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Synthesis of Silver Nanoparticles Using *Setaria italica* (Foxtail Millets) Husk and its Antimicrobial Activity

B. Venkataramana, S. Siva Sankar, A. Sai Kumar and B. Vijaya Kumar Naidu
Department of Materials Science and Nanotechnology, Yogi Vemana University, Kadapa, 516 003, India

Corresponding Author: B. Vijaya Kumar Naidu, Department of Materials Science and Nanotechnology, Yogi Vemana University, Kadapa, 516003, India Tel: +918562225451 Fax: +918562225419

ABSTRACT

In this study, we report a simple and eco-friendly synthesis of silver nanoparticles using natural product, *Setaria italica* husk. The formation of silver nanoparticles was confirmed and characterized by using UV-Vis spectrophotometer, X-ray diffraction, Fourier transform infrared spectroscopy, scanning electron microscopy, energy dispersive X-ray analysis, particle size analyzer and transmission electron microscopy for their size, distribution and morphology. Average particle size of the synthesized silver nanoparticles was found to be 30 nm. Antimicrobial activity of the synthesized silver nanoparticles was studied against Gram positive and Gram negative bacteria, *Staphylococcus* and *Escherichia coli* sps., respectively.

Key words: Natural product, *Setaria italica* husk, silver nanoparticles, antimicrobial activity

INTRODUCTION

Nanotechnology is a multidisciplinary research field that emerges from physical, chemical, engineering and materials science with novel techniques and produces material at nanoscale (Narasimha *et al*., 2011; Vanaja *et al*., 2013). This technology is mainly concerned with the synthesis of nanoparticles of variable size, shape, chemical composition and controlled dispersity and their potential use for human benefits (Vanaja *et al*., 2013). Generally, particles less than 100 nm are considered as nanomaterials (Thombre *et al*., 2013). Nanoparticles are very important and they have unique properties when compared to bulk materials, i.e., large surface area to volume ratio. Due to the high surface volume and smaller size, nanoparticles are involved in a many applications such as catalysis (Santos *et al*., 2012; Venkatesham *et al*., 2014), drug delivery (Khan *et al*., 2014; Sripriya *et al*., 2013), biosensors (Ma *et al*., 2005), biolabelling (Jaidev and Narasimha, 2010), medical (Vahabi and Dorcheh, 2014), water purification (Pradeep and Anshup, 2009), electrical (Chen *et al*., 2009), optics (Murphy *et al*., 2005) etc. It is a well-known fact that silver ions and silver-based compounds are highly toxic to microorganisms includes major species of bacteria. This aspect of silver makes it an excellent choice for multiple roles in the medical field. Silver is generally used in the nitrate form to induce antimicrobial effect but when silver nanoparticles are used, there is a huge surface area available for the microbe to be exposed. Silver and silver nanoparticles have been found to be very useful in preventing infection in burns and open wounds. Silver nanoparticles have also been reported to possess antifungal, antiviral and antiplatelet activity (Mie *et al*., 2014) along with excellent antimicrobial activity (Prabhu and Poulose, 2012; Mie *et al*., 2014; Jaidev and Narasimha, 2010). Though silver nanoparticles find use in many antibacterial applications the action of this metal on microbes is not fully known. It has

been hypothesized that silver nanoparticles can cause cell lysis or inhibit cell transduction. There are various mechanisms involved in cell lysis and growth inhibition (Pradeep and Anshup, 2009). Silver nanoparticles are being used as antimicrobial agents in many public places such as railway stations and elevators in China and they have showed good antimicrobial action. Silver ions are used in the formulation of dental resin composites; in coatings of medical devices; as a bactericidal coating in water filters as an antimicrobial agent in air sanitizer sprays, pillows, respirators, socks, wet wipes, detergents, soaps, shampoos, toothpastes, washing machines and many other consumer products as bone cement and in many wound dressings to name a few. Though there are various benefits of silver nanoparticles, there is also the problem of nanotoxicity of silver. There are few reports which suggest that the nanoparticles can cause various environmental problems, though there is a need for more studies to be conducted to conclude that there is a real problem with silver nanoparticles (Prabhu and Poulose, 2012). Recent studies have focused on the use of silver nanoparticles as anticancer activity against breast cancer cells (Rashidipour and Heydari, 2014; Rath *et al*., 2014). Elechiguerra *et al*. (2005) have demonstrated that silver nanoparticles undergo a size dependent interaction with HIV-1, especially in the range of 1-10 nm when attached to the virus. It has been suggested that silver nanoparticles interact with the HIV-1 virus via preferential binding to the gp120 glycoprotein knobs. Due to this interaction, silver nanoparticles inhibit the virus from binding to host cells as demonstrated *in vitro* studies (Elechiguerra *et al*., 2005).

Silver nanoparticles are generally produced by chemical methods including reduction of silver salts such as silver nitrate, silver sulphate by different reducing agents such as hydrazine hydrate, ethanol, isopropyl alcohol and polyvinyl alcohol (Malina *et al*., 2012; Lu *et al*., 2015) and also by using other methods such as the sol-gel route (Perumal *et al*., 2014) and chemical precipitation. Silver nanoparticles can also be produced by physical methods including, γ-radiation (Zhu *et al*., 1997), microwave irradiations (Yin *et al*., 2004) and ultra sonication vibrations (Jiang *et al*., 2004). In recent years, the approach of production of silver nanoparticles was shifted towards less harmful and eco friendly biological methods which involve extracts from plants such as *Capsicum annuum*, *Coriandrum sativum* (Sathyavathi *et al*., 2010), *Grewia flaviscences* (Sana *et al*., 2015) and *Ocimum sanctum* (Rao *et al*., 2013). In plant extract biomolecules like proteins, phenols and flavonoids not only play a role in reducing the ions to the particles but also play an important role in stabilizing the nanoparticles (Ahmad *et al*., 2011). Synthesis of nanoparticles can be brought about by using microorganisms such as bacterial species like *Bacillus subtilis* (Yoon *et al*., 2007) and fungi like *Penicillium* sps. (Kathiresan *et al*., 2009). Biosynthetic methods utilize either biological microorganisms or plant extracts which have emerged as a simple and possible alternative to chemical and physical methods.

In this study we are reporting the synthesis of silver nanoparticles by a 'Eco-Friendly' route using extract of *Setaria italica* husk. *Setaria italica* is commonly known as Foxtail millet. In India it is chiefly cultivated in Andhra Pradesh and Tamilnadu. Due to the presence of high fiber content, it is suggested as a food for diabetic patients in India. The aqueous extract of *Setaria italica* seeds have excellent antihyperglycemic and hypolipidemic activities and thus have great potential as a source for natural health products (Sireesha *et al*., 2011). The synthesized silver nanoparticles are characterized by using UV-vis spectrophotometer, Fourier Transform Infra Red spectroscopy (FTIR), X-ray Diffractometer (XRD), Particle Size Analyzer, Field Emission Scanning Electron Microscopy (FESEM) and Transmission Electron Microscopy (TEM). Antimicrobial activity of the silver nanoparticles is also studied.

MATERIALS AND METHODS

Materials: Silver nitrate ($AgNO_3$) was purchased from Sd-Fine Chemicals, Mumbai, India. *Setaria italica* husk was collected from crops near the places of Pathikonda, Kurnool (Dist), Andhra Pradesh, India. Double distilled water was used throughout our studies.

Methods

Preparation of *Setaria italica* husk extract: *Setaria italica* husk was washed several times with distilled water to remove dust and 5 g of husk was added to 100 mL distilled water and kept for boiling at 80°C for 20 min. Then the solution was cooled and filtered through Whatman No. 1 filter paper. The extract solution obtained was stored at 4°C for further use.

Synthesis of silver nanoparticles: In a typical reaction procedure, 10 mL of husk extract was added to 90 mL of 1 mM $AgNO_3$ solution and kept for stirring at dark condition for about 6 h. The reduction of Ag^+ ions to silver nanoparticles was monitored by measuring UV-Vis spectra at regular time intervals. After reduction, silver nanoparticles were isolated by centrifugation at 5000 rpm for 20 min and then washed with water repeatedly under centrifugation. Finally the nanoparticles were dried and stored.

Characterization studies: The UV-Vis spectroscopy is primary method to confirm the formation of silver nanoparticles. UV-visible spectra were recorded on a UV-Visible spectrophotometer (Lab India, Mumbai, Model 3092) between 300-600 nm. The X-ray diffraction technique (XRD) is used to analyze the metallic nature of silver particles. The XRD pattern of dried silver nanoparticles was recorded on Rigaku mini 600 using Cu Kα radiation X-ray diffractometer. Dried silver nanoparticles were grinded with KBr to make pellet and Fourier Transforms Infra-Red spectroscopy (FTIR) spectra was recorded using Perkin Elmer Spectrophotometer in the region of 4000-400 cm^{-1}. Field emission scanning electron microscopy with energy dispersive X-ray analysis (FESEM EDAX) was carried out on SUPRA^{TM} 55 with co-relatively microscope SEM machine. Dried powdered sample was placed on the SEM grid and the images were taken for size and morphology. Energy dispersive X-ray spectroscopy (EDAX) spectra were also taken along with SEM images to find out the chemical composition. The synthesized silver nanoparticles were dispersed in distilled water and a drop of aqueous dispersion was placed on 200 mesh carbon coated copper grids and dried at ambient conditions for 10-12 h. Transmission electron microscopy images were taken using a JEOL 3010 at 200 Kv microscopy. Average particle size and distribution of silver nanoparticles was measured by using Zeta Sizer model Nano-S90 (Malvern U.K) using nanoparticle dispersion.

Anti microbial activity: Antimicrobial activity of silver nanoparticles was examined against the *Staphylococcus* and *E. coli* sps., bacteria. The study was carried out with 24 h active cultures of the selected bacterial strains. The bacterial strains were inoculated into nutrient agar medium. Four cavities of wells was made in a each plate and well No. 1 is filled with 10 µL of aqueous silver nanoparticles, well No. 2 is filled with *Setaria italica* husk, well No. 3 is filled with 20 µL of 1 mM $AgNO_3$ solution and well No. 4 is filled with 20 µL of 1% of streptomycin (antibiotic) solution. The plates were incubated in an incubator at 37°C overnight, after incubation, Zone Of Inhibition (ZOI) around the well were measured.

RESULTS AND DISCUSSION

The first instance one can identify the formation of silver nanoparticles is the characteristic color change when silver ions (Ag^+) are reduced to silver particles (Ag^0) (Narasimha *et al*., 2011;

Vanaja *et al*., 2013; Malina *et al*., 2012; Sathyavathi *et al*., 2010). In the present study as the reaction proceeds, the color of the reaction mixture changes to watery yellow and then to brown color at the end of reaction after 6 h. This is the primary indication that silver ions are reduced to fine silver nanoparticles. This change in color may be due to the excitation of the surface plasmon resonance. The formation of silver nanoparticles from its ions was monitored by measuring UV-Vis spectra of the reaction mixture at different intervals of time during the reaction and the same has been presented in Fig. 1a (Malina *et al*., 2012).

It is reported that, every metal nanoparticles has a characteristic absorption at particular wave length when exposed to UV-vis radiation. The peak in between 420-480 nm is very specific and characteristic for the presence of silver nanoparticles (Narasimha *et al*., 2011; Vanaja *et al*., 2013; Malina *et al*., 2012; Sathyavathi *et al*., 2010). At the beginning of the reaction no absorption was observed in the range of 420-480 nm. As the time progress absorption between 420-480 nm was observed and increased upto 6 h of the reaction. This suggests that silver ions were reduced to silver particles over a period of 6 h.

The stability of silver particle dispersion is very important for various applications. The synthesized silver nanoparticles dispersion in water showed stability for longer period of time. Even after six months, the silver particles are highly stable at room condition as evidenced by UV-vis spectra shown in Fig. 1b.

The XRD pattern of the silver nanoparticles shown in the Fig. 2. The XRD pattern shows peaks at 2θ values of 37.86, 44.08, 64.31 and 77.33° correspond to 111, 200, 220 and 311 planes, respectively. This confirms that silver is in pure crystalline form. The data obtained was compared with the database of Joint Committee on Powder Diffraction Standards (JCPDS file No. 04-0783) which is in good agreement with standard values.

The FTIR spectra of silver nanoparticles synthesized by using *Setaria italica* husk extract presented in Fig. 3. The band at 3420.41 cm^{-1} corresponds to O-H groups and also the H-bonded alcohols of millet extract. The band at 2936.78 cm^{-1} indicates presence of alkanes. The band at 2462.78 cm^{-1} corresponds to -C-H stretching present in millet extract. The band at 1651.73 cm^{-1} corresponds to primary amines. The band at 1384.49 cm^{-1} indicates C-H rock alkenes and

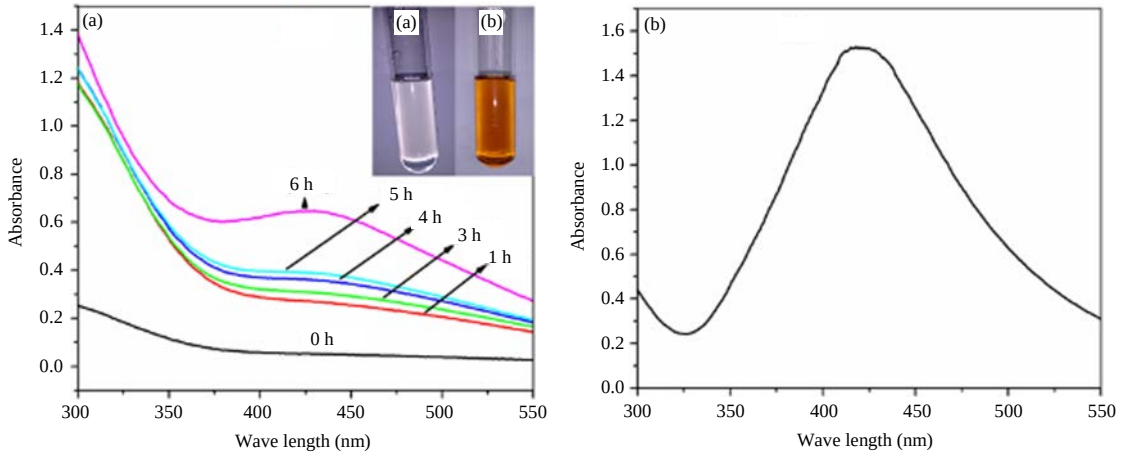

Fig. 1(a-b): UV-vis Spectra (a) Monitoring the formation of silver nanoparticles and (b) Nanoparticle dispersion after 6 months

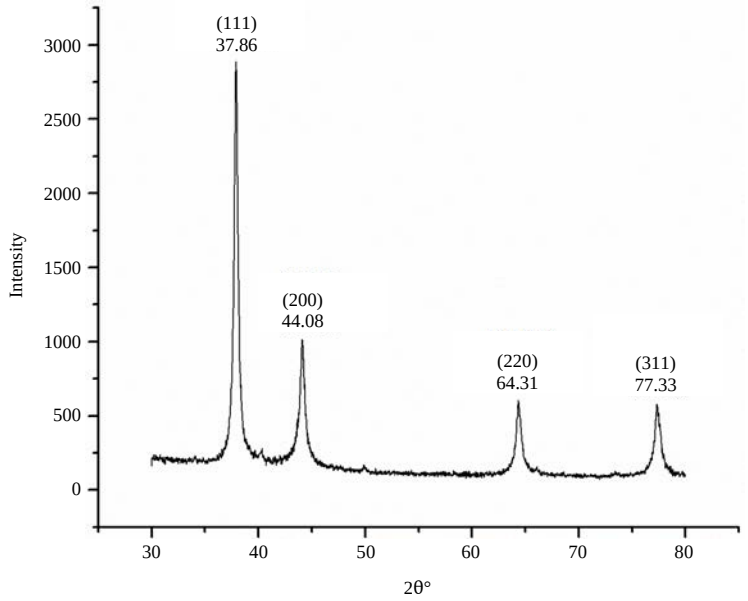

Fig. 2: XRD pattern of synthesized silver nanoparticles by using *Setaria italica* husk extract

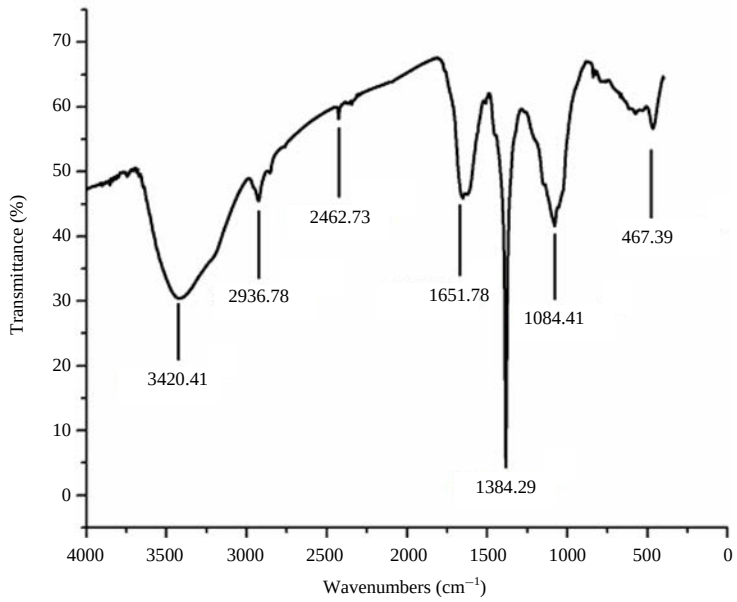

Fig. 3: FTIR spectra of silver nanoparticles synthesized by using *Setaria italica* husk extract

1084.84 cm^{-1} indicates that C-O stretching alcohols, carboxylic acids, esters and ethers. This analysis provides evidence for the presence of proteins and metabolites such as terpenoids having functional groups of alcohols, ketones, aldehydes and carboxylic acids which act as reducing as well as stabilizing agent and helps in increasing the stability of the synthesized silver nanoparticles (Mallikarjuna *et al*., 2012).

Scanning electron microscopy will provide further insight into the morphology and size of the nanoparticles. The SEM micrograph of the synthesized silver nanoparticles presented in Fig. 4. The

SEM image suggests that the silver nanoparticles were dispersed well without much aggregation and possessing spherical shape. The average particle size of the silver nanoparticles is found to 30 nm. The energy dispersive X-ray spectroscopy (EDAX) shows (Fig. 5) strong signal at the energy of 3 keV and also some of the weak signals are obtained for Cl, K, O, Ca, Mg, Na and Si elements. The major emission energy at 3 keV indicates the presence of silver nanoparticles.

Figure 6 shows TEM images of silver nanoparticles. This reveals that the prepared silver nanoparticles are spherical in shape and dispersed well with a few agglomerated particles at some places. Average size of the silver nanoparticles was about 30 nm. A Selective Area Electron Diffraction (SAED) pattern depicted in the inset of Fig. 6 confirms the crystallinity of silver nanoparticles. Particle size and distribution of synthesized nanoparticles were also measured using particle size analyzer and the results are displayed in Fig. 7. The histogram showed that most of the particles are in the range of 25-40 nm. However, the particles are ranging from 15-50 nm. It further supports the results showed in SEM and TEM studies.

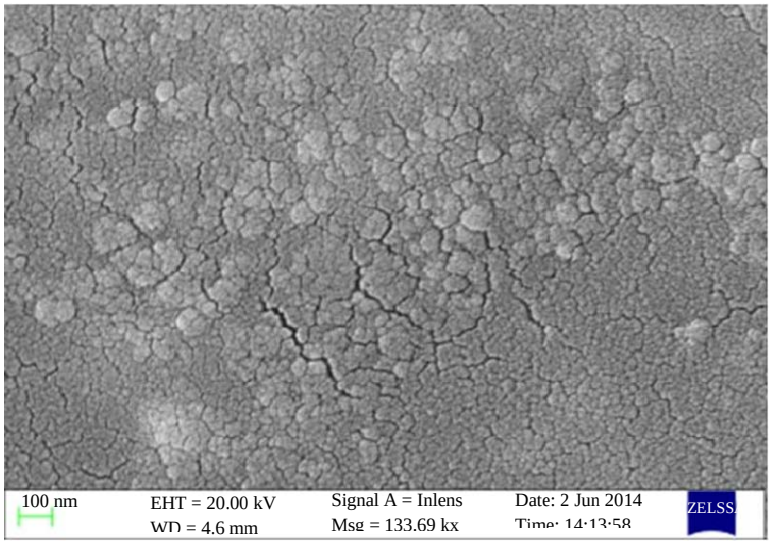

Fig. 4: SEM image of synthesized silver nanoparticles by using *Setaria italica* husk extract

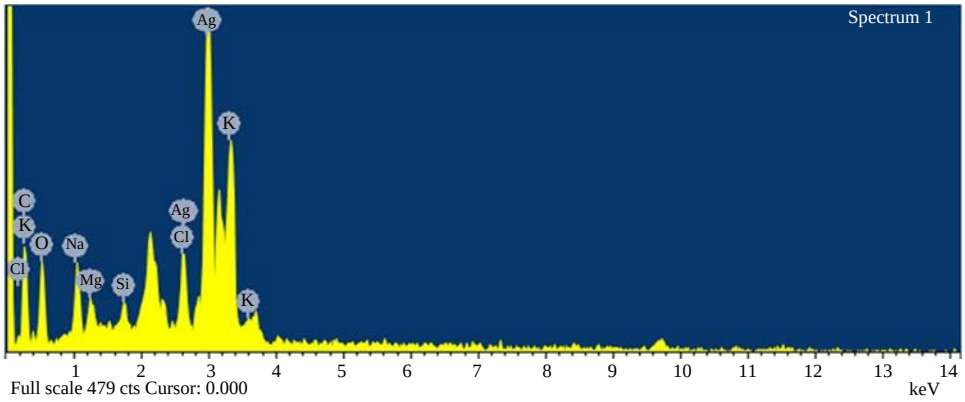

Fig. 5: EDAX spectrum of synthesized silver nanoparticles by using *Setaria italica* husk extract

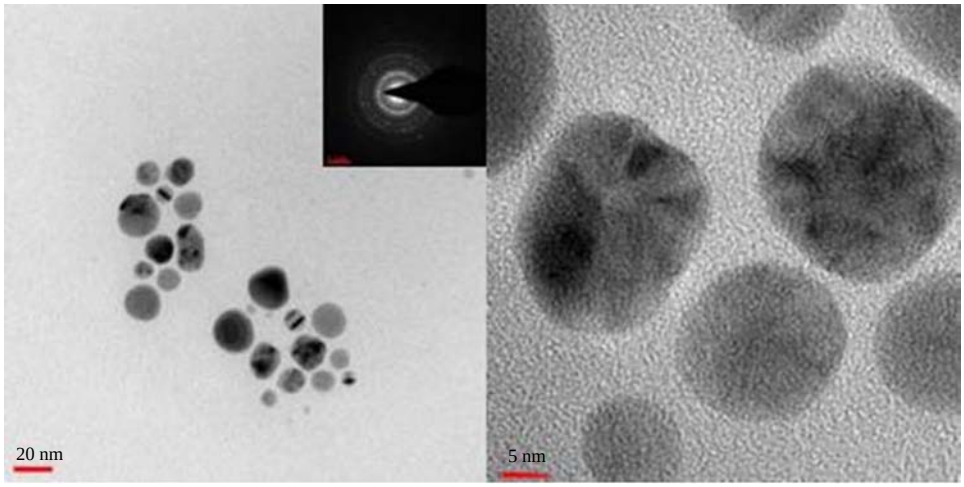

Fig. 6: TEM images of synthesized silver nanoparticles by using *Setaria italica* husk extract. Inset shows the SAED pattern of silver nanoparticles

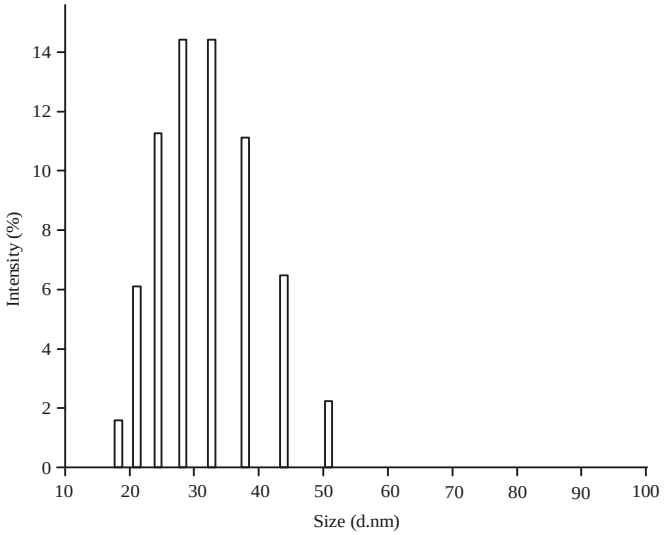

Fig. 7: Histogram showing particle size distribution of silver nanoparticles synthesized using *Setaria italica* husk extract

Antimicrobial activity of synthesized silver nanoparticles against both Gram-positive, *Staphylococcus* sp. (A) and Gram-negative, *E. coli* sps. (B) bacteria was tested and the results are presented in Fig. 8. Four cavities of wells were made in each plate. Well No. 1 is filled with 10 µL of aqueous silver nanoparticles, well No. 2 is filled with Setaria italica husk, well No. 3 is filled with 20 µL of 1 mM $AgNO_3$ solution and well No. 4 is filled with 20 µL of 1% streptomycin (antibiotic) solution. Compared to well No. 3 and 4, well No. 1 which is filled with silver nanoparticles, has shown better inhibition towards both bacterial cultures. No inhibition was observed in well No. 2 which is filled with pure extract. When compared, the synthesized silver nanoparticles showed higher inhibition towards *Staphylococcus* sps. (ZOI~1.2 cm) than *E. coli* sps. (ZOI~0.9 cm).

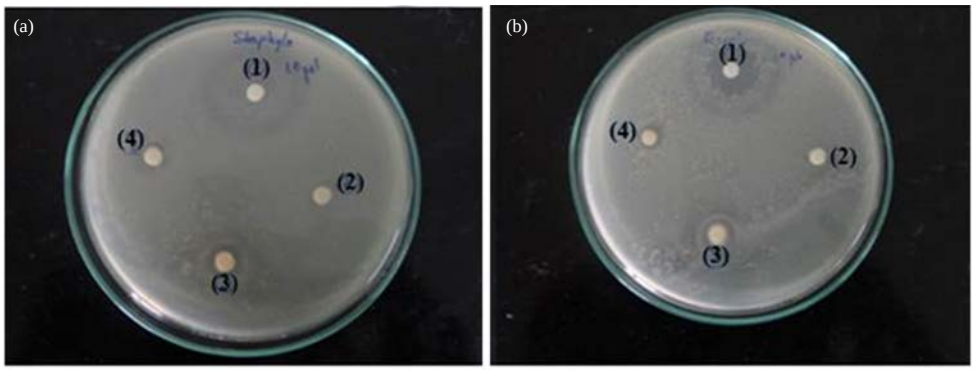

Fig. 8(a-b): Antimicrobial activity studies against (a) *Staphylococcus* sps. (b) *E. coli* sps. bacterial culture

CONCLUSION

We have synthesized silver nanoparticles by using natural product *Setaria italica* husk extract. Extract is capable of producing silver nanoparticles by simple, safe, low cost, less time and efficient methodology. The synthesized silver nanoparticles are spherical in shape and dispersed well and average size is found to be 30 nm as confirmed by SEM, TEM and particle size analyzer. Silver nanoparticles showed good antimicrobial activity towards *Staphylococcus* sp. and *E. coli* (ZOI~0.9) bacterial sps.

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