were obtained in 2 theta configuration and the range was selected from 20° and 80°. These were performed by using Panalytical X'pert Powder X' Celerator Diffractometer. The alterations in the chemical group were confirmed by Fourier transform infra red spectroscopy (FT-IR, Jasco) by employing KBr pellet technique. The FT-IR spectra were taken at a resolution of 4 cm^1 in the transmission mode (4000-400 cm^{-1}).

Fig. 1. Fresh and healthy fruits of *Capsicum frutescence.*

2.3.2. Antibacterial activity of fruit extract and nanoparticles

The bacterial strains used were *E. coli* and *B. subtilis.* The strains were obtained from the Department of Microbiology, Sri Paramakalyani College, Alwarkurichi, Tamil Nadu. Stock cultures were maintained at 4 °C on slopes of nutrient agar. Dynamic cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller Hinton Broth (MHB) for bacteria. The cultures were incubated for 24 hours at room temperature. Agar well diffusion method [10] for bactericidal susceptibility was carried out according to standard method to assess the presence of antibacterial activity of the synthesized silver nanoparticle. The concentration of the nanoparticle used in the experiment was 20, 40, 60 and 80 µL. Well of about 6 mm diameter were made aseptically using gel puncture instrument. The plates were swabbed with gram negative strains like *E. coli* and a gram positive strain *B. subtilis.* Then the plates were incubated at 37 °C for 24 hours. Antibacterial activity was evaluated by measuring the diameter of the zone of inhibition around the well.

3. Results and discussion

3.1. Visual observation and UV-vis spectral analysis

The optical properties of silver nanoparticles were studied by absorption spectroscopy. The structural change of the particles can be easily examined by the UV-Visible absorption spectrum, which can help us to know the complex formation. It is the primary method to indicate the bioreduction of silver from aqueous silver nitrate solution to silver nanoparticles. Surface Plasmon resonance bands play a vital role in size, shape, morphology [11]. The synthesized Ag NPs exhibit reddish brown color due to the excitation of surface Plasmon resonance in Ag NPs. The optical absorption spectra of metal nanoparticles is dominated by the SPR, which shows a shift toward the red end or blue end depending upon the particle size, shape, state of aggregation and the surrounding dielectric medium [12]. After 24 hr the settling of synthesized silver nanoparticles at the bottom of the Erlenmeyer flask reveals the reduction of silver metal into silver nanoparticles was completed.

The secondary metabolite capsaicin alkaloid and other antioxidants present in the fruit extract acting both as reducing and stabilizing agent to form the nanoparticles. The reduction of silver ions and the development of nanoparticles occurred within hour due to excitation of surface Plasmon vibrations in the nanoparticles [13]. Different concentrations of silver nitrate were taken for the study to synthesize silver nanoparticles were analyzed using UV spectra of resonance band at around 380–450 nm but 1 mM shows the band at around 385 nm may be due to the strong activity of capsaicin. 2 mM- 5 mM concentration Plasmon band were similar to previously reported literature [14]. If we increase the silver nitrate concentration simultaneously, there is increase in wavelength up to 450 nm shown in Fig. 2. The slight variation leads to slight changes in shape and particle size. The results were similar to the reported literature with slight variations [15]. The intensity of the SRP peaks increases as reaction time increases, which designates the increase in concentration of the silver nanoparticles. The result reflects that the Ag nanoparticles prepared by *Capsicum frutesence* fruit extract

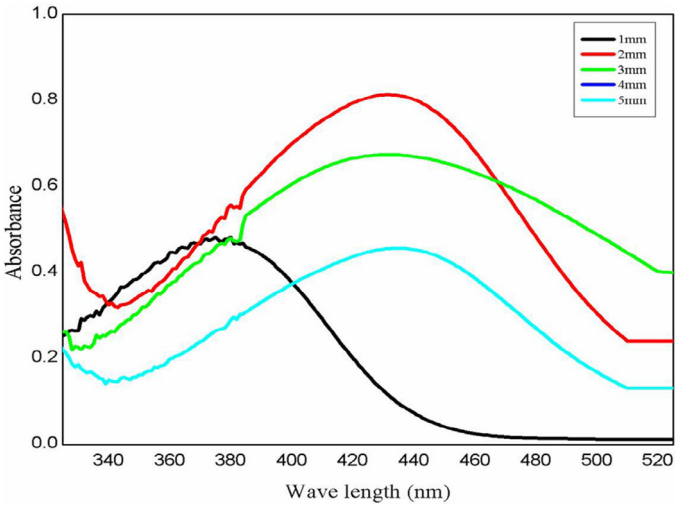

Fig. 2. UV-Vis spectra of biosynthesized silver nanoparticles at different concentrations from 1 mM to 5 mM.

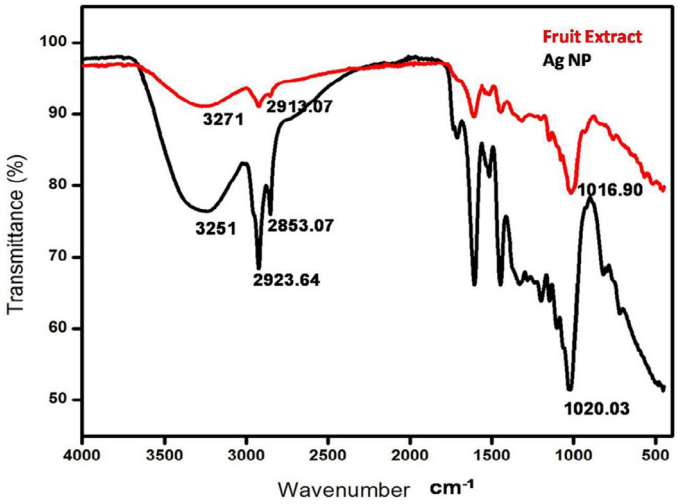

Fig. 3. FT-IR spectra of the aqueous fruit extract and biosynthesized silver nanoparticles.

are constant without aggregation This similar report were matched with *Psidium guajava* leaf extract [16].

3.2. Fourier transform infrared analysis

FTIR spectroscopic studies were carried out to find out the possible chemical changes present in the extract. The spectra were recorded before and after addition of silver nitrate solution. The broad and narrow peaks of the fruit extract and nanoparticles shown in Fig. 3. The peak at 3271 cm^{-1} belongs to N–H stretching of amine and the weak band at 2913.07 cm^{-1} indicates the H–C–H symmetric stretching of alkanes. Capsaicin which is alkaloids having N–H stretch, this specific compound involved in the synthesis and act as a backbone for the nanoparticles formation. The band at 1016.90 cm^{-1} corresponds to C–O stretching in fruit extract. Simultaneously in nanoparticles the band at 3251 cm^{-1} shows the hydrogen bonded O–H stretch phenols and alcohols. The band at 2923.64 cm^{-1} and 2853.07 cm^{-1} represents the H–C–H symmetric stretching of alkanes. The strong peak at 1020.03 cm^{-1} denotes the C–O stretching of ethers. Some of the secondary metabolites, proteins may also bind with the silver ions to the formation of silver nanoparticles.

3.3. XRD analysis

The crystalline character of silver nanoparticles confirmed from the X-ray diffraction (XRD) pattern. The prominent peaks [38.48°, 44.39°, 64.92°,77.67°] are indexed as (111), (200), (220), (311) shown in Fig. 4. These peaks indicates that the crystals are anisotropic reported by Daizy Philip [17]. The average size of nanoparticles is calculated by Scherrer's equation by determining the width of the prominent peak which was found to be 19 nm also which was agreed with the SEM measurement. Generally, the broad peak in this pattern will attribute the size of the particles [18]. The obtained XRD pattern was compared and matched with the joint committee powder diffraction standards JCPDS file No. 04–0783. It might be considered that the unassigned peaks are owing to the crystallization of bioorganic phases that occur on the surface of the silver nanoparticles [19].Our results were well agreed with *Calliandra haematocephala* leaf extract [20].

3.4. Energy dispersive X-ray spectroscopy

This technique was to verify the presence of specific element and it showed some small peaks along with the specific element as C and O. The peak observed around 3 KeV shown in Fig. 5 predicts the binding energy of AgL which proves the confirmation of pure silver due to the surface plasmon resonance [21]. Some weaker elements like C and O also appeared due to the minor impurities. The amount of energy released by transferring electrons depends on which shell its transferring from as well as which shell it is transferred to furthermore, the atom of every element releases x-ray with its unique amount of energy during the transferring process.

3.5. Scanning electron microscopy

SEM Analysis is used to visualize the size and shape of the nanoparticles. SEM micrographs of silver nanoparticles are given in Fig. 6 with different magnifications. The absorption of Ag NPs shows the broad peak which represents that the particles were in monodispersed in nature. In this, the secondary metabolites

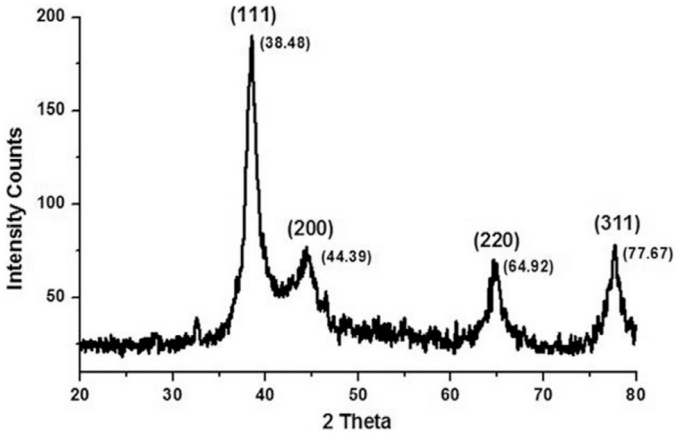

Fig. 4. XRD pattern of biosynthesized silver nanoparticles using fruit extract of *Capsicum frutescence.*

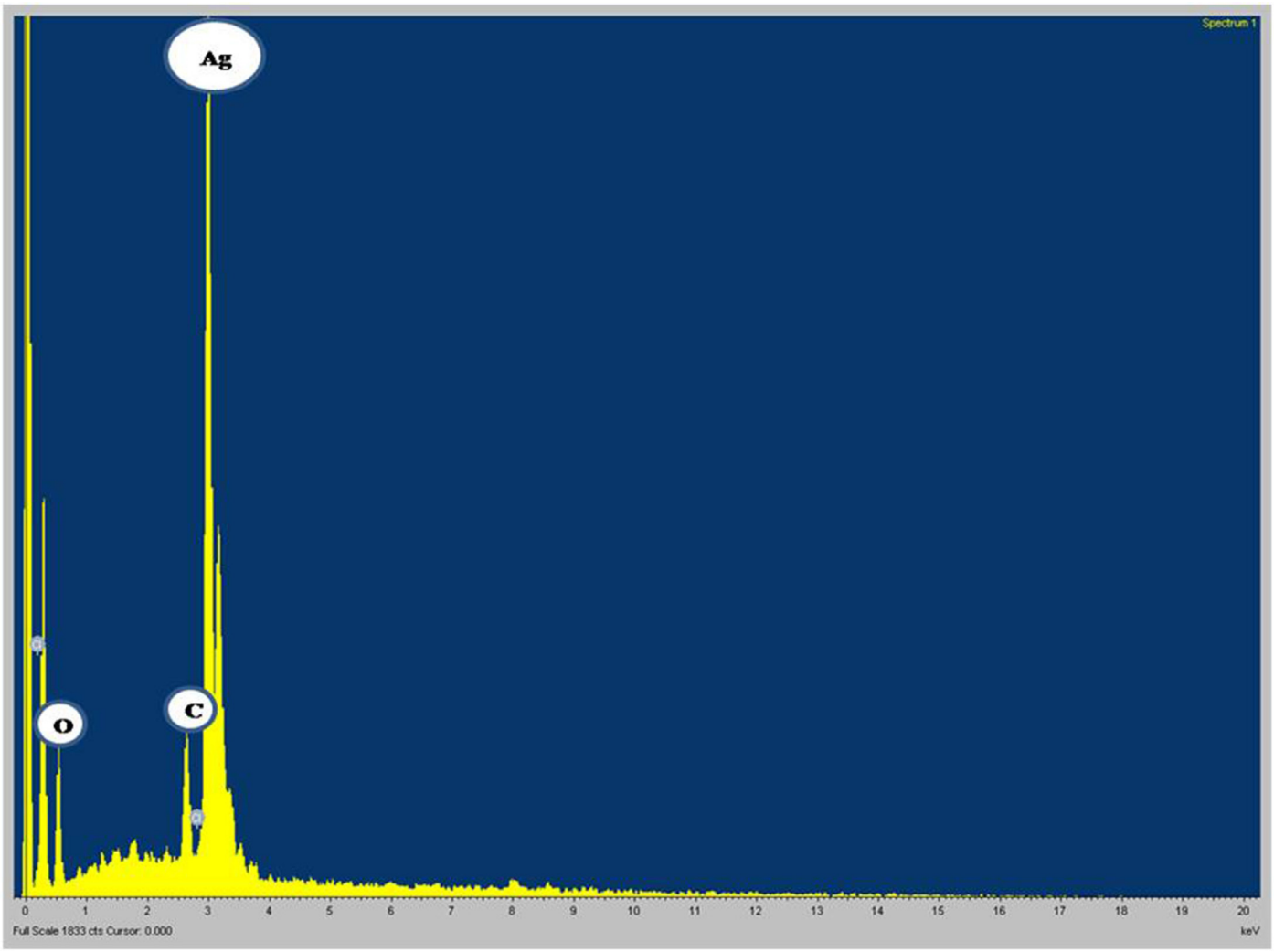

Fig. 5. EDX profile of biosynthesized silver nanoparticles at 3 KeV.

present in the plant also plays a vital role in the morphological changes. Among the secondary metabolites capsaicin, this was the major compound present in the fruit extract of *C.frutescence* involved in the synthesis part. The particles get aggregated with one another and the image was taken after 24–48 hr, this similar result was discussed by Chandran et al. [22]. However the particles aggregate may be due to cross linking. The particle size obtained from SEM images is well correlated with the particle size determined from XRD using according to the Scherrer formula and the average of the synthesized nanoparticles was in the range of 15–20 nm.

3.6. Bactericidal efficacy of fruit extract and silver nanoparticles

Silver nanoparticles interactions toward the clinical pathogens are depends on the size and shape of the nanoparticles [23]. Antibacterial activity is investigated against *E. coli* and *B. subtilis* for silver nanoparticles and fruit extract by well diffusion and disc method. It is well known *E. coli* is the common clinical pathogen causes intestinal infection includes diarrhea, abdominal pain, and fever. More severe cases can lead to bloody diarrhea, dehydration, or even kidney failure. So the specific strain was selected.

Table 1
Antibacterial activity of fruit extract and silver nanoparticles with positive and negative controls against the human clinical pathogens (*E. coli and B. subtilis*).

S. no	Antibacterial agents	*Escherichia coli*	*Bacillus subtilis*
1	Fruit extract	8.8 ± 1	7.4 ± 1
2	Silver nitrate	9.9 ± 1	8.5 ± 1
3	Silver nanoparticles	14.5 ± 1	10.5 ± 1
4	Antibiotic disc	11.1 ± 1	9.8 ± 1

The increased zone of inhibition was good at gram negative bacteria *E. coli* when compared to *B. subtilis*. The zone of inhibition around each well with silver nanoparticles and fruit extract for both strains represented in Figs. 7 and 8. The zone of inhibition is also clearly reported in Table 1. Positive and negative control was used against both strains. The mechanisms of antibacterial activity of silver nanoparticles are by binding on the membrane of microorganisms through electrostatic interactions, cell wall disruption andaffecting the intracellular processes such as DNA, RNA and protein synthesis [24–27]. It shows immense possibilities in biomedical applications. Similar observations were found with *Allium cepa* [28].

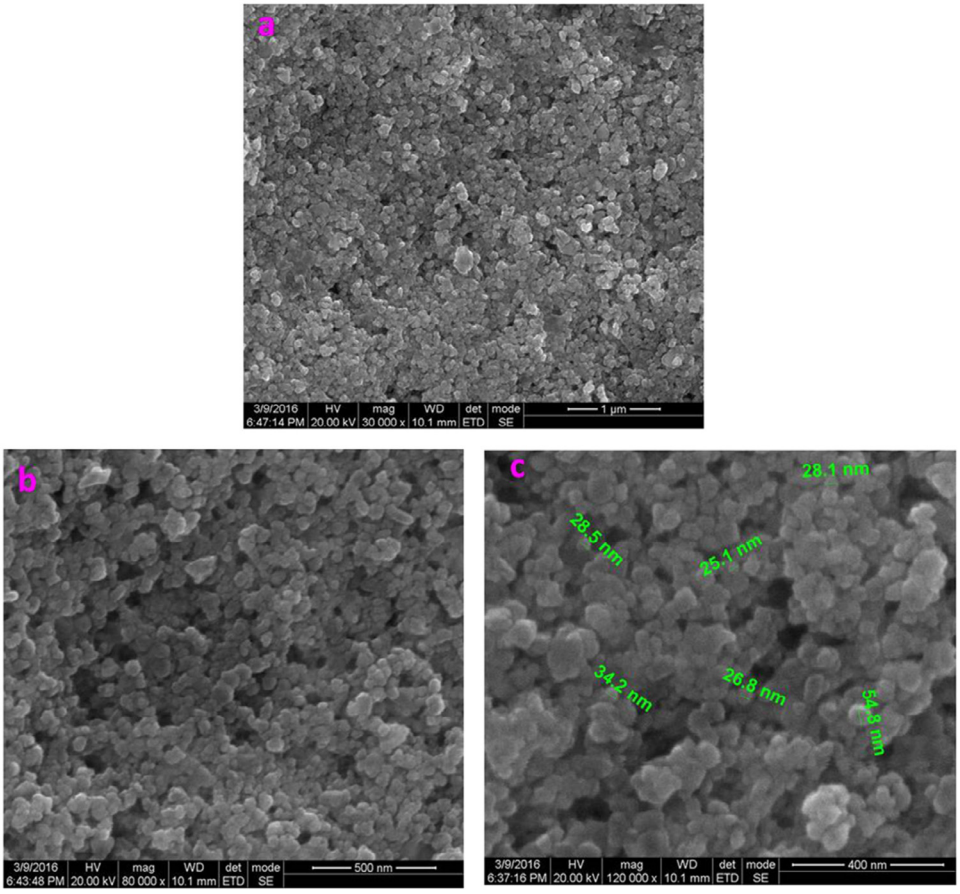

Fig. 6. SEM images of silver nanoparticles synthesized using *Capsicum frutescence* (a) 1 µm (b) 500 nm and (c) 400 nm.

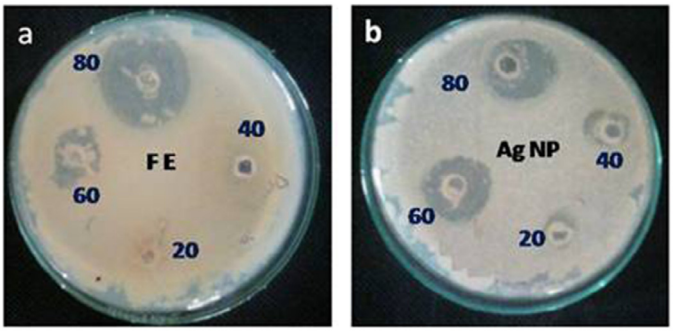

Fig. 7. Antibacterial activity of fruit extract FE (a) and silver nanoparticle Ag NP (b) against *Escherichia coli.*

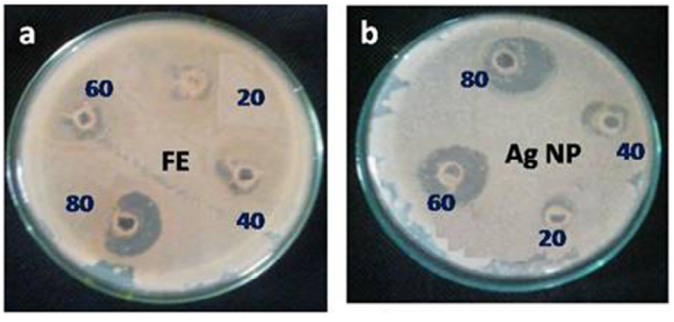

Fig. 8. Antibacterial activity of fruit extract FE (a) and silver nanoparticle Ag NP (b) against *B. subtilis.*

4. Conclusion

In the present study we found that fruits also act as a best source for the formation of silver nanoparticles. This green chemistry approach toward the nanoparticles has immense merits like economic viability. The green synthesized silver nanoparticles show excellent bactericidal activity against the gram negative bacteria and moderate activity against the gram positive bacteria. Our findings indicating that biosynthesized silver nanoparticles using the plant source will afford unique opportunities toward the growth of nanomedicine and thus has the budding for utilize in biomedical applications.

Acknowledgement

Author, PK gratefully acknowledges the UGC-PDFWM, University Grants Commission, NewDelhi, India for funding research development (Ref No: F. 15-1/2012-13/PDFWM-2012-13-SC-TAM-21783) (SA-II) – dated 18.04.2014. We would like to acknowledge the support of Dr. G.Annadurai, Centre for Environmental Sciences, Alwarkurichi, for microbiological studies.

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