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# Exploiting Pear Leaves In Biosynthesis Of Silver Nanoparticles

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**Abstract**

Biosynthesis techniques have a number of advantages over other methods for producing silver nanoparticles (AgNPs), which provide a wide range of applications. The present work highlights the biosynthesis of AgNPs by mixing pear leaf aqueous extract with silver nitrate, and the formation of AgNPs was observed by the change of mixture color from yellow to dark brown and visible spectrophotometry. Moreover, the effect of pH, reaction time, $AgNO_3$ concentration, extract volume and temperature on the suggested approach was also studied. The results showed that pear leaf aqueous extract is an excellent material for the biosynthesis of AgNPs, and by controlling the mentioned parameters that influence synthesis, a large number of AgNPs with small sizes may be produced.

**Keywords**: silver nitrate, silver nanoparticles, pear leaves, biosynthesis

## 1. Introduction

Silver nanoparticles (AgNPs) have wide applications because of their unique physical, chemical, and biological properties [1, 2]. Among these applications are antimicrobial activity [3, 4], antifungal activity [5, 6], antioxidant and cytotoxic activity [7, 8], and wound healing [9, 10]. Many techniques, including vapor condensation, laser ablation, chemical reduction, and electrochemical methods [11, 12] have been used to produce AgNPs. However, these methods have some limitations, such as the use of costly equipment, applying high pressure and temperature, and employing hazardous reducing reagents [3, 13]. Accordingly, researchers have focused their efforts on developing alternative methods, and they have recently proposed the biosynthesis approach to circumvent those limitations. This approach, is simple, less costly, and eco-friendly [14, 15]. Biosynthesis techniques are often based on the use of fungi algae, yeasts, bacteria, and plants [16, 17]. In particular, plant-mediated synthesis of AgNPs is attained by miscellaneous phytochemicals present in plant extracts, such as terpenoids, flavonoids, polyphenols, saponins and tannins, which act as reducing agents for silver and stabilizers for AgNPs [7, 10]. Numerous researches have revealed that pH, reaction time, concentration of silver nitrate ($Ag^+$ ions source), plant extract volume and temperature all play crucial roles in plant-mediated of AgNPs as well as their shape and size [7, 14, 15]. AgNPs biosynthesis using aqueous leaf extract has been reported such as Aloe vera [6], Premna integrifolia [7], Melia azedarach [10], Eriobotrya japonica (Thunb.) [18], almond [19], Artemisia turcomanica [20], lavender [21] and Pteris tripartita Sw [22].
The purpose of this study was to exploit the aqueous extract of pear leaves, and silver nitrate as a source of silver ions, in biosynthesis of AgNPs. Furthermore, the influence of several parameters, including pH, reaction time, concentration of silver nitrate, temperature, and the volume ratio of pear leaves extract, were investigated.

## 2. Materials and methods

### 2.1 Chemicals:

Silver nitrate solution with a concentration of 0.1 molar, was obtained from Winlab in the United Kingdom, and from this solution all silver nitrate solutions were prepared by dilution. To adjust the pH of the reaction medium, solutions containing 0.1 M sodium hydroxide (NaOH) or 0.1 M hydrochloric acid (HCl) were prepared using sodium hydroxide and hydrochloric acid bought from BDH Chemicals Ltd in the UK. All solutions were prepared by deionized water.

**2.2 Preparation of the aqueous extract of pear leaves:**

Pear leaves were collected from a farm in Gharyan, Libya, in September 2021. The collected sample was transferred to the laboratory in a clean plastic bag, where pear leaves were washed with deionized water and air dried. In a clean beaker, 2.5 grams of pear leaves were added to 50 ml of deionized water, and the mixture was heated at 100 °C for 5 minutes.
The mixture was allowed to cool before being filtered using a Whatman No. 1 filter paper. The filtrate was collected in a clean Erlenmeyer flask and stored at 4 °C before being utilized for the biosynthesis of silver nanoparticles.

**2.3 Biosynthesis of silver nanoparticles:**

One ml of pear leaves aqueous extract was added to 9 ml of 1 mM silver nitrate solution, and then the pH of the mixture was adjusted to 9 with 0.1 M NaOH, and 0.1 M HCl. After that, the solution was kept at room temperature for 30 minutes without being stirred, and the color change was observed. To confirm the formation of silver nanoparticles, the visible spectrum of the solution was recorded using a visible spectrophotometer (Jenway 6300 spectrophotometer, Staffordshire, UK).

**2.4 Effect of pH:**

Five solutions were prepared to investigate the influence of pH on the production of silver nanoparticles by the aqueous extract of pear leaves: 1 ml of the aqueous extract of pear leaves was added to 9 ml of a solution of silver nitrate (1 mM) to five sample bottles, then the pH of these solutions was set to 6, 7, 8, 9, 10 by adding drops of NaOH (0.1 M). The resultant solutions were then mixed and left at room temperature for 30 minutes, and the color change of each solution was examined after half an hour. Also, the visible spectrum of each solution was recorded using the visible spectrophotometer.

**2.5 Effect of reaction time:**

Five ml of the aqueous extract of the pear leaves were added to 45 ml of silver nitrate solution of concentration 1 mM, then the pH was adjusted to 9 by adding drops of NaOH solution (0.1 M). To record the visible spectrum, 3 ml were withdrawn by the pipette after 15, 30, 60, 120, 180 minutes, as well as after 48, 72 hours, and scanned by the visible spectrophotometer.

**2.6 Effect of silver nitrate concentration:**

One ml of pear leaves aqueous extract was added to 7 sample bottles, which contained 9 ml of silver nitrate solution of different concentrations: 0.5, 1, 1.5, 2.5, 5, 7.5, 10 mM. After setting the pH of each solution to 9 by adding drops of NaOH solution (0.1 M), all solutions were kept at room temperature for half an hour. Following that, the color change of each solution was observed, and the optical properties of all solutions were studied using the visible spectrophotometer in the field of visible region.

**2.7 Effect of extract volume:**

Different volumes of pear leaf aqueous extract (0.5, 1, 2, 2.5 and 3 ml) were added to 5 sample bottles, each contained 9 ml of 1 mM silver nitrate solution. After mixing each solution, the pH was adjusted to 9 using NaOH solution (0.1 M), then all solutions were left at room temperature for 30 min. The color change was observed after 30 min, and the optical properties of all solutions were studied using the visible spectrophotometer.

**2.8 Effect of temperature:**

One ml of the aqueous extract of pear leaves was added to 5 sample bottles, each contained 9 ml of 1 mM silver nitrate solution, and then the pH was adjusted to 9 by adding drops of NaOH (0.1 M). These solutions were heated at different temperatures (20, 40, 50, 60, 70 °C) for 20 min. Subsequently, the color change of each solution was observed, and also the optical properties of the prepared solutions were studied using the visible spectrophotometer.

**3. Results and discussion:**

**3.1 Synthesis of AgNPs by aqueous extract of pear leaves:**

The interaction of silver nitrate with the aqueous extract of pear leaves resulted in the reduction of silver ions as well as a change in the color of the solution from yellow to dark brown, indicating the production of silver nanoparticles as shown in Fig (1).

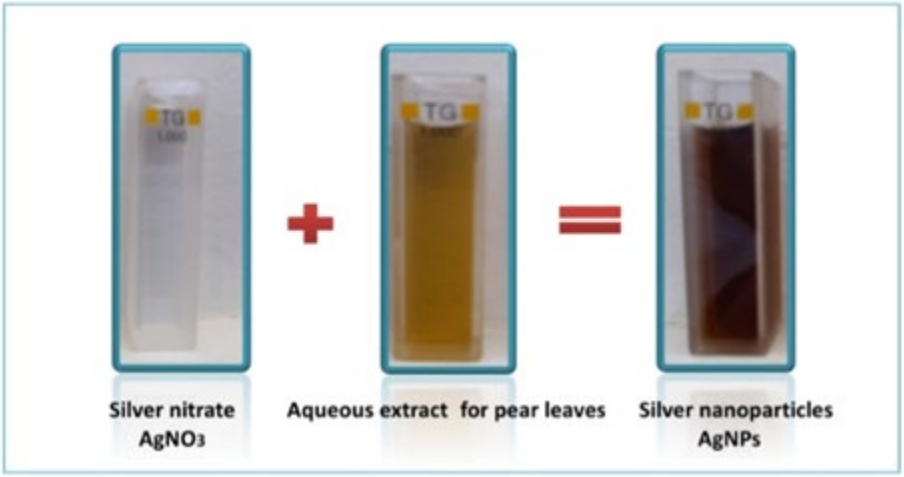


**Fig.1** Color change due to AgNPs formation

The properties of silver nanoparticles were investigated using the visible spectrophotometer, and a peak appeared at 420 nm (Fig.2), which was not observed in the visible spectrum of the aqueous extract of pear leaves. This peak was attributed to the surface Plasmon resonance (SPR). The strong interaction between silver nanoparticles and the light led to collective oscillations of the surface electrons when excitation of the light occurs at a particular wavelength. Many prior researches have reported similar findings about the color shift and the appearance of the SPR peak as an evidence for AgNPs production [23-25].

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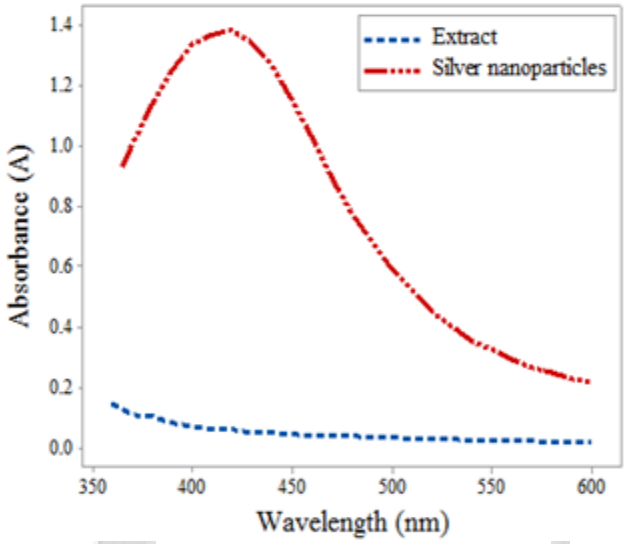


**Fig.2** Proof of AgNPs formations by Vis spectrum

### 3.2 Effect of pH:

Figure 3 shows the variation in the color of silver nanoparticle solution from colorless to yellow to reddish brown by changing the pH values and the color difference can be attributed to the aforementioned surface Plasmon resonance.

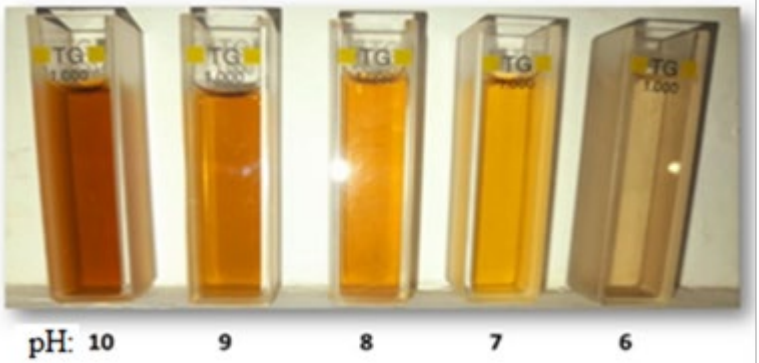


**Fig.3** Changes in the color of silver nanoparticle solution with pH changes

It was observed that the solution became darker with increasing pH, which suggests that the green synthesis in this study favors the basic medium. Moreover, the visible spectrum of these solutions showed that the higher the pH, the greater the absorbance of the SPR beam, and the beam became sharper, indicating that the formed nanoparticles became smaller, and the highest absorption for the SPR peak was recorded at pH 10 as shown in Fig. 4.

Several prior investigations have shown that pH influences green AgNPs production, with basic medium enhancing the process and acidic medium suppressing it [24, 26].

It is important to note that at pH 6 there was no color change, and the SPR peak did not appear indicating that silver nanoparticles was not formed in the acidic medium. This may be attributed as follows: in the acidic medium, some functional groups, such as OH in polyphenols, did not ionize; therefore they were unable to play their part in reducing $Ag^{+}$ ions and converting them into AgNPs.

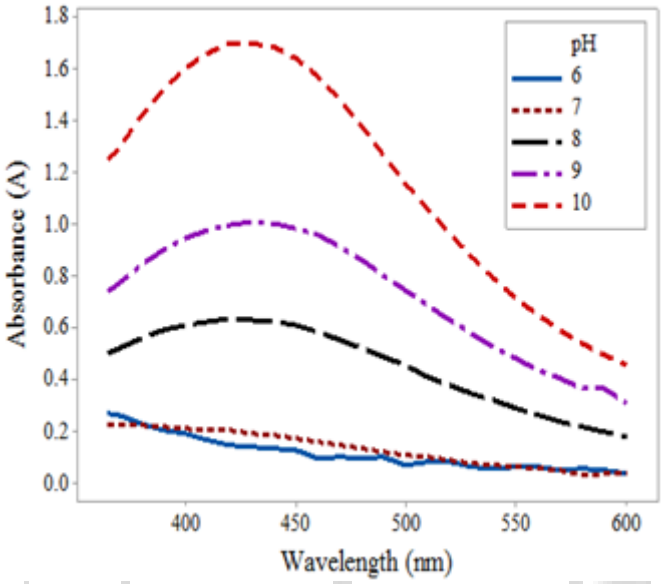


**Fig.4** Increasing absorbance of SPR peak as the pH of the solution increased.

### 3.3 Effect of reaction time:

Reaction time is one of the important factors that control the size of silver nanoparticles in biosynthesis. The SPR peak was broad after 15 minutes of reaction time due to the gradual transformation of silver $Ag^{+}$ ion into silver nanoparticles, as shown in Figure 5. And with increasing the reaction time, a significant increase in the absorbance of the SPR peak was noticed, and the peak became sharper. After 48 hours, the maximum absorbance of the SPR peak was observed, indicating the completion of the reaction. Additionally, after 72 hours there was no clear change in the visible spectrum, reflecting the stability of the formed AgNPs [25].

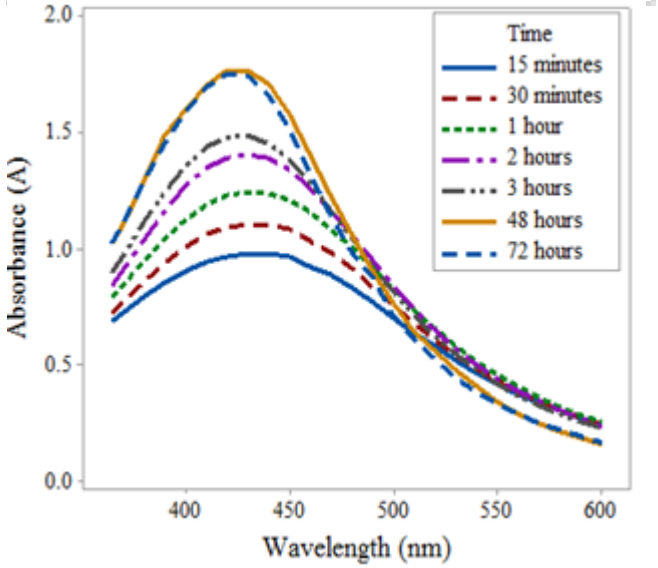


**Fig.5** Increasing absorbance of SPR peak as the time increased.

### 3.4 Effect of silver nitrate concentration:

From the visible spectrum (Figure 6) it was observed that the absorption peak of the SPR band shifted to higher values of absorbance (A), and the wavelength region became narrower when the concentration of silver nitrate solution increased (1-10mM). These indicated a change in the size of silver nanoparticles by changing the concentration of the silver nitrate solutions, also it was concluded that increasing the concentrations of the silver nitrate solution produced silver nanoparticles with a smaller size, while lowering concentrations produced silver nanoparticles with a larger size in the solution.

The color of silver nanoparticles was green at a concentration of 10 mM of silver nitrate, and the possible explanation for this is the development of more silver nanoparticles of various sizes, which is related to the high increase in silver nitrate concentration. At this high concentration, the number of active compounds available in the aqueous extract of pear leaves could be insufficient to reduce the excess number of silver ions compared to the other concentrations.

The SPR band did not appear when the silver nitrate concentration was 0.5 mm, and the color did not change, and this could be attributed to the small number of silver $Ag^{+}$ ions available for reduction, hence silver nanoparticles did not form. [26,28, 30].

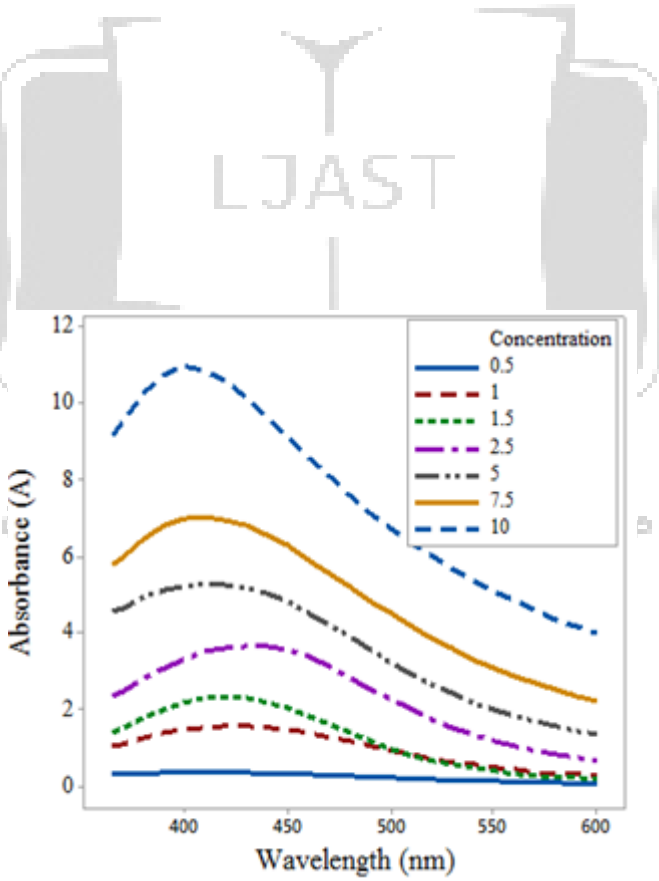


**Fig.6** Effect of $AgNO_3$ Concentration (mM)

### 3.5 Effect of extract volume:

As shown in Figure 7, increasing the volume of the pear leaf extract led to increasing the absorbance of the SPR band, which is a sign of the formation of more amounts of silver nanoparticles. Additionally, the appearance of SPR bands at the same wavelength indicated that different amounts of extract were adjusted to synthesis the AgNPs, and that formation increased as the volume of extract increased without a significant change in the size of the silver nanoparticles. The

absorbance of SPR peak was significantly increased when the extract volume increased without any shift in wavelength, with 3 ml of extract resulting in the highest reduction of the constant volume of silver nitrate solution (9ml) [27-29].

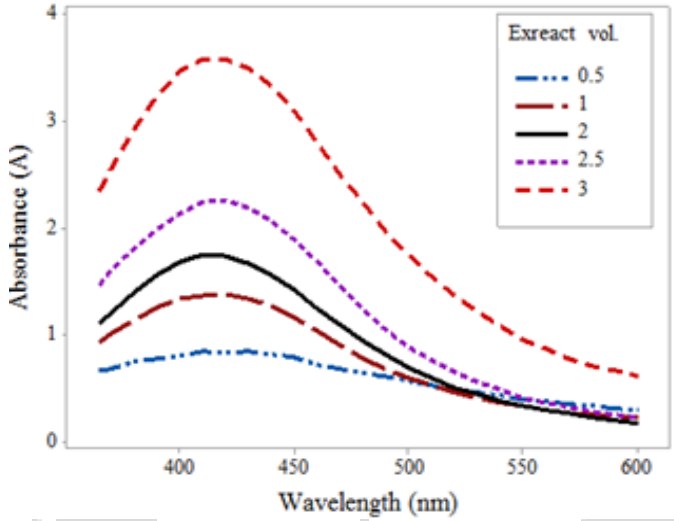


**Fig.6** Increasing absorbance of SPR peak with increasing of pear leaf extract volume (ml).

**3.6 Effect of temperature:**

According to similar research [27, 30] that studied the effect of temperature on the biosynthesis of silver nanoparticles from other plants, it was found that nanoparticles grow better and larger at lower temperatures, and this means that lower temperature helps growth. However, it was observed that the overall reaction rate increases with the increase in the reaction temperature, and from this we conclude that the increase in temperature helps nucleation.

Based on what was mentioned above, it was noticed in the current study (Figure 7) that at a temperature of 20ºC, the SPR band was broad, indicating the large size of the formed silver nanoparticles. Also, the absorbance of the SPR band was low, reflecting that the reaction was slow at low temperatures. This led to the formation of a few silver nanoparticles but with a large size.

With the increase in the reaction temperature (40-60°C), the peak became more intense, and the absorbance of the SPR peak increased due to the formation of silver nanoparticles with a smaller size and a larger number, that is to say, the nucleation rate increased. Furthermore, when the temperature increased to 70°C, there was no noticeable increase in the absorbance of the SPR band, which was due to the crystal growth around the nucleus only without the nucleation process taking place.

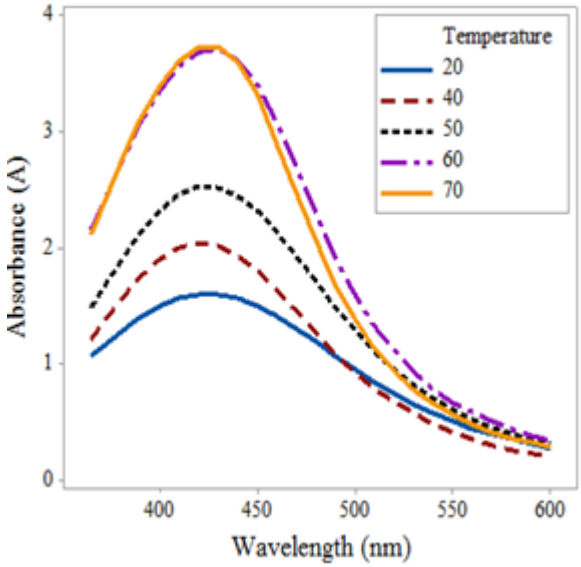


**Fig.7** Increasing absorbance of SPR peak as temperature (°C) increased.

**4. Conclusion:**

Pear leaf aqueous extract is an excellent material for the biosynthesis of AgNPs, and by controlling the elements that influence synthesis, such as time, temperature, and volume of pear leaf aqueous extract, a bigger number of AgNPs with small particle sizes may be produced. The proposed method in this study is simple, cost-effective and ecofriendly and can be applied to produce AgNPs with different particle sizes, which might broaden the applications. Other studies are required to characterize AgNPs formed by the aqueous extract of pear leaves, as well as using this pear leaves extract to biosynthesis other metal nanoparticles.

**5. Limitations of the study:**

This study did not include a surface analysis, which may have offered useful information regarding the shape and size of the created objects if it had been done.

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