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Comparative analysis of antibacterial activity of Silver nanoparticles synthesized using leaf extract of wheat varieties

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ABSTRACT: In present study, it has been reported the biological synthesis of silver nanoparticles (AgNPs) using aqueous leaf extract prepared using hot percolation treatment of wheat varieties (PBW343, *Triticum durum* and *Aegilops tauschii*)for the reduction of silver ions to silver nanoparticles. Bioreduction of Ag^{+} to Ag^{0} was observed when an aqueous extract of wheat leaves augmented with silver nitrate ($AgNO_3$) was incubated for 2 hours at 100 °C. The formation of silver nanoparticles was confirmed by surface plasmon resonance as determined by UV-Vis spectra at 430 nm. Silver nanoparticles of size ranging from 5-14 nm of spherical shape were characterized using transmission electron microscopy (TEM). These nanoparticles were found to possess potential antibacterial activity against *(Escherichia coli, Staphylococcus aureus, Klebsiella*) using disc diffusion method and macro dilution (tube) broth method. This environmental friendly method provides a simple, easy, fast and cost effective method for nanoparticles synthesis and can be used in several areas of medicines and industries.

Keywords: *A. Tauschii; Antibacterial; Macrodilution (Tube) broth method; Silver Nanoparticles; T. Durum; TEM (Transmission Electron Microscopy); Wheat varieties (PBW343).*

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INTRODUCTION

Nanobiotechnology deals with the synthesis of nanostructures using living organisms. This field helps to indicate the merger of biological research with various fields of nanotechnology [1]. Nanotechnology applications are highly suitable for biological molecules because of its exclusive properties, providing the tools and platform for the investigation and transformation of biological system. Initially, Chemical and Physical methods were used which were usually fast but also have some problems like high consumption of energy is required to maintain high pressure and temperature required for these methods. Due to these problems of non biological methods, biological methods have been developed for the synthesis of nanoparticles [2]. The three major components involved in the preparation of nanoparticles using biological methods are the solvent medium for synthesis, the environmentally friendly reducing agent, and a nontoxic stabilizing agent [3]. Nanoparticles have been utilized as a therapeutic tool against microbes and in various infections, so for clinical applications it is very essential to understand their effect on microbes. Nanoparticles (Nps) of noble metals like silver and gold also have potential application in various fields including Biomedicine, where they can be used for drug and gene delivery systems and treatment of some cancers [4]. From centuries till date, various medicinal plants have been extensively utilized in Ayurveda. Recently, many such plants have been gaining importance due to their unique constituents

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and their versatile applicability in various developing fields of research and development. Nanobiotechnology is presently one of the most dynamic disciplines of research in contemporary material science whereby plants and different plant products are finding an imperative use in the synthesis of nanoparticles (NPs) [5]. Among noble metal nanoparticles, silver nanoparticles have received considerable attention due to their attractive physiochemical properties (At present, a number of living organisms have been known to synthesize nanoparticles such as cyanobacteria, bacteria, fungi (*Aspergillus parasiticus, Aspergillus terrus*), acitomycetes, biomolecular and various plant materials such as *Cinnamomum camphora, Medicago sativa, Avene sativa, Azardirchta indica, Tamarindus indica, Parthenium hysterophorus, Acanthella elongata, Sesuvivm potulacastrum, Crataegus douglasii, Centella asiatica, Phyllanthusniruri* [6,7.8,9,10]. Leaf extracts of Neem, Geranium*, Hibiscus, Cinnamon, Tamarind, Coriander, Brassicaceae* spp. and many plant and seeds such as Gram, Maize, and Moong have been used for development of nanoparticles [11]. In previous studies, Biofabricated Silver Nanoparticles from various plants and organisms act as a Strong Fungicide against various diseases of wheat for example, silver nanoparticles from *Serratia* sp. against Bipolaris sorokiniana Causing Spot Blotch Disease in Wheat have been used but wheat has not been as such used for biosynthesis of silver nanoparticles in previous studies [12].

In the present study, leaf extracts of different varieties of wheat was used as reducing agent for reduction of silver nitrate into silver nanoparticles. Wheat is a common food grain in all over the world and the leaves of wheat have also medicinal importance, so study related to silver nanoparticles synthesis using different varieties of wheat has been conducted.

EXPERIMENTAL

Preparation of wheat leaf extract

The leaves of wheat varieties (*T. aestivum, T. durum, A. tauschii)* were collected from Punjab Agricultural University (PAU), Ludhiana. The leaves of wheat varieties were grinded to form a clear paste. 20ml of crude paste was diluted 5 times in double distilled water and was subjected to hot percolation treatment. In hot percolation treatment, diluted wheat leaf paste was stirred at 40^{0}C for 2 hrs till resultant mixture boils completely. The resultant mixture was then subjected to 60 ^{0}C and was kept undisturbed till reduced volume of resultant mixture was obtained. The reduced volume of resultant was filtered out using Whattman filter paper no. 1 in conical flask. The filtrate so obtained was used as raw extract for synthesis of silver nanoparticles.

Biosynthesis of Silver Nanoparticles

For each experimental setup, two flasks were prepared, in each flask 50ml of 1mM $AgNO_3$ was added and no leaf extract was added in first flask and considered as control, in second flask along with 50ml of 1mM $AgNO_3$ 2.5ml of leaf extract was added, maintaining pH11 and was kept undisturbed at 100^{0}C for 2 hours. In 2010 Dubey and his co workers used temperature variations from 25 ^{0}C to 150 ^{0}C and noted increase in peak sharpness due to increasing temperature and concluded that increase in absorbance peak sharpness may be due to size of the synthesized nanoparticles with high temperature, particle size may be small which results into sharpness of plasmon resonance band of Silver nanoparticles [13, 14].

Characterization of silver nanoparticles

UV-Visible spectrophotometry analysis (preliminary test): Absorbance was recorded at 430nm using UV-Visible spectrophotometer and a graph using the readings was plotted by graph pad prism software 5.0.

Transmission Electron Microscopy (confirmatory analysis): TEM analysis was carried out to confirm the synthesis of silver nanoparticles. Pellet was prepared by centrifugation at 10,000 rpm for 15 minutes. The pellet formed was carried to the Sophisticated Analytical Instrumentation Facility, Panjab University, Chandigarh to carry out TEM analysis.

Comaparative analysis of antibacterial activity

Comparative analysis of antibacterial activity of synthesized silver nanoparticles was determined using two methods:

Kirby Bauer Disc Diffusion method: This is one of the most common method to determine antimicrobial activity of given silver nanoparticle sample or antibiotic. Mueller Hinton Agar (MHA Agar) was used for antimicrobial activity test. Under ascetic conditions in the biosafety chamber, 15ml of MHA

medium was dispensed into pre-sterilized petridishes to yield a uniform depth of 4mm and inoculated with the bacterial culture, respectively. The discs were placed on MHA medium in petri dishes which were prepared using whatmann filter paper no. 1 with the help of punching machine and were sterilized. The discs were marked as A, B, C, and D [15].

A: Silvernanoparticles solution (Temperature variant)
B: Silvernanoparticles suspension (pellet)
C: Raw Plant extract only (positive control)
D: Distilled water (negative control).

The discs were placed on MHA agar surface with the help of red hot forcep. The discs were placed far enough to avoid reflection wave from the edges of the petri dishes and overlapping zone of inhibition. Finally the petridishes were incubated for 24 hours at 37^{0}C for all different strains used. The occurrence of zone of inhibition as indicated by clear area which was devoid of growth of microbes was measured. Macro dilution (Tube) Broth Method: In this method the dilutions of a given silvernanoparticles solution was prepared to determine its activity. The 1:2 dilution of the each sample of silver nanoparticles was prepared in peptone water. The solution was further serially diluted i.e. 10^{-1}, 10^{-2} and 10^{-3} in 10ml of broth to determine up to which concentration it was actively acting as a antimicrobial agent. Each tube containing dilution and a control (in which no silvernanoparticles were added containing only broth and was used for comparative analysis) was inoculated with 1ml of inoculums of given bacterial culture and were incubated at 37 ^{0}C for 24 hours [16].

Table 1: Absorbance observed using UV-Visible spectra of $AgNO_3$ treated leaf extracts of wheat varieties at 430nm after 2 hours incubation at specific temperature(100^{0}C) :

Name of wheat variety	Absorbance at 430nm
T. durum	1.75
PBW343	2.00
A. tauschii	1.09

RESULTS AND DISCUSSION

UV-Visible spectrophotometric analysis:

The samples of each variety were treated at different reaction conditions and a strong change in color from green to dark pink was observed. This color change was a preliminary result showed the presence of silvernanoparticles in the suspension or reduction of Ag^+ of $AgNO_3$ to Ag^o. After observing the change in color the absorbance was recorded between 230 to 280nm and maximum absorbance was observed at 430nm due to Surface Plasmon Resonance of silver nanoparticles (Fig. 1a-c), Table 1, and Fig. 2).

Transmission Electron Microscopy Analysis

The confirmation of the biosynthesis of silver nanoparticles from wheat leaf extract was done using Transmission Electron Microscope. The pellets of the

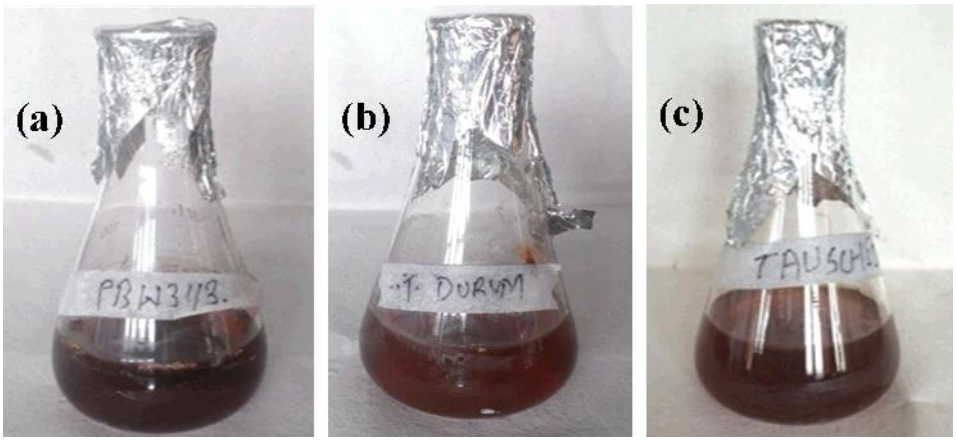

Fig. 1: Biosynthesized silver nanoparticles resulted in change of color in leaf extract treated with $AgNO_3$ after incubation of 2 hours at 100^{0}C.

(a): Synthesized silver nanoparticles from leaf extract of PBW343 when augmented with $AgNO_3$
(b): Synthesized silver nanoparticles from leaf extract of *T. durum* when augmented with $AgNO_3$
(c): Synthesized silver nanoparticles from leaf extract of *A. tauschii* when augmented with $AgNO_3$

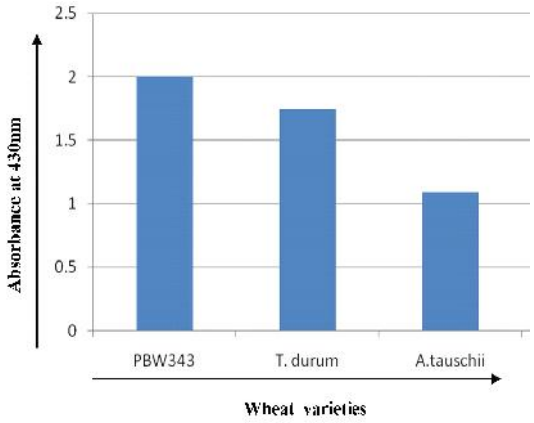

Fig. 2: Comparative bar diagram of absorbance observed from different wheat leaf extracts at specific temperature (100 ^{0}C). X-axis indicates the different wheat varieties and Y-axis indicates absorbance at 430 nm.

suspension were prepared and the pellets were taken to sophisticated and Analytical Instrumentation Facility, Panjab University, Chandigarh. Analysis was performed using drop method and TEM images were obtained. The nanoparticles produced are spherical in shape and size varies from 5-14nm (Fig. 3a-c).

***Comparative analysis of antibacterial activity**:*

Two methods were used to determine the antibacterial activity of produced silver nanoparticles.

1) Kirby Bauer Disc Diffusion method: Comparative analysis of the antibacterial activity of silver nanoparticles produced using leaf extract of different wheat varieties was performed. The nanoparticles so produced showed antimicrobial activity against *Escherichia coli* (Fig. 4a-c)*, klebsiella pneumonia* (Fig. 5a-c) and *Staphylococcus aureus* (Fig. 6a-c).

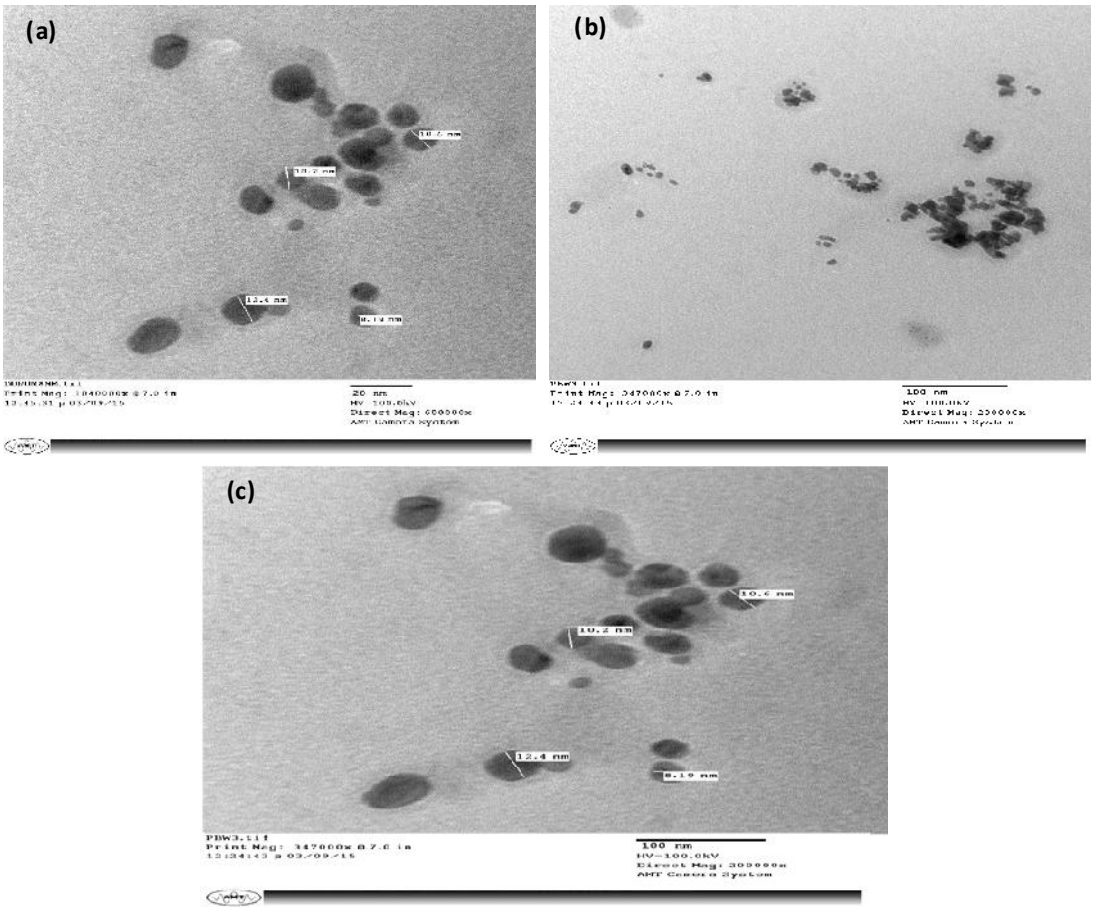

Fig. 3: Magnified images of silver nanoparticles size ranging from 5-14nm observed through Transmission Electron Microscope.
a) Magnified images of silver nanoparticles of *T.durum*; b) Magnified images of silver nanoparticles of PBW343;
c) Magnified images of silver nanoparticles of *A.tauschii*.

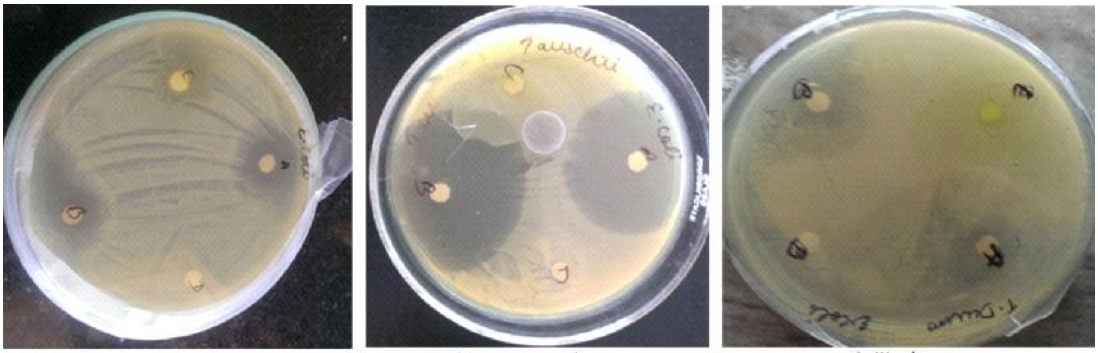

Fig. 4: Comparative analysis for the antibacterial activity of synthesized silver nanoparticles from leaf extract of a)PBW343, b)*A. tauschii*, c)*T. durum* wheat varieties against *E. coli*. Positive results(Zone of inhibition) were observed with all the varieties against *E.coli*. In each plate zone of inhibition were observed in wells A and B(SNP suspension). No zone of inhibition were obtained in well C(raw extract only) and D(distilled water only).

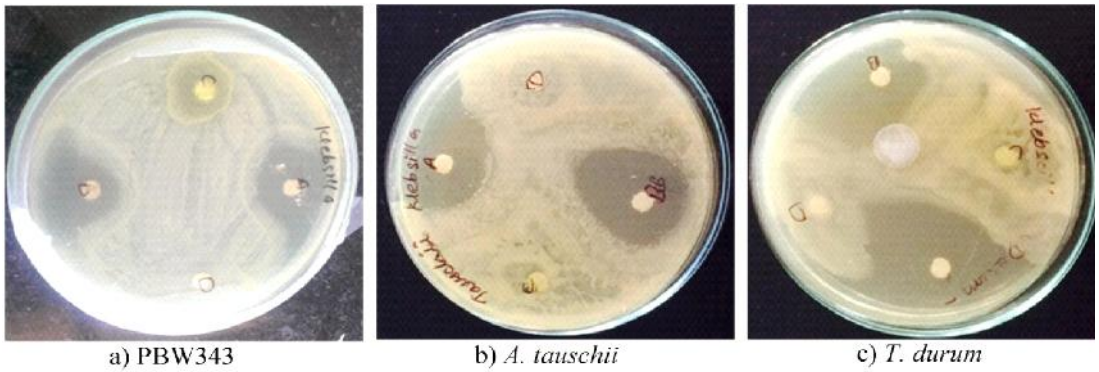

Fig. 5: Comparative analysis for the antibacterial activity of synthesized silver nanoparticles from leaf extract of a)PBW343, b)*A. tauschii*, c)*T. durum* wheat varieties against *Klebsiella*. Positive results(Zone of inhibition) were observed with all the varieties against *Klebsiella*. In each plate zone od inhibition were observed in well A and B(SNP suspension). No zone of inhibition were obtained in well C(raw extract only) and D(distilled water only).

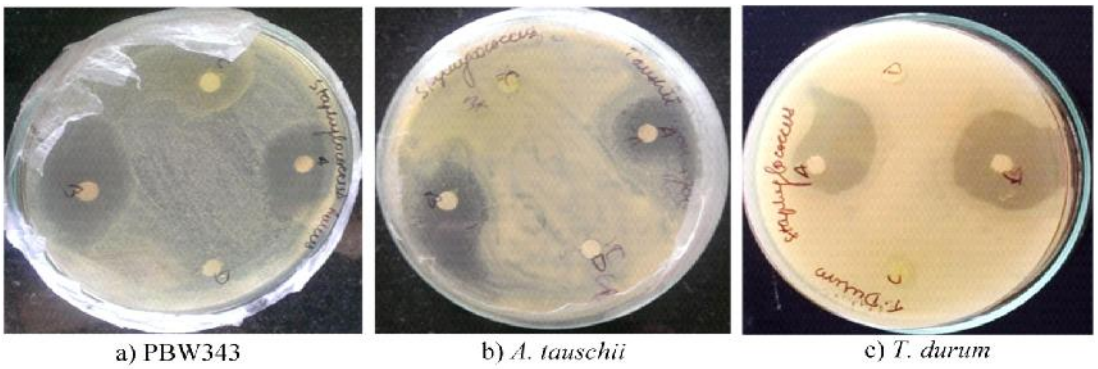

Fig. 6: Comparative analysis for the antibacterial activity of synthesized silver nanoparticles from leaf extract of a)PBW343, b)*A. tauschii*, c)*T. durum* wheat varieties against *S. aureus*. Positive results(Zone of inhibition) were observed with all the varieties against *S. aureus*. In each plate zone od inhibition were observed in well A and B(SNP suspension). No zone of inhibition were obtained in well C(raw extract only) and D(distilled water only).

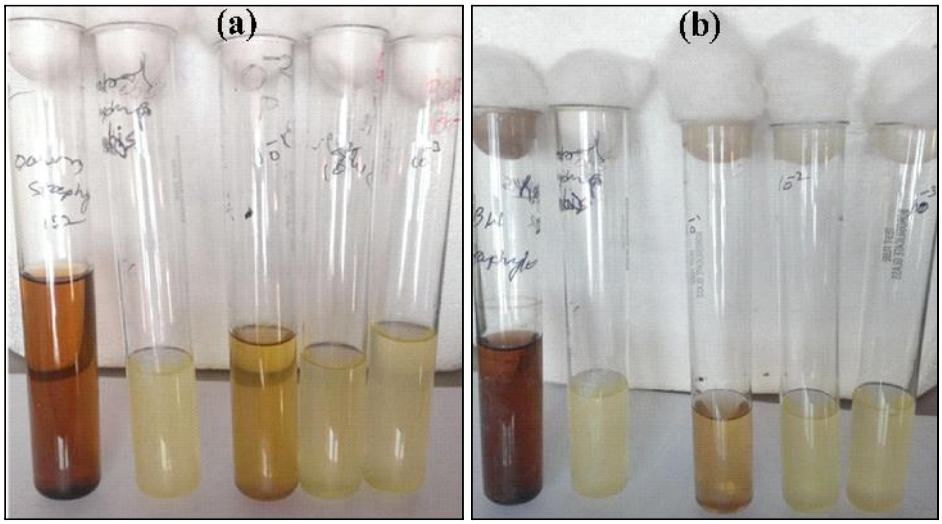

Fig. 7: Determination of concentration of silver nanoparticles i.e. minimum concentration of silver nanoparticles that is effective for antimicrobial activity against pathogenic microorganism *(S.aureus*).

a: Dilutions prepared using synthesized silver nanoparticles from leaf extract of *T.durum* and inoculated with *S.aureus* and incubated for 24 hrs at 37 °C. There was no growth in 10^{-1} but in 10^{-2} and 10^{-3} there was growth of bacteria that means concentration of silver nanoparticles present in 10^{-1} is minimum concentration that is required for the inhibition of growth of bacteria.

b: Dilutions prepared using synthesized silver nanoparticles from leaf extract of PBW343 and inoculated with *S.aureus* and incubated for 24 hrs at 37 °C. There was no growth in 10^{-1} but in 10^{-2} and 10^{-3} there was growth of bacteria that means concentration of silver nanoparticles present in 10^{-1} is minimum concentration that is required for the inhibition of growth of bacteria.

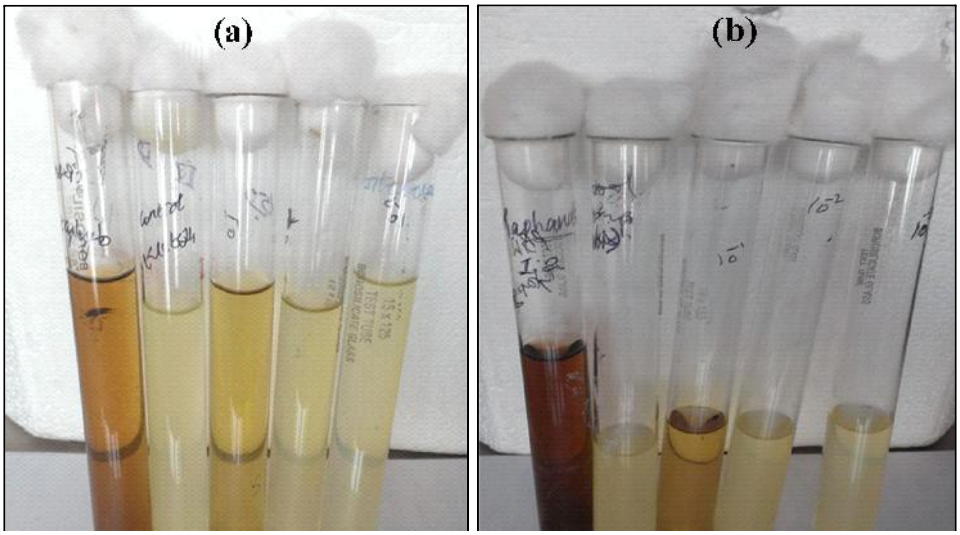

Fig. 8: Determination of concentration of silver nanoparticles i.e. minimum concentration of silver nanoparticles that is effective for antimicrobial activity against pathogenic microorganism *(Klebsiella*).

a: Dilutions prepared using synthesized silver nanoparticles from leaf extract of *T.durum* and inoculated with *Klebsiella* and incubated for 24 hrs at 37 °C. There was no growth in 10^{-1} but in 10^{-2} and 10^{-3} there was growth of bacteria that means concentration of silver nanoparticles present in 10^{-1} is minimum concentration that is required for the inhibition of growth of bacteria.

b: Dilutions prepared using synthesized silver nanoparticles from leaf extract of PBW343 and inoculated with *Klebsiella* and incubated for 24 hrs at 37 °C. There was no growth in 10^{-1} but in 10^{-2} and 10^{-3} there was growth of bacteria that means concentration of silver nanoparticles present in 10^{-1} is minimum concentration that is required for the inhibition of growth of bacteria.

2) Macrodilution Analysis: Macrodilution analysis for synthesized silver nanoparticles was carried out to study up to which concentration these nanoparticles were showing their antibacterial activity against clinically isolated pathogens. Comparative analysis for each pathogen with respect to the nanoparticles synthesized from leaf extract of wheat varieties was performed against *Klebsiella pneumonia* (Fig. 7a-b) and *Staphylococcus aureus* (Fig. 8a-b).

CONCLUSION

The approach which has been used to reduce silver nitrate solution to form silver nanoparticles is cost effective. Leaf extracts of three wheat varieties *i.e.* PBW343*, T.durum, A.tauschii* for the synthesis of silver nanoparticles was used. The synthesis of silver nanoparticles was characterized using UV-Visible spectrometric analysis absorbance at 430nm. Maximum wavelength was observed in PBW343 as compared to *T.durum* and *A.tauschii.* Confirmatory analysis using Transmission Electron Microscopy was performed from SAIF, PU, Chandigarh**.** Varying size (5-14nm) of silver nanoparticles was observed in TEM analysis. Comparative analysis among three varieties of wheat against clinically isolated pathogens (*E.coli, S.aureus,* and *Klebsiella)* was performed. Positive results were obtained against all the pathogenic organisms for the silver nanoparticles of all three varieties of wheat.

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