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SYNTHESIS AND CHARACTERIZATION OF ANTIBACTERIAL SILVER NANOPARTICLE USING LILIUM CANDIDUM FLOWERS EXTRACT

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Abstract*: *In the present study, silver nanoparticles were synthesized using extract of Lilium candidum flowers. Formation of the silver nanoparticles was confirmed by UV–Vis spectrum. Transmission electron microscopy （TEM） image revealed that the silver nanoparticles are mostly spherical and separated with an average size of 20nm. The silver nanoparticles are crystalline in nature on the basis of X–ray diffraction （XRD ）measurement. Fourier transform infrared (FTIR) analysis was used to confirm possible compounds involved in the formation and stabilization of the obtained silver nanoparticles. Antibacterial assessment shows that the silver nanoparticles exhibit great antibacterial activities against test bacteria.
Keyword : silver nanoparticles; *Lilium candidum* flowers extract; antibacterial activity

1. INTRODUCTION

Owing to its simplicity, cost–effectiveness and eco–friendliness, bio–synthesis has become a critical method to fabricating silver nanoparticles[1].Various biological methods such as bacteria[2], fungi[3], Enzyme[4], and plant extract[5] have been used to synthesize silver nanoparticles by researchers from different areas. These methods have been proved to be very efficient in preparing the silver nanopaticles. Among the above–mentioned methods, the method using plant extract can be preferred over other biological methods as a process of maintaining cell cultures can be eliminated and it can be scaled up for a large scale synthesis of the silver nanoparticles.

The synthesis of the silver nanoparticles by plant extracts is currently under exploitation. A variety of plant extracts have been exploited to synthesize the silver nanoparticles. Leaf extract[6], seed extract[7], bark extract[8] and stem extract[9] of plants are frequently used as reducing agents for fabrication of the silver nanopaticles. However, some flower extracts may also be novel alternatives for this purpose. Researchers have synthesized the silver nanoparticles from silver salt using the flower extract. For example, Baharara et al. showed that *Achillea biebersteinii* flowers extract can be used to prepare silver nanoparticles from silver nitrate solution[10]. Padalia et al. also reported that synthesis of silver nanoparticles was from silver nitrate solution using the extract of *marigold* flower[11].

Lilium candidum flower is native to the Balkans and West Asia. It was reported that *Lilium candidum* flower was used as an antiviral to treat shingles[12]. In the present study, the silver nanoparticles were synthesized by reducing silver ions in the presence of *Lilium candidum* flowers extract for the first time. The present study may provide a novel route to fabricating the silver nanoparticles .

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2. MATERIALS AND METHODS

2.1. Materials

For the synthesis of silver nanoparticls, *Lilium candidum* flowers (Figure 1) were obtained from a local flower shop. The flowers were washed 3 times with distilled water to remove dust particles and other pollutants before drying at room temperature. Silver nitrate ($AgNO_3$) was from Aladdin Industrial Inc in Shanghai, China. Distilled water was used throughout the experiments.

2.2. Synthesis of the Extract

25g of *Lilium candidum* flowers was placed into a 250mL round bottom flask containing 100mL distilled water to form mixture, and then boiled for 15min. The resulting solution was filtered with filter paper to obtain extract. The extract was stored at 4^{o}C for further experiment.

2.3. Synthesis of the silver nanoparticles

0.1M aqueous solution of Silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 100mL of distilled water and 5mL of extract were taken into 250mL round bottom flask to form mixture and heated under stirring till the mixture was boiled. Then, 2mL of silver nitrate solution was added to the mixture. After refluxed for 5 min, reaction media turned brown, indicating formation of the silver nanoparticles (Figure 2).

2.4. Characterization of silver nanoparticles

UV–*Vis* adsorption spectra (UV–1601 Shimadzu spectrophotometer, Kyoto, Japan) were used for measuring the characteristic plasma band of Ag NPs. XRD patterns were recorded with an X–ray diffraction (Rigaku D/Max 2200PC) with a graphite monochromator and Cu–*K*α radiation (λ=0.15418nm).The size and morphology of silver

nanoparticles were characterized by transmission electron microscope (TEM) [JEOL, JEM–2100, 200 kV]. The infrared (FTIR) spectra were measured on a Fourier transform infrared spectrometer (Nicolet 5DX FTIR) using a KBr pellet technique.

2.5. Antibacterial assessment

For zone of inhibition test, nutrient agar was poured onto disposable sterilized Petri dishes and was allowed to solidify. 100µL of the bacterial water (106CFU/mL) was streaked over the plate and was spread uniformly. Filter paper discs (12mm in diameter) loaded with 20µL of silver nanoparticles solution (318µg/mL), and extract were placed on the surface of each cultured plate. The same process was done for all the bacterial strains. Plates were incubated at 37^{o}C for 12h. After incubation, the zone of inhibition was measured.

For minimum inhibitory concentration (MIC) test, 50µL of silver nanoparticles solution was transferred into 96 well microtitre plates containing 50µL of broth for bacterial. Dilution was performed by the two–fold serial dilution method. Later, 50µL of tested bacterial were inoculated to all wells. The total volume of suspension in each well was adjusted to 200µL by adding the broth. The microtitre paltes were incubated at 37^{o}C for 24h. After the incubation, optical densities of cultures were measured at 600nm using a microplate reader. The inhibition of bacterial growth was evaluated by comparing the optical density (OD) of samples with controls that were not treated with the silver nanoparticles. The minimum inhibitory concentration (MIC) of was determined as the lowest concentration of the silver nanoparticles that inhibit growth of the bacterial.

3. RESULTS AND DISCUSSION

UV–*Vis* spectroscopy was used to confirm the formation of silver nanoparticles by reducing silver ions using *Lilium candidum* flowers extract as a reducing agent. UV–*Vis* spectrum of silver nanoparticles is shown in figure 3. The surface Plasmon band in the silver nanoparticles is close to 417nm, indicating that silver nitrate is reduced into the silver nanoparticles by the extract of *Lilium candidum* flowers [13].

TEM provided a further insight in morphology and size details of the silver nanoparticles. It is shown that most of the silver nanoparticles are spherical in shape (Figure 4). All the silver nanoparticles are well separated and no agglomeration was observed. From the TEM image, size of the silver nanoparticles varies from 12 to 26nm. Moreover, the mean diameter and standard deviation of silver nanoparticles is 19±4nm. The TEM image also reveals that the silver nanoparticles are covered by some plant organic compounds which stabilize the silver nanoparticles.

The formation of silver nanoparticles was further supported by X–ray diffraction (XRD) measurement (Figure 5). A number of Bragg reflections peaks are observed at 2θ values of 38.05^{o}, 44.30^{o}, 64.33^{o} and 77.30^{o} which can be indexed to (111), (200), (220) and (311) planes of pure silver based on the face-centered cubic structure (JCPDS, file No. 04-0783). The XRD results suggest that the as–prepared silver nanoparticles are crystalline in nature[14].The average particle size of silver nanoparticles can be calculated using Debye–Scherer's equation. From the Scherer's equation, an average size of the silver nanoparticles is found to be 27.7 nm which is also approximately consistent with the TEM result.

The *Lilium candidum* flower contains many components, such as amino acids, ribonucleic acids, flavonoids, glycoside, nitrogenous compounds and steroids[15]. In the FTIR spectrum recorded from the *Lilium candidum* flower extract (Figure 6a), the adsorption band around 3397 cm^{-1} is attributed to the –NH_2 and –OH groups [16]which are derived from amino acids molecules. So it can be confirmed that amino acids are present in the Lilium candidum flower extract that act as a reducing agent. The FTIR spectrum also reveals presence of O–H (3445cm^{-1}) and C=O (1632cm^{-1}) functional groups on the surface of silver nanoparticles (Figure 6b). So flavonoids also can be effective in silver ion reduction. Moreover, the carbonyl groups in amino acids molecules have a strong binding ability to the silver nanoparticles, and form covering layers on the surface of the as–prepared silver nanoparticles. The covering layers prevent the silver nanoparticles from aggregating in the aqueous medium.

A preliminary investigation of antibacterial activities of the obtained silver nanoparticles was performed through measurement of zone of inhibition and minimum inhibitory concentration (MIC). Two bacteria were chosen as test microorganisms: *S. aureus* as gram positive bacteria, and *E. coli* as a representative of gram negative bacteria. Antibacterial activity of the silver nanoparticles was observed in Figure 7. No zone of inhibition was noticed when only extract of *Lilium candidum* flowers was added, indicating that the extract does not possess any antibacterial property against those two bacteria. The zone of inhibition and minimum inhibitory concentration (MIC) are summarized in Table 1 which shows that Sizes of the zone of inhibition ranges from 8.91 mm to 5.72 mm while the minimum inhibitory concentration (MIC) ranges from 0.13µg/mL to 0.26µg/mL. On the basis of the results, it is evident that as–prepared silve nanoparticles are a good candidate using as antibacterial drugs.

4. CONCLUSIONS

In the present investigation, the silver nanoparticles have been synthesized by using *Lilium candidum* flower extract as a reducing agent. The average size of as–prepared silver nanoparticles is 19±4 nm. The biological technique developed in this investigation for producing the silver nanoparticles is advantageous over chemical method due to simplicity, cost–effectiveness and eco–friendliness. Moreover, the silver nanoparticles show an excellent antibacterial activity against the test bacteria, which may find a potential application in the area of drugs.

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Figure 1. Image of *Lilium candidum* flowers

Figure 2. Image of the silver nanoparticles

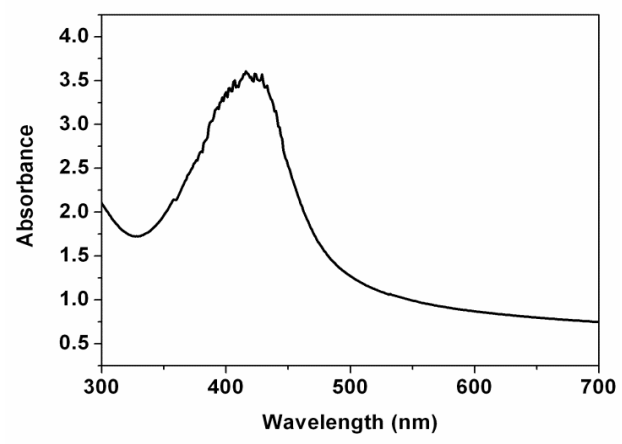
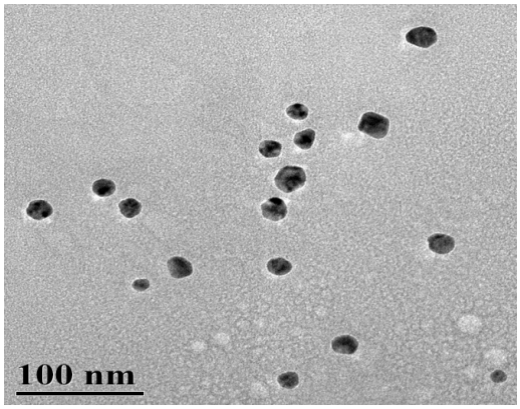


Figure 3. UV–Vis absorption spectrum of the silver nanoparticles



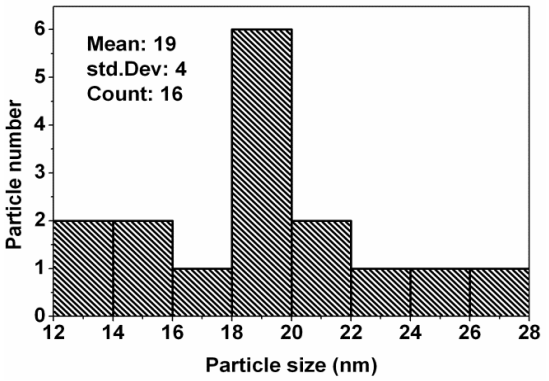


Figure 4. TEM image and size distribution histogram of the silver nanoparticles

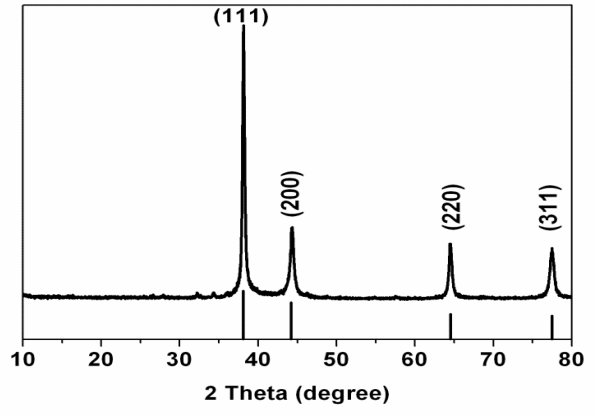
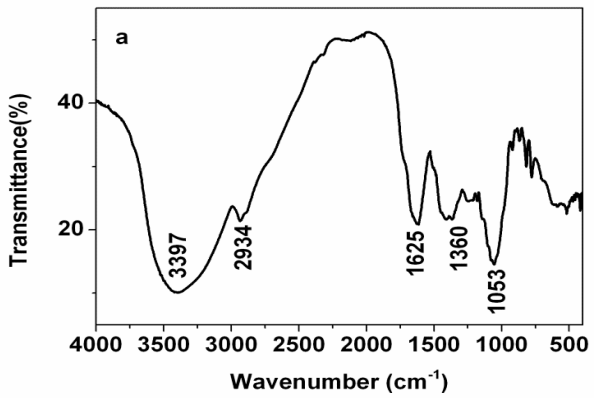


Figure 5. XRD pattern of the silver nanoparticles



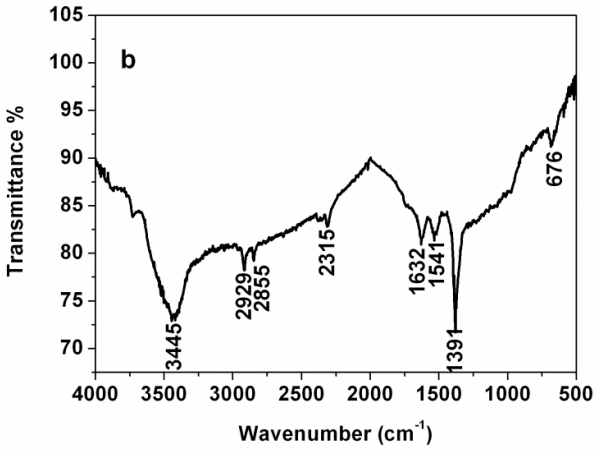


Figure 6. FTIR spectra of *Lilium candidum* flowers extract (a) and as–prepared silver nanoparticles (b)

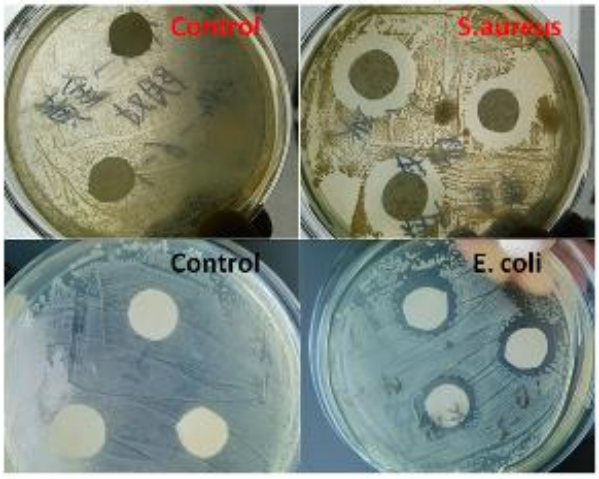


Figure 7. Antibacterial activity of the resulting silver nanoparticles against the test bacterial

Table 1. The antibacterial activity of the resulting silver nanoparticles

Test bacteria	Zone of inhibition (mm)	MIC (µg/mL)
E. coli	8.72±1.00	0.13
S.aureus	10.44±0.73	0.26