Biosynthesis of Silver Nanoparticles using Wheat Straw Biomass under Light Radiation and their Antibacterial Activity

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Silver nanoparticles (AgNPs) were synthesized with wheat straw biomass at room temperature using light irradiation. The reaction conditions were optimized, including the light intensity, biomass concentration, NaCl addition, and reaction time. The silver nanoparticles fabricated at the optimum conditions (light intensity 60,000 lx, biomass concentration 2 mg/mL, and reaction time 90 min) were characterized by UV-Vis spectroscopy, X-ray diffraction (XRD), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), and zeta potential. The TEM results showed that the silver nanoparticles were mostly spherical, with an average diameter of 17.2 nm. The zeta potential of AgNPs reached -21.6 mV. The XRD spectra showed that the AgNPs were highly crystalline, with four characteristic peaks. The FTIR of nanoparticles implied that alcohols and proteins may have a vital role in the formation and stability of AgNPs. The silver nanoparticles synthesized by wheat straw biomass revealed antimicrobial activity against *Escherichia coli* and *Bacillus subtilis* strains.

Keywords: Silver nanoparticles; Wheat straw biomass; Biosynthesis; Characterization; Antimicrobial activity

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INTRODUCTION

Silver nanoparticles (AgNPs) are important metallic nanoparticles (NPs) because of their widely reported commercial applications, including biological tagging (Dhas *et al.* 2014), electronic sensing (Borase *et al.* 2013b), photonics, therapeutics, and catalysis (Dhand *et al.* 2016). Several chemical and physical methods are used to synthesize AgNPs, while the use of unsafe chemicals or employment of high pressure and temperature is considered not to be environmentally friendly (Dhas *et al.* 2014). In recent years, various eco-friendly sources of organisms such as bacteria, fungus, and plant extracts have been used to synthesize AgNPs (Akhtar *et al.* 2013; Gade *et al.* 2013; Ng *et al.* 2013). Several plants extracts can synthesize AgNPs, such as extracts of *Lawsonia inermis* (Gupta *et al.* 2013), *Cacumen platycladi* (Huang *et al.* 2011), and aqueous sorghum bran (Njagi and Huang 2011) and as-synthesized AgNPs can strongly inhibit the growth of common human pathogens (Gupta *et al.* 2013). Rajasekharreddy *et al.* also synthesized highly crystalline AgNPs by sunlight irradiation of leaf extracts from seven plants, including *Jatropa curcas*, *Tridax procumbens*, *Solanum melongena*, *Datura metel*, *Carica papaya*, *Citrus aurantium*,

Calotropis gigantea (Rajasekharreddy *et al.* 2010). In conclusion, the synthesis of nanoparticles from plant extracts is usually easier than that from microbial extracts, which require complex culture procedures (Dhas *et al.* 2014). In comparison, plant sources are much easier to be acquired and safer to handle.

Since 2010, China has produced approximately 530.8 million tons of crops on the average per year, and a large mass of crop straw has been formed as a residual. Approximately 80.5% of the crop straw is from corn, wheat, and rice; among them, wheat straw accounts for 15.7% of the theoretical resources. Only 18.7% of straw is recycled for bioenergy (Jiang *et al.* 2012; Chen 2015). Río *et al.* analyzed the complete structural characteristics of the lignin of wheat straw to extend the utilization for biorefinery products (Río *et al.* 2012). Batidzirai *et al.* assessed the feasibility of wheat straw for large-scale bioenergy application and demonstrated the sustainable bioenergy potential from wheat straw (Batidzirai *et al.* 2016). Wheat straw has a higher content of cellulose and hemicelluloses, and lower crude protein content than other straws, leading to a low utilization rate (Del Rio *et al.* 2012). Over 31.3% of straw is wasted, and the current methods for wheat straw utilization lack effectiveness and stability (Lu *et al.* 2010). Therefore, more novel and efficient methods were strongly needed for the utilization of wheat straws.

The objective of this study was to utilize wheat straw to synthesize silver nanoparticles. The biosynthesis conditions were investigated, and the main properties of silver nanoparticles were characterized. Biosynthesis of AgNPs using wheat straw will contribute not only to wheat straw treatment, but also will help to produce valuable nanoparticles.

EXPERIMENTAL

Preparation of Wheat Straw Biomass

Wheat straw was obtained from the experimental foundation of Chengdu Institute of Biology, Chinese Academy of Sciences, Wenjiang, China. The wheat straw was dried under sunlight for 5 days, and fine powders were prepared with a pulverizer (XFB-200, Zhongxiang Pharmaceutical Machinery, Hunan, China) at 25,000 rpm for 15 min. The extract solution was prepared according to a previous method with slight modifications (Rajasekharreddy *et al.* 2010). Approximately 5 g of wheat straw powder was added to 200 mL of deionized water. The sample was then mixed by ultrasonication (KQ-400KDB, Kunshan Ultrasound Instrument, Inc., Suzhou, China), followed by standing for 10 min at room temperature. The supernatant solution was filtered through filter paper to obtain clear extracts immediately. The filtered biomass was stored at 4 °C for further experiments and used within 10 days.

Biosynthesis of AgNPs

To synthesize nanoparticles, 20 μL $AgNO_3$ solution (50 mM) was added to 980 μL of wheat broth, and then the reactor was exposed to light radiation directly at room temperature. To evaluate the effect of various reaction conditions on AgNP biosynthesis, the samples were set at different light intensity conditions (30,000; 40,000; 50,000; 60,000; and 70,000 lx). The light intensity was detected with a Digital Lux Meter AR823 (Smart Seneor, HK, China). Control tests were conducted in the dark. Effects of various wheat

straw biomass concentrations (0, 0.5, 1.0, 2.0, and 3.0 mg/mL), NaCl additions (0.5, 1.0, and 1.5 mM), and reaction times (30, 60, 90, and 120 min) were also determined.

UV-Vis Spectra Test

Diluted reaction samples were immediately placed into a UV-cuvette for recording the UV-Vis spectrum from 330 to 700 nm on a spectrophotometer (Analytikjena/Biometra ScanDrop 100, Analytik Jena AG Corp., Jena, Germany) at room temperature.

Transmission Electron Microscopy (TEM)

The AgNPs were synthesized at 60,000 lx, with the addition of 2 mg/mL of wheat straw and 1 mM $AgNO_3$ at a reaction time of 90 min. The AgNPs were collected by centrifugation at 15,500 ×g for 30 min. The samples were prepared by dipping one drop of AgNPs solution on carbon-coated copper grids, and analysis was performed using a Tecnai G^2 F20 TEM (FEI Co., Hillsboro, USA) with an energy-dispersive spectroscopy (EDS) (Oxford) system. The average size and size distribution were estimated by measuring the diameters of 100 AgNPs with SmileView 2.0 software (JEOL Software Informer, TKY, Japan).

X-Ray Diffraction (XRD) Analysis

The AgNPs were lyophilized, and the resulting powders were characterized using a Rigaku Smart Lab X-ray diffractometer (Rigaku Corp., TKY, Japan) equipped with CuK_α radiation (λ = 1.54 Å).

Fourier Transform Infrared Spectroscopy (FTIR)

The samples of AgNPs and wheat straw were lyophilized, and pellets were prepared by dispersing them in KBr. The FTIR analysis was conducted using a Bruker Vecter X70 FTIR spectrometer (Bruker Corp., Karlsruhe, Germany).

Zeta Potential Analysis

The samples were centrifuged at 15,500 ×g for 30 min, washed twice with distilled water, and then suspended in distilled water for zeta potential measurement with a Malvern Zetas sizer Nano ZSP (Malvern Instruments Ltd. Malvern, UK).

Antimicrobial Studies

Dry powders of AgNPs were first diluted in deionized water. After inoculating with 0.15 mL of *Escherichia coli* (ATCC 25922) and *Bacterial subtilis* (ATCC 6633) (10^9 CFU/mL) into 5 mL of LB broth containing AgNP concentrations of 0, 16, or 32 μg/mL. The culture without the addition of AgNPs was used as the control. The reactors were incubated at 37 °C for 4 h, and then OD_{600} was measured. The assays were performed in triplicate, and the inhibitory effect (%) was calculated by the formula (Lee *et al.* 2013):

$$\text{Inhibitory effect (\%)} = (CFU_{control} - CFU_{treatment})/CFU_{control} \times 100\% \quad (1)$$

The well-diffusion method was used to determine the antimicrobial activity in the solid LB agar plate. Approximately 0.1 mL of test bacteria (10^9 CFU/mL) was inoculated into 100 mL of LB agar. Approximately 50 μL of AgNP solution with concentrations of 80 or 120 μg/mL was added to the wells.

Statistical Analysis

All experiments were repeated at least three times. The results were expressed as the average values with standard deviations. A t-test was carried out to assess the mean comparison. Differences in analysis between means were performed in SPSS software (version 19.0) for windows (SPSS Inc., USA) using one-way analysis of variance (ANOVA). The adopted level of significance and high significance were 5% ($P < 0.05$) and 1% ($P < 0.01$), respectively.

RESULTS AND DISCUSSION

Optimization of Parameters

Effect of light radiation on AgNPs synthesis

Silver nitrate was added to the wheat straw solution (4 mg/mL) to attain a final concentration of 1 mM. The reactor was exposed to light radiation for 90 min to observe the effects of light intensity on AgNP synthesis. A color change of the reaction mixture from yellow to brown was observed, whereas no such changes were observed for samples incubated in the dark. The color changes were attributed to the surface plasmon resonance of AgNPs in the visible region (Wei *et al.* 2012). All the reaction products displayed maximum absorption at 410 nm in the UV-Vis spectra test (Fig. 1A), which indicated that AgNPs with similar particle sizes and shapes were produced. It can be deduced that the light stimulated the AgNP synthesis (Raut *et al.* 2014) when compared with the control sample kept in the dark. The absorption peak heights increased with increasing light intensity, and thus the synthesis efficiency was substantially affected by the light intensity ($P<0.05$).

Effect of wheat straw concentration

The effect of wheat straw concentration on the synthesis of AgNPs is shown in Fig. 1B. No absorbance and color change was seen in the control tests without wheat straw biomass, which indicated a colorless and transparent liquid during the reaction. Along with the increased concentration of the biomass, the reaction mixtures became red and the UV peak absorbance intensity increased. The maximum absorption peaks in the visible light region were similar, indicating that the particle sizes of AgNPs were similar with various concentration of wheat straw. The maximum absorption was found at 2 mg/mL, which was lower than that of *Ficus carica* (2015) and *Withania somnifera* (2014) (Fig. 1B) biomass samples. These results suggested that the AgNP synthesis efficiency from wheat straw in this study was higher than that from other two plant sources.

Effect of NaCl addition

Different concentrations of NaCl were added to the reaction mixtures. The results showed that NaCl did not increase the quantity of AgNPs. It has been reported that the synthesis of AgNPs using bacterial extracts requires the addition of NaCl (Wei *et al.* 2012), but our results suggested that NaCl did not increase the quantity of AgNPs with wheat straw biomass (Fig. 1C), indicating that no additional chemicals such as NaCl are needed in this biosynthesis method.

Effect of reaction time

To investigate the effect of reaction time on the synthesis of AgNPs, AgNPs were synthesized with 60,000 lx light intensity, 2 mg/mL biomass concentration, and 1mM $AgNO_3$ solution. After 30 min, the reaction mixtures immediately became dark brown. The maximum AgNPs synthesis was achieved after 90 min (Fig. 1D), and the reaction rate was more rapid than that reported in previous studies (Wei *et al.* 2012; Das *et al.* 2013; Ng *et al.* 2013; Aziz *et al.* 2015; Dhand *et al.* 2016).

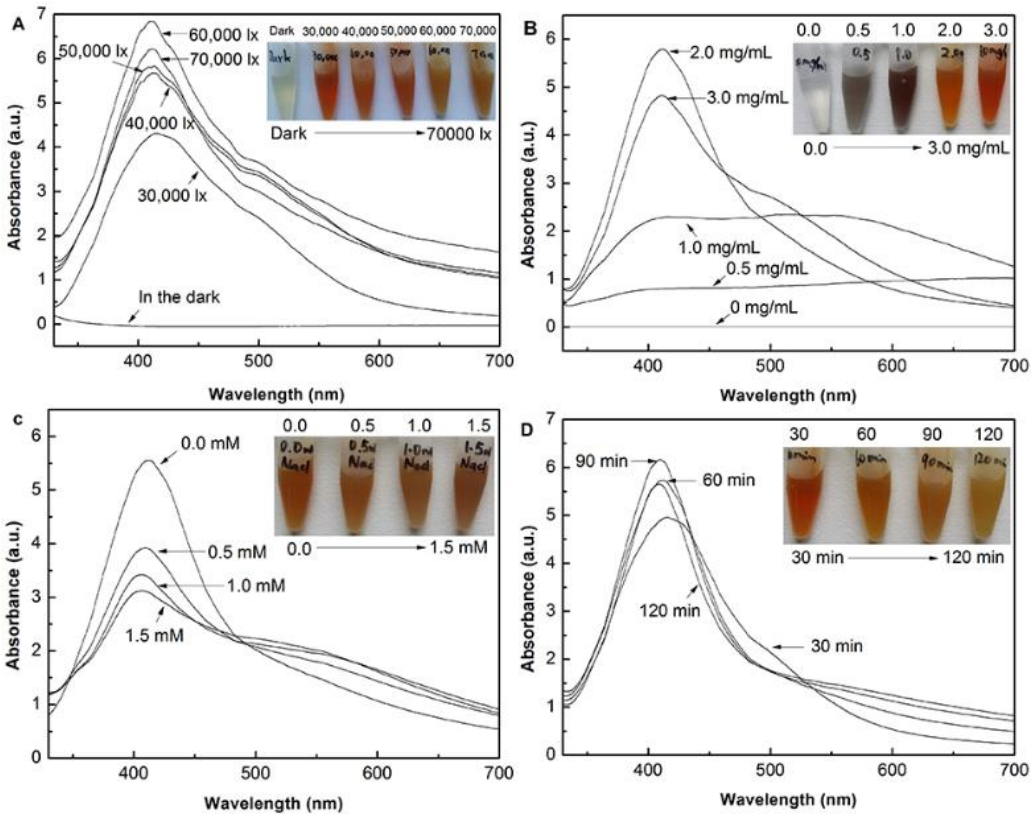

Fig. 1. Effects of synthesis conditions on AgNPs with wheat straw biomass: (A) light intensity; (B) wheat straw concentration; (C) NaCl addition; and (D) reaction time

Characterization of AgNPs

TEM and zeta potential analysis

The AgNPs was well dispersed before detection. The TEM analysis showed that the AgNPs were ellipsoidal and nearly spherical (Fig. 2A).

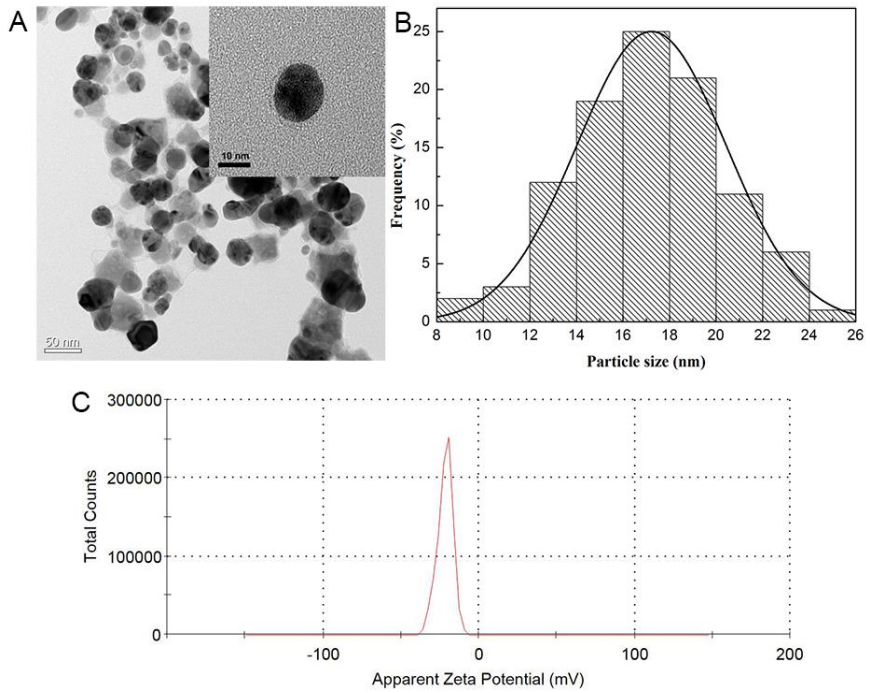

Fig. 2. Characterization of AgNPs by (A) TEM analysis; (B) particle size calculation; and (C) zeta potential analysis

The diameter distributions of 100 nanoparticles ranged from 9.0 to 24.0 nm, with a mean size of 17.2 nm (Fig. 2B). The zeta potential of AgNPs was -21.6 mV at pH 7.0 (Fig. 2C), which facilitated the stability of AgNPs. These patterns demonstrated that the AgNPs synthesized from wheat straw were face-centered cubic (FCC) structures, which coincides with previous studies (Borase *et al.* 2015; Dhand *et al.* 2016).

X-ray diffraction analysis

The dried powders of AgNPs were characterized by X-ray diffraction (XRD) analysis. The XRD data were compared with the pure crystalline sliver structure from the database of the Joint Committee on Powder Diffraction Standards (JCPDS, file nos. 04-0783) (ASTM 1999). Four intense diffraction peaks at 2θ values of 38.0°, 46.13°, 64.43°, and 76.61° were indexed to the 111, 200, 220, and 311 planes of silver reflections, respectively (Fig. 3) (Ganachari *et al.* 2012). These results demonstrated that the nanoparticles synthesized from wheat straw were composed of nanocrystals with the structure of FCC (Lokina and Stephen 2011). Other peaks shown in the figure might be due to metabolites of wheat straw that were still attached to the surface of silver nanoparticles (Borase *et al.* 2013a; Raut *et al.* 2014).

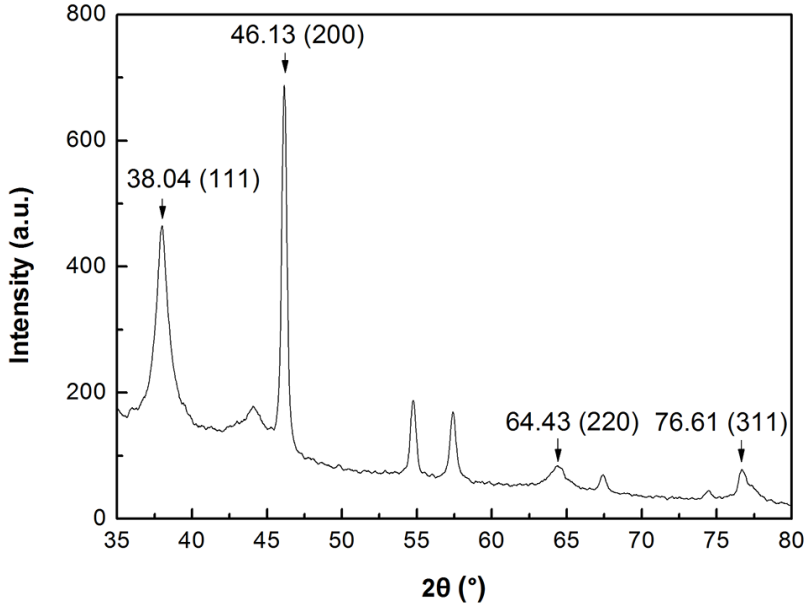

Fig. 3. Characterization of AgNPs by XRD. Four intense diffraction peaks at 2*θ* values of 38.0°, 46.13°, 64.43°, and 76.61° corresponded to (111), (200), (220), and (311) planes of silver reflections, respectively (Ganachari *et al.* 2012).

Fourier transform infrared spectroscopy (FTIR) analysis

The FTIR spectrum showed that there were a variety of functional groups present on the surface of silver nanoparticles (Fig. 4). The peaks found at 3201 and 1071 cm^{-1} correspond to the -OH groups and could be attributed to phenol (Dhand *et al.* 2016). The peaks at 2921, 1544, 1400, and 2852 cm^{-1} were assigned to C-H bonds. The peak at 1238 cm^{-1} denoted the presence of an amino group (-C-N streching) (Golińska *et al.* 2015). These results indicate that compounds such as alcohol and protein may have contributed to the reduction of Ag^{+} ions and the stabilization of AgNPs, which is consistent with the results reported by Ghaedi *et al*. (2015).

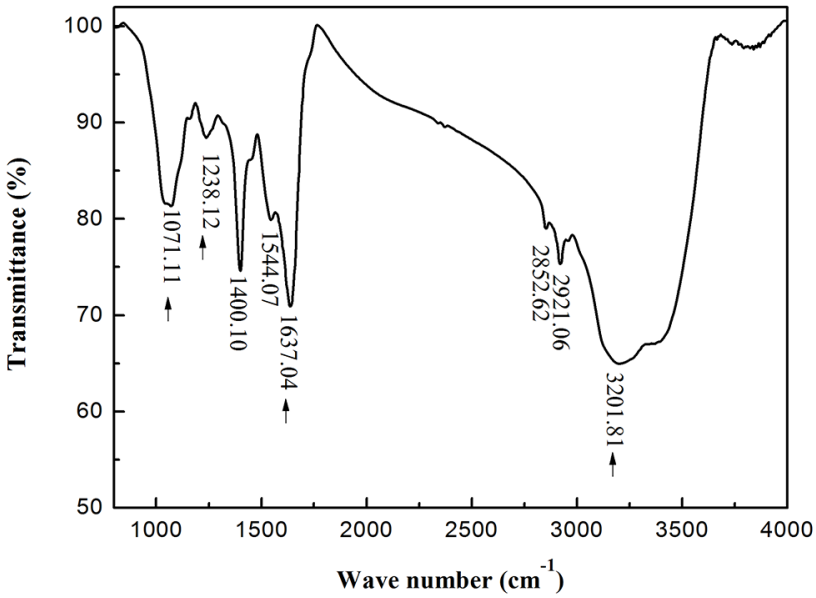

Fig. 4. Characterization of AgNPs by FTIR. Typical absorption peaks at about 1071.11, 1238.12,

1637.04, 3201.81 cm^{-1} are indicated by arrows.

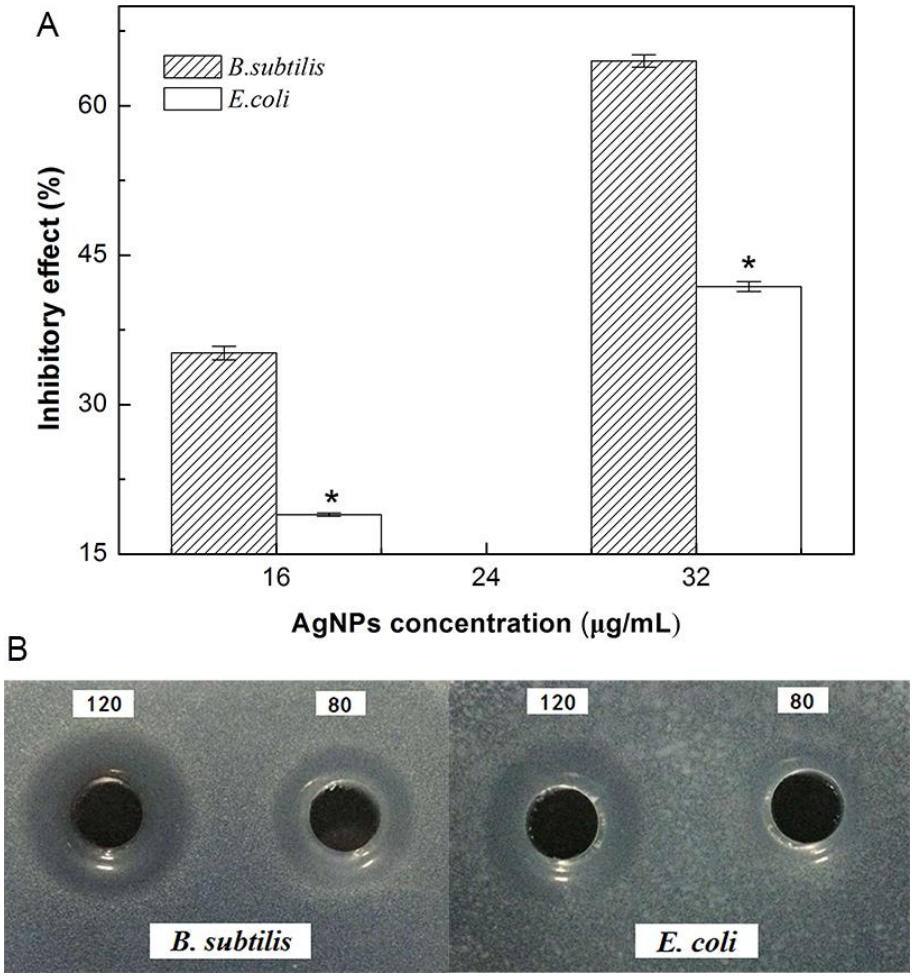

Fig. 5. Inhibitory effect of AgNPs on the growth of *B. subtilis* and *E. coli* in (A) LB broth and (B) LB agar. * indicates a significant difference between the inhibitory effect on *B. subtilis* and *E. coli* at the same concentration of AgNP ($P < 0.05$).

Antimicrobial Activity Analysis

The antibacterial activity of AgNPs toward *B. subtilis* (Gram-positive) and *E. coli* (Gram-negative) strains was tested using the broth dilution method and agar well diffusion method. Some researchers have found that the inhibition of silver nanoparticles on *B. subtilis* was stronger than that on *E. coli* (Arun *et al.* 2015; Dhand *et al.* 2016). In our study, the inhibitory effects of AgNPs on *B. subtilis* were much higher than that of *E. coli* at AgNP concentrations of 16 and 32 μg/mL (Fig. 5A). The bacterial inhibition of AgNPs was found to be concentration-dependent (Wei *et al.* 2012). The agar well diffusion assay also showed that *B. subtilis* was more susceptible to AgNPs (Fig. 5B), which was consistent with the results of inhibition tests in liquid culture, as well as previous reports (Arun *et al.* 2015; Dhand *et al.* 2016). All results implied that the AgNPs synthesized by wheat straw biomass showed a substantial antimicrobial activity and possess promising application potential in antibacterial medicine and food preservation.

CONCLUSIONS

1. Stable AgNPs were synthesized using wheat straw biomass by a green chemistry biosynthesis method. The green method for the AgNP synthesis is low cost, eco-friendly, safe, and highly efficient.
2. The utilization of wheat straw can reduce the accumulation of agricultural wastes and can promote the recycling of resources.
3. The present study evaluated the use of light irradiation instead of using high temperature and pressure to save energy. The AgNP crystalline lattice showed FCC structures, with an average diameter of 17.2 nm and zeta potential of -21.6 mV. The synthesized AgNPs showed substantial antibacterial activity against *E. coli* and *B. subtilis*.
4. These results suggest that wheat straw biomass can be used efficiently to synthesize highly crystalline and spherical AgNPs by a light irradiation process.

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