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Biosynthesis of Silver Nanoparticles Using *Malva Sylvestris* Flower Extract and Its Antibacterial and Catalytic Activity

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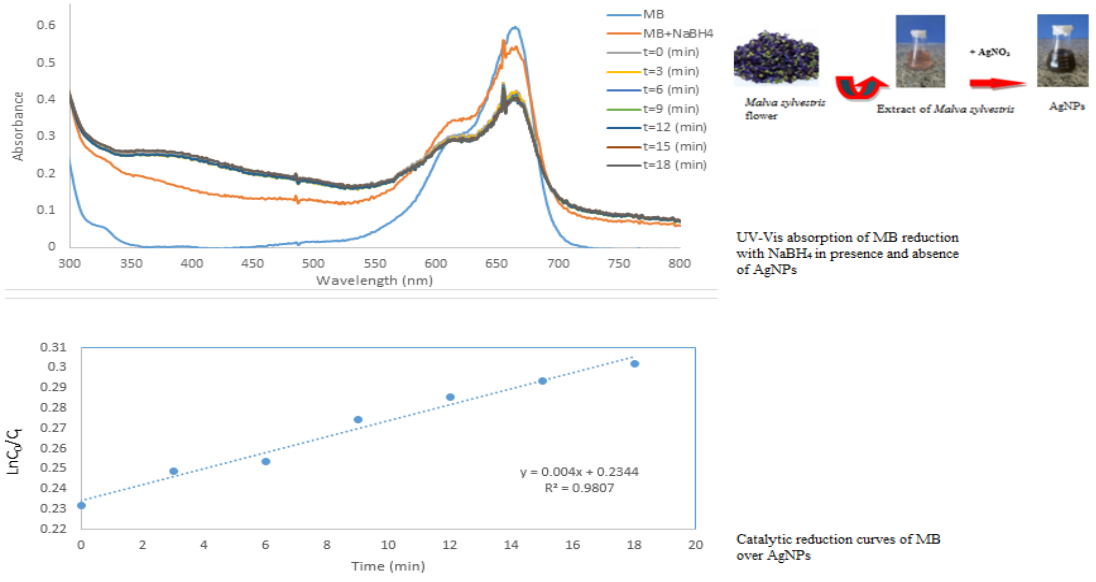
ABSTRACT

In this study, silver nanoparticles (AgNPs) were synthesized by using *Malva sylvestris* flower extract. Different parameters such as, pH, extract volume, silver nitrate concentration, temperature and reaction time were controlled correctly and the effect of them on the synthesis of nanoparticles were investigated. Some of techniques like, UV-Vis, FTIR, SEM, TEM, and XRD were used to analyze the characteristics of AgNPs. According to our results, the shape and size of nanoparticles were spherical and about 20 - 30 nm range, respectively. In addition, the surface plasmon resonance (SPR) of nanoparticles occurred in 445 nm. The effect of catalytic activity AgNPs synthesized on the reaction Sodium Borohydride ($NaBH_4$) with Methylene Blue (MB) and its antibacterial activity on (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Salmonellatyphy murium*) as a references bacteria have been studied. Also, the effect of *Malva sylvestris* flower extract on them was measured and analyzed precisely. The results showed ability of catalytic activity on the reduction of MB. Further, synthesized nanoparticles have significant ability antibacterial activity against gram negative bacteria (*Escherichia coli and salmonella typhy murium*).

GRAPHICAL ABSTRACT



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Introduction

Nowadays, nanotechnology is categorized among new field of science that is bound to numerous fields to solve some major problems about health and environment. Nanotechnology connects knowledge of different sciences like, chemistry, biology, physics, and engineering [1, 2]. Nanotechnology is so important field of the modern science, because it deals with the synthesis and modification of particles with small sizes, from 1 to 100 nm. Nanoparticles can be synthesized by different approaches like chemical, physical and biological methods. The shape of many materials will change when formed as nanoparticles, because nanoparticles have greater surface to volume ratio; therefore, it gives them a chance to be more active than other materials [3]. Several studies have proven that different types of nanoparticles, particularly nanomaterial such as zinc, gold, silver and other types of nanomaterial have a significant ability to control the growth of some bacteria, viruses and other type of microorganisms. Increasing the rate of resistant bacteria to available antibiotics is the strict challenge for every country, so finding an alternative therapy against pathogenic bacteria can be a greatest revolution in medical science [4, 5]. AgNPs are playing as an important tool in the field of medical science and industrial technology, particularly nanotechnology.

Different methods are used to synthesize the AgNPs such as, electrochemical, photochemical, radiation chemical reduction and other methods, but finding the best method without negative impact on the environment should be considered as a primary necessity [3]. It is interesting that some plants and their products are used to synthesize nanoparticles, because most of them are nontoxic [6]; this method for synthesizing nanoparticles is called green synthesis [7]. Therefore, using plant extract can be an eco-friendly method for synthesizing AgNPs. Different studies have shown that, AgNPs are prepared by using the leaf extract of some plants like, *Lantana camara*, *Camellia sinesis and Eucalyptus chapmaniana*. As we said before, the whole parts of plants such as fruit, flower, root, are used to nanoparticle synthesis. For example, the extract of the seed powder of *Cuminum cyminum* and fruit extract of *Securinega leucopyrust* can be used to synthesize the AgNPs as well. Some advantages like, high speed, low costs and simple method are the reasonable reason for using plants to synthesize AgNPs. Other cases are the use of plants related to the speed of reduction of metal ions that is faster and the shape and size of nanoparticles is controllable by various parameters such as changing pH. All in all, regarding this fact that metal nanoparticles have some noticeable features like, SPR optical features, suitable catalytic feature, antibacterial and antiviral activity and other advantages, these compounds attract more attention of scientists to deal with them in different fields [8-13].

According to different studies about nanoparticles synthesis by plants extract, in this study, AgNPs was synthesized by using the flower extract of *Malva sylvestris* plant. The genus *Malva* includes different species. *Malva sylvestris* is categorized among *Malva* genus. This plant has stem with nearly 10 to 15 cm height, heart-shaped leaves, purple flowers and perennial root. *Malva sylvestris* is originated from the southern Europe and Asia, but it can be found in numerous parts of the world. In this study, effects of different factors on the synthesis of nanoparticles were measured precisely. Catalytic properties on reduction of MB by $NaBH_4$ were calculated, as well as, antibacterial effect of synthesized nanoparticles at optimum conditions on gram positive and negative bacteria by using disc diffusion method was measured.

Material and methods

The *Malva sylvestris* plant was prepared by local market in Iran (Arak), also chemicals were bought by Merck Company.

Apparatus

AgNPs was synthesized through using the flower extract of *Malva sylvestris* plant; nanoparticles were synthesized under mentioned conditions and analyzed by UV-Vis**,** FTIR, XRD, SEM, TEM

techniques. UV-Visible spectroscopic (UV-Vis Agilent 8541) analysis was utilized as a primary technique to characterize the nanoparticles. Fourier transform infrared Spectroscopy (Perkinelmer Spectrum 2 spectrophotometer) was used to recognize the potential of functional group in *Malva sylvestris* flower extract. XRD analysis was used to establish the metallic nature of particles. The crystalline structure of the silver nanoparticles was identified by using Philips company χ' pert. Pro X-ray diffractometer by monochromatic Cu kα radiation (λ=1.54 Å) were maintained at 40 kV, 30 mA. Scanning electron microscopy (SEM, EM 3200 Model, KYKY Company) was applied to determine the size, shape and structure of our samples and transmission electron microscopy (TEM, CM120 Model, Philips Company) was used in our study.

Preparation of Malva sylvestris extract

In the first step, the *Malva sylvestris* plant was washed twice with distilled water and dried at 25°C. 10 g of *Malva sylvestris* flower was added to 100 mL of distilled water and boiled for 5 min. Then, it was filtered by the use of Whatman filter paper. The solution was centrifuged for 30 min at 4000 rpm and kept at 4 °C for the future studies.

Synthesis of AgNPs

In the first step, 250 mL of silver nitrate ($AgNO_3$) 0.01 M was prepared and used for preparing diluted concentration 1, 3, 5 and 10 mM). By adding the extract to these solutions, the color of aqueous solution changed from pink to dark brown Figure 1. The change of color occurred by the reaction of metal ions with reducing agents in the flower extract and finally, the formation nanoparticles. The color of the colloidal silver solution attributed to surface plasmon resonance (SPR) is due to the mass fluctuations of free electrons induced by interaction with the electromagnetic field. This is specific to each type of nanoparticle in any size and is due to plasmonic phenomenon ability in absorbing light in the region of the ultraviolet-visible absorption spectrum [14]. The optimization of variables in this step is so crucial and important. The size of nanoparticles is influenced mainly by different parameters such as, pH, salt solution concentration, volume of *Malva sylvestris* flower extract, temperature and reaction time. They were surveyed correctly and the effect of them on the synthesis of nanoparticles was measured.

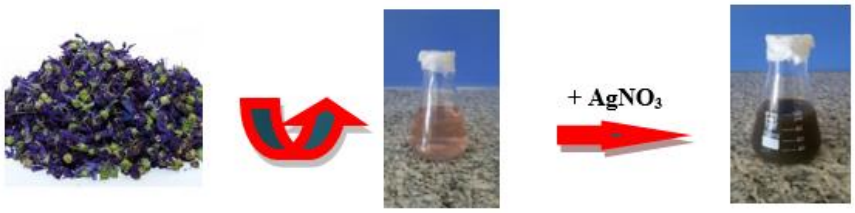


Figure 1: Schematic bio-synthesis procedure of AgNPs

Procedures

5 mL of *Malva sylvestris* extract was blended with 95 mL of silver nitrate 0.001M, after shaking 30 min at different pH (2, 4, 6, 8 and 10) for adjusting pH, HCl and NaOH 0.1M is used. Adjusting pH with 0.1 M acid and base was based on other researcher's studies that had reported [15]. The pH was measured with an EDT GP 353 ATC pH meter. Then, it was centrifuged 30 min at 4000 rpm. All the steps listed were applied for extract volume (2, 4, 6 and 8 mL), salt concentration (1, 3, 5 and 10 mM), temperature (25, 35, 60, 80 and 100 °C) and time (10, 20, 35, 60, 80 and 120 min) of reaction. The absorbance of colloidal solutions was measured by using UV-Vis spectroscopy in the range of 330-800 nm.

Catalytic study

0.005 g of AgNPs was reached 25 mL volume and solved by using ultrasonic device. Then, the

absorbance of 1 mL extract with 0.1 mL of $NaBH_4$ and 3 mL MB 10^{-5} M was studied and measured in different times (0, 3, 6, 9, 12, 15 and 20 min).

Measurement of antibacterial activity

Escherichia coli (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633) and *Salmonella typhimurium* (ATCC 19430) were used as reference bacteria for analyzing the antimicrobial activity.

Antibacterial activity by disc diffusion method

Antibacterial activity was measured by using paper discs. The dilution of 0.5 Mac Farland was prepared for every reference bacterium separately. 500 µL of each bacterium was cultured separately on the each nutrient agar plate as spread culture. Then, 20 µL, 30 µL and 40 µL values of each sample (AgNPs by *Malva sylvestris* flower extract) were used to saturate every paper disc. The saturated discs were placed on the surface of agar that reference microorganism was cultured on it previously. The plates were located in the incubator from 48 to 72 h at 37 °C. Eventually, the diameter of inhibition zone around each paper disc was measured precisely.

Result and Dissection

Effects of pH, extract volume, salt concentration, temperature and reaction time

The UV-Vis spectral analysis was applied to monitor the formation of AgNPs. As a general rule, the characteristic part of SPR bond of AgNPs falls in the range of 330-800 nm. According to Figure 2, the SPR bond for AgNPs was at 445 nm. The highest value of the absorbance of wavelength was proved by SPR of the metallic silver in visible region and similar results were reported by Govindarajan et al., using leaf of the plant for extraction [16].

According in previous section, the optimized pH is defined by the use of UV-Vis spectroscopy. The results showed the maximum value of absorbance is seen at pH 10 Figure 3a. This blue shift depicts a reduction in the size of the AgNPs [17].

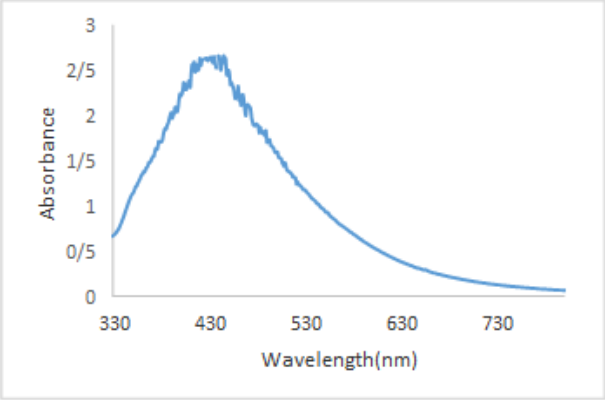


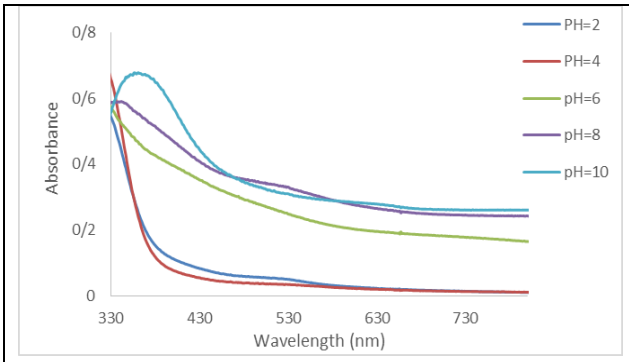
Figure 2: UV-Vis spectrum analysis of synthesized AgNPs

After the determination pH, the optimal silver nitrate concentration should be determined. As previously mentioned in this section, different concentrations of silver nitrate were added after the adjustment optimum pH, and put on the shaker at room temperature. The maximum value of absorbance was determined in the range of 330 to 800 nm. According to Figure 3b, 10 mM was recognized as the optimized value of silver nitrate concentration.

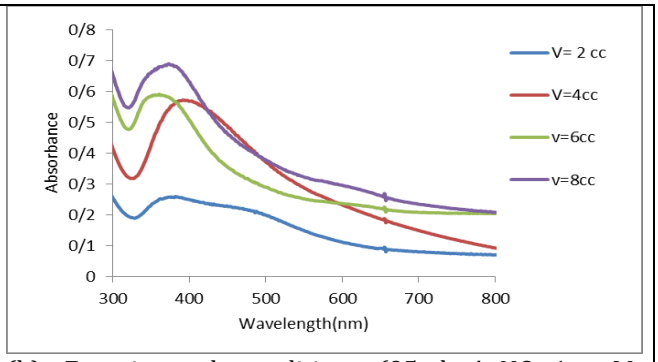
Different values of flower extract in the optimal pH, and silver nitrate were used to find the optimized value of flower extract. The results showed, the highest value of wavelength is related to optimized value. According to Figure 3c, 8 mL was selected as the optimized value of silver nitrate. Optimized temperature is so important to synthetize silver nanoparticles. In this stage, we applied optimum conditions that were obtained in previous stages at different temperatures. The highest value of absorbance was in coincidence with 80 °C; as a result, it was introduced as the optimized temperature (Figure 3d).

The effect of the time on this reaction was evaluated for reaching the best status; different times were selected and the role of them on AgNPs synthesis at optimum conditions was surveyed. According to Figure 3e, time has

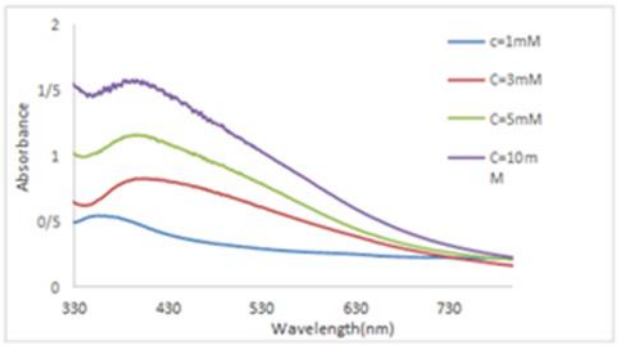
significant effect on the rate of AgNPs synthesis. AgNPs were analyzed between the ranges of 10 to 120 min regarding to the peak intensity; 120 min was selected as the optimum time to synthesize the AgNPs. According to coincident peaks in this figure, it seems that, time does not have noticeable effect on the size of AgNPs, but, was effective only on the number of AgNPs and the stability of them in colloidal environment.



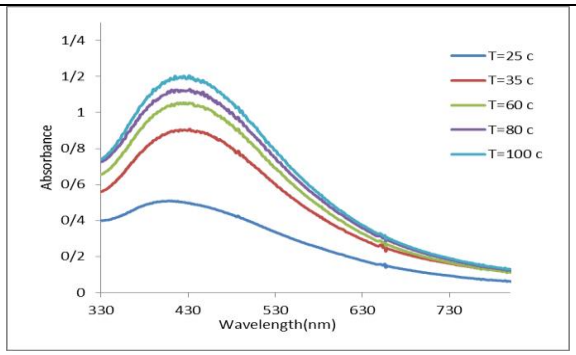
(a) Experimental condition: (5ml of volume extract, 95ml $AgNO_3$ 1 mM, various pH, shaker :30 min, at room temperature)



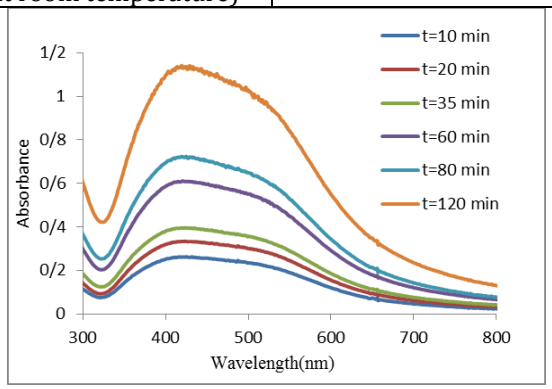
(b) Experimental condition: (95ml $AgNO_3$:1 mM, pH=10, shaker: 30 min, at room temperature)



(c) Experimental condition: (8ml volume extract, 95ml $AgNO_3$, pH=10, shaker: 30 min, at room temperature)



(d) Experimental condition: (8ml volume extract, 95ml $AgNO_3$:10 mM, pH=10, shaker: 30 min)



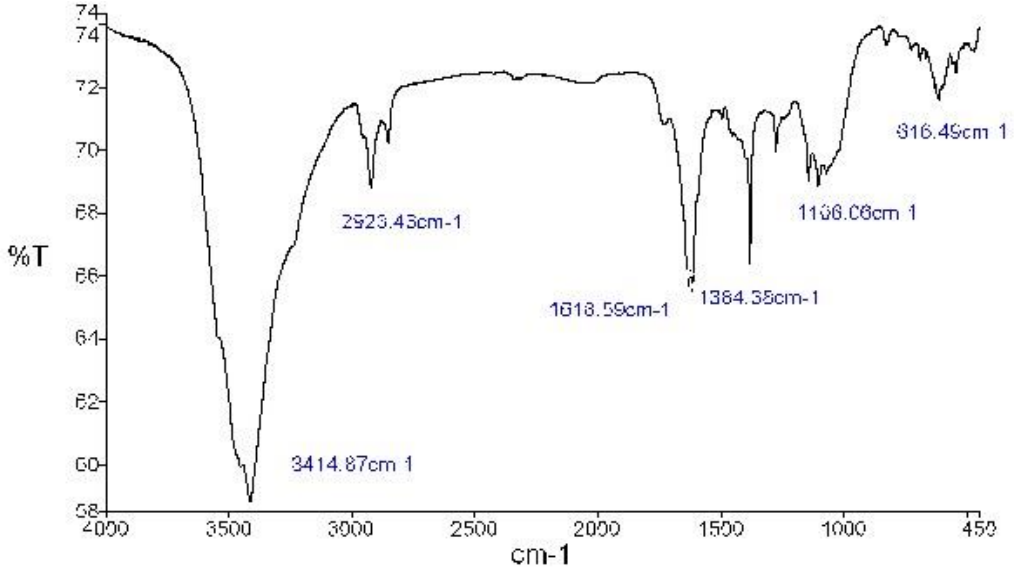
(e)Experimental condition: (8ml volume extract, 95ml $AgNO_3$: 10mM, pH=10, shaker: 30 min, temperature: 80 °C)

Figure 3: UV-Vis spectra of AgNPs: (a) pH, (b) Malva sylvestris extract volume, (c) $AgNO_3$ concentration, (d) temperature, (e) reaction time

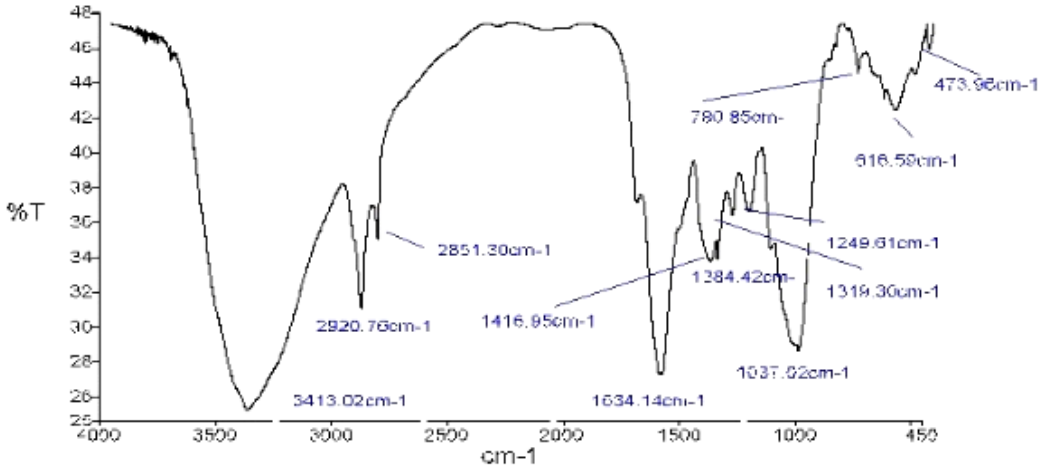
The characterization of Malva sylvestris extract and AgNPs

The FTIR spectrum of AgNPs and flower extract is shown at Figure 4. Since functional groups are effective in reduction silver ions, it is necessary to be investigated. So, FTIR technique is used to identify the functional groups. The spectrum of FTIR in nanoparticles at optimal conditions

shows several results: Sharp peak at 3413 cm^{-1} is related to the stretching vibration of OH (OH-stretching) in alcohols and phenols, peak at 2920 cm^{-1} is related to (C-H) the stretching vibration of alkanes, peak at 2851 cm^{-1} is resulted by the presence of aldehyde group, strong peak at 1634 cm^{-1} is influenced by the presence of stretching and bending vibration of carbonyl amide, and peak at 1318 cm^{-1} is related to the stretching vibration of (C-N) and bending vibration of (OH) bond [18-20].



(a)



(b)

Figure 4: FTIR analyses (a) *Malva sylvestris* extract, (b) AgNPs using *Malva sylvestris* extract in the optimum conditions

It was reported that formation of AgNPs was gained by the presence of amide, amino, carbonyl, phenol and flavonoid groups. According to FTIR results, All 5 groups can be used for the reduction of silver ions and the stability of formed nanoparticles.

The pattern of XRD in nanoparticles showed that it was completely coincident with reference pattern of metal AgNPs (Figure 5). Four main peaks at 2θ values were, 38.4589, 44.5207, 64.7785, 77.7046 that were coincident with 1 1 1, 2 0 0, 2 2 0 and 3 1 1, respectively, and the structure was as cubic. Peak 1 1 1 had the highest length and other two peaks were represented at 3 1 1 and 2 2 0. The average of the size of particles was 20.19 nm that was obtained by the use of Deby-Scherrer (Equation 1).

$$D = K\lambda / \beta \cos\theta \quad (1)$$

in which the parameters are as follows, respectively: D is the size of the crystal, its unit is equal to λ unit and is usually angstrom or nm; λ is the X-ray wavelength; K=0.9 is a dimensionless shape factor, with a value close to unity; β is the full width at half maximum (FWHM); and θ is the peak position on the horizontal axis [21].

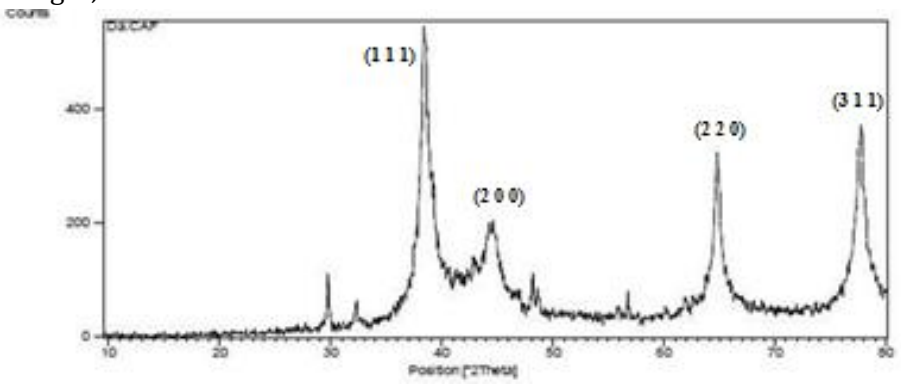


Figure 5. XRD pattern of the AgNPs powder obtained for the optimum conditions

The SEM image of AgNPs at optimized condition is shown in Figure 6 and the size of distribution ranging from 32-54 nm. According to Figure 6, it is obvious that synthesized AgNPs in optimized conditions were spherical and the average diameter of them was 30 nm.

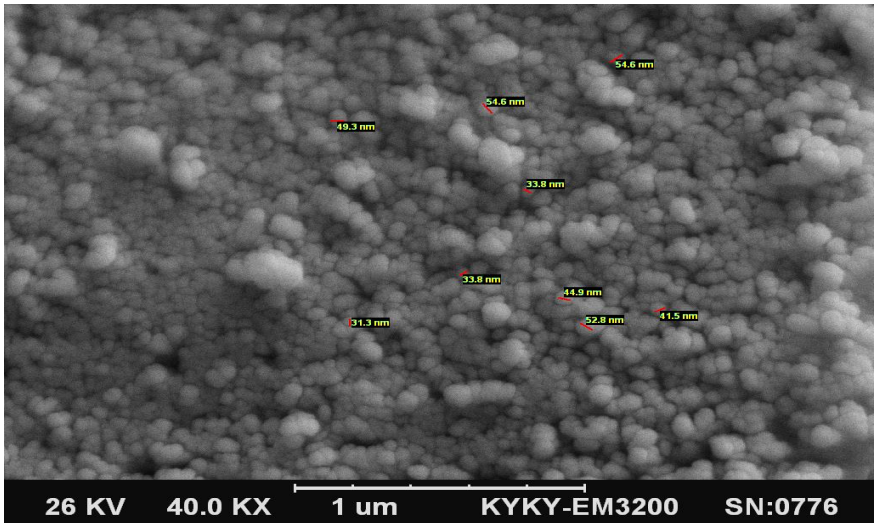


Figure 6. SEM image of AgNPs

The TEM images of AgNPs synthesized at optimized condition is depicted in Figure 7 and it detected that the synthesized AgNPs are spherical in shape and size ranged from 20 -30 nm. The size of nanoparticles that was resulted by SEM, TEM and XRD methods was close together.

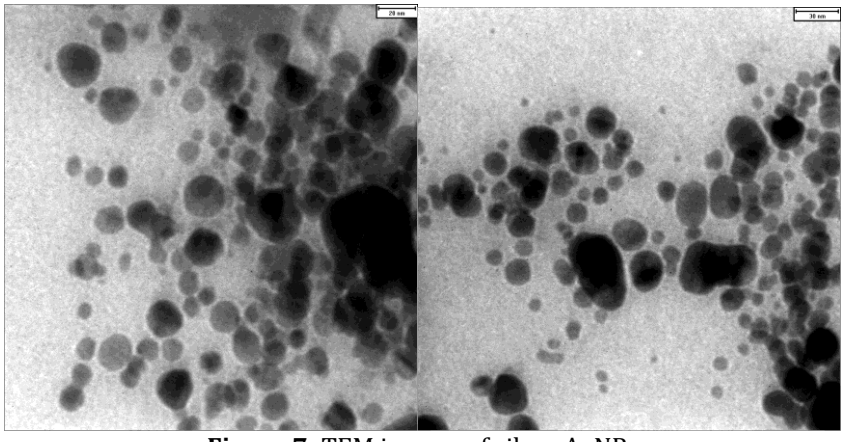


Figure 7: TEM images of silver AgNPs

A comparison of the characterization results in present study with some extracts of the flower of plants is shown in Table 1.

Table 1: Comparison of characterization results of the present study with some flowers extract research studies

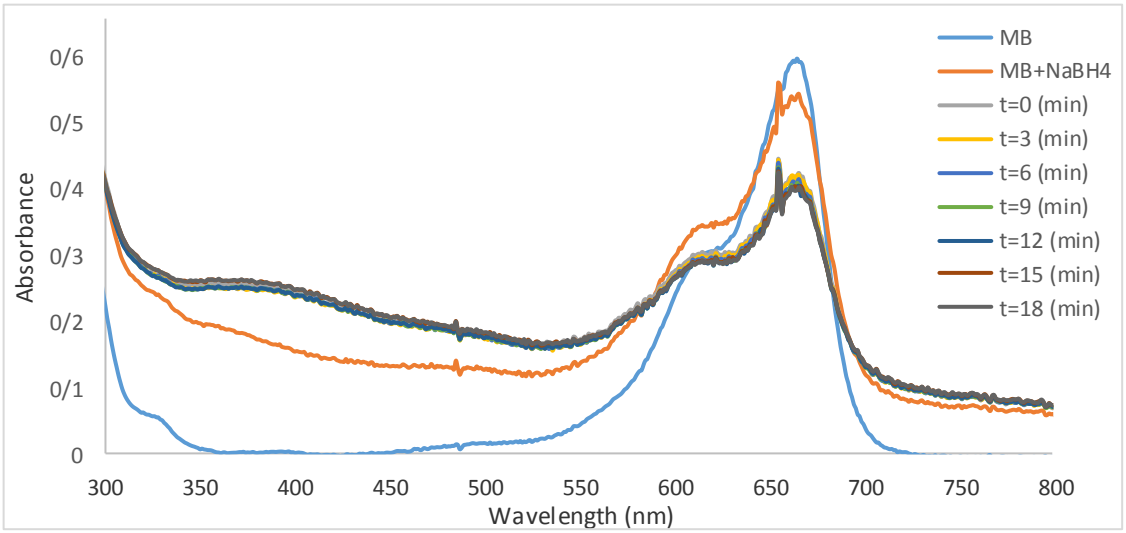
Reducing and Stablizing agent	Absorbance maximum (nm)	Morphology of silver nanopartieles		characterization	References
		Size (nm)	shape		
Marigold	430	10-90	Spherical, hexagonal, irregular	UV-Vis, FTIR, TEM XRD, SAEM, EDX	[22]
Catharanthus roseus	460	6-25	spherical	UV-Vis, FTIR, TEM	[3]
Chrysanthemum indicum	435	37.71-71.99	spherical	UV-Vis, XRD , EDX , TEM	[23]
Hibiscus rosa sinensis	422	5-40	spherical	UV-Vis , FTIR, SEM	[13]
Erythrina indica	401	10-40	spherical	UV-Vis, FTIR, SEM	[5]
Malva sylvestris	445	20-30	spherical	UV-Vis, FTIR, XRD, SEM, TEM	This study

Catalytic activity

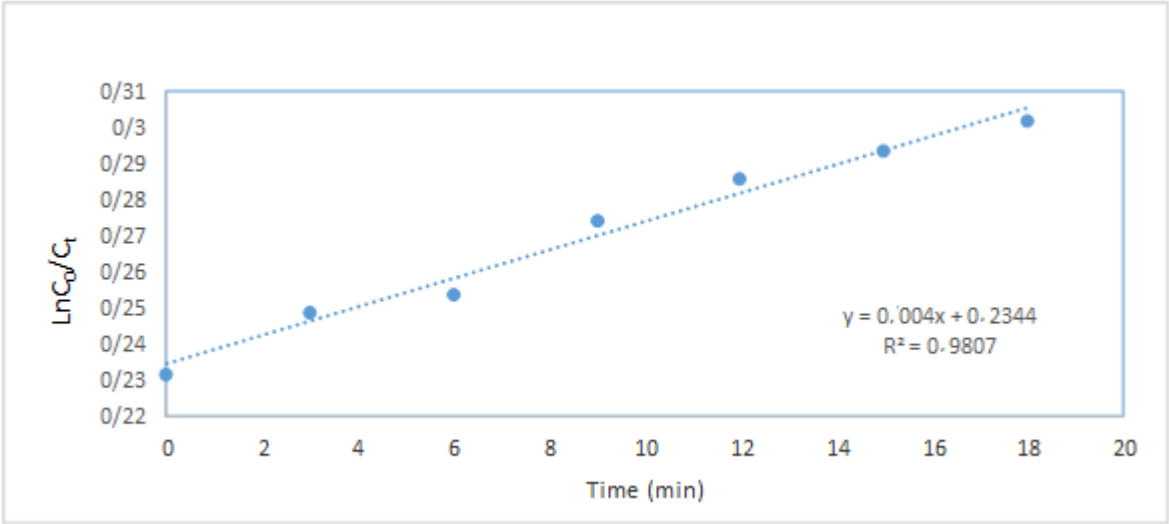
AgNPs can be used as effective catalyst. The kinetics of catalytic conversion of MB by the use of $NaBH_4$ in the presence of synthesized AgNPs at optimum condition is followed by the UV-Vis spectroscopy. The absorbance intensity is shown at 664 nm Figure 8a. The reduction of MB along with AgNPs or without it showed that improvement of the reduction was sufficient [24, 25]. The pseudo-first order kinetics with the respect to the concentration of MB was used to calculate the rate of constant (k). Pseudo-first order can be described by the following equation 2 [16, 26].

$$\mathrm{Ln}C_0/C_t = Kt \qquad (2)$$

where C_o (M) is the concentration of MB at t =0 min, C_t is the concentration of MB at time t, k (min^{-1}) is the pseudo first order rate of constant and t is reaction time. Figure 8b shows that $\mathrm{Ln}C_0/C_t$ into t is acceptable linear and slope of the linear graph gives the rate of constant that was received 0.004 min^{-1}.



(a)



(b)

Figure 8: (a) UV-Vis absorption of MB reduction with $NaBH_4$ in presence and absence of AgNPs, (b) Catalytic reduction curves of MB over AgNPs

Antibacterial assay

The results of antibacterial assay were obtained by the measurement of the inhibition zone around each paper disc. First of all, the minimum concentration of our sample (AgNPs by *Malva sylvestris* flower extract) that references bacteria were inhibited in this concentration was obtained and identified. 30 µL was the best minimum concentration for inhibiting the growth of references bacteria. At 20 µL the inhibition of the growth was not seen and at 40 µL the results were nearly similar to 30 µL. As a result, 30 µL was selected as the best concentration. After the measurement of inhibition zone around each paper disc (Figure 9), the results were interpreted according to the following instruction (Table 2).

Table 2: The results of antibacterial assay

Sample	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus subtilis*	*Salmonellatyphy murium*
*	+++	-	-	+++

(-) without inhibition zone, Mm 0-3(+) , Mm 3-5(++) , Mm > 5 (+++)

The results showed that *Bacillus subtilis* and *Staphylococcus aureus* were completely resistant to our sample; these bacteria grew entirely in the presence of the prepared sample. In contrast, *Escherichia coli* and *Salmonella typhy murium* were sensitive to our extract. On the whole, the gram negative bacteria were inhibited but gram positive bacteria grew well. According to survey the bacterial structure, the structure of cell wall between gram positive bacteria and gram negative bacteria is completely different. Most of the gram positive bacteria are sensitive to common antibiotics, because the structure of the cell wall among gram positive bacteria consist of few peptidoglycan layers; therefore, they cannot be resistant against antibiotics, but gram negative bacteria have lipopolysaccharide in their structure; it helps them to be resistant against antibiotics. Most of the dangerous and pathogenic bacteria are categorized among gram negative bacteria. As a result, finding the compounds with antibacterial activity is so important and vital nowadays. The results show that our sample has noticeable activity for inhibiting the growth of gram negative bacteria. It brings a good prospect to control of some dangerous bacteria and it may be so beneficial and helpful in the field of medical science.

Conclusion

The wide variety of nanoparticle size in nanometric dimension is considered as one of the important restrictions to synthetize nanoparticles; we can overcome these restrictions by using changing parameters such as different salt concentration, pH variety, different volume of plant extract and times and temperatures ranges. Therefore, changing parameters is used to reduce nanoparticle size and reach optimized condition. In this research, we could reach and illustrate a simple method to synthesize AgNPs. By using the flower extract of *Malva Sylvestris,* different types of techniques were applied to characterize the nanoparticles like UV-Vis, FTIR, XRD, SEM and TEM. UV-Vis spectroscopy was used to form nanoparticles and survey the effect of different parameters on biosynthesis. The use of FTIR showed the functional groups in the extract before and after of synthesis of nanoparticles, which caused Ag^{+} reduction. The analyses of SEM and TEM showed that synthesized nanoparticles were spherical in shape and the average size of them was about 20-30 nm. Additionally, XRD was applied to prove the existence of AgNPs with the average size of 20.19 nm, which was coincident with the results of SEM and TEM. The results of catalytic activity of synthesized nanoparticles on the reaction of MB and $NaBH_4$ showed the reaction followed pseudo-first order kinetics reaction. On the whole, green synthesis AgNPs by using the flower extract of *Malva sylvestris* is an ecofriendly and economical method. It can be used to remove colorful pollutants in water and antibacterial properties in synthesized AgNPs on gram positive and negative bacteria showed that it can be applied as antibacterial agent and the antibacterial effect of synthesized AgNPs on gram negative bacteria is so higher than gram positive bacteria.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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