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Research article

Green Synthesis of Silver Nanoparticles and their Antibacterial Activity Enhancement

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Abstract
In this study, a green facile method was implemented for the synthesis of silver nanoparticles (AgNPs). The effect of pH, temperature, content of sage extract, ionic solution concentration, and reaction time was investigated using aqueous extract of Salvia officinalis as a bioreducing and capping agent in a single step biosynthesis. All these factors showed a significant influence on the synthesis rate, size and shape of the AgNPs. The optimum synthesis conditions were at pH 10, 10mM [Ag^+], 5 days reaction time, 2:5 volume ratio extract: [Ag^+] and 60°C. The prepared silver nanoparticles were characterized using UV-Vis, scanning electron microscopy (SEM), FTIR and X-Ray Diffraction. According to the observed results, the synthesized AgNPs were confirmed and showed a spherical morphology and a particle size of 30.9 nm. Furthermore, the AgNPs showed significant antibacterial activity against gram-positive and gram-negative bacteria and enhancement for antibiotic activity. Further research is needed to develop more this approach and to investigate the effects of AgNPs.

Keywords: facile method, aqueous extract, silver nanoparticles, *Salvia Officinalis*, antibacterial

Introduction

Nanotechnology is one of the most important technologies applied in numerous fields (Visweswara *et al*., 2015: Mohd, *et al.*, 2024). In recent years, interest has increased in the production of metallic nanomaterials such as gold, platinum, silver, titanium, zinc, cerium, iron, and thallium because of their versatile uses in different fields such as biomedical, agricultural, environmental and industrial (Singh *et al*., 2016; Kunle *et al*., 2021). Of the entire metallic nanomaterials, silver nanoparticle is exceptional because of its versatility, simplicity of synthesis, adaptability, morphology, high catalytic property, high conductivity, high thermal stability, antibacterial effect and its extreme surface area that paves way for the coordination of a vast number of ligands. Silver in many of its forms has been historically used as an antimicrobial agent exclusively or combined with other technologies (Silva *et al*., 2017). Due to its inhibitory property of bacterial growth, silver has been studied in advance by incorporating it as silver nitrate or silver sulfadiazine in creams and dressings to treat burns and ulcers, as anti-contaminant in food packaging technology, in home appliances as refrigerators and washing machines, and several applications in the industrial area (Bruna *et al*., 2021). In addition to their significant biomedical applications such as anticancer (Habibeh, 2020), antibacterial (Monira *et al*., 2023: Zaraei, *et al.*, 2024), mosquitocidal activity, antifungal, anti-inflammatory and anti-viral (Okaiyeto *et al*., 2021). Silver nanoparticles (AgNPs) can be prepared by physical, chemical and biological methods (Sehnal *et al*., 2019). One of the disadvantages of the physical and chemical methods used in the manufacture of nanoparticles is that they take longer, and use harmful substances and dangerous solvents that may be difficult to get rid of, and their effects remain in the environment, in addition to their high expense (Irshad *et al*., 2020). Biological methods are more favorable for synthesizing silver nanoparticles, which may involve the use of bacteria, fungi and plant extract using green synthesis route (Chhangte *et al*., 2021). This method is ecofriendly and of low cost and silver nanoparticles formed are stable and well dispersed with limited aggregation and good size control. Working conditions such as concentration, volume ratio, contact time, temperature and pH affect the synthesis of silver nanoparticles. (Dada *et al*., 2018).

In pharmaceutical and biomedical fields, human pathogen resistance is a big challenge. Antibiotic resistance profiles arose concern about the emergence and reemergence of multidrug-resistant (MDR) pathogens and parasites (Tenover *et al*., 2016). Once an individual is infected with MDR bacteria, a multiple treatment of broad-spectrum antibiotics is required where he/she has to spend more time in hospital and in addition to that such treatment is more expensive, more toxic to patient and less effective (Webb *et al*., 2005).

To solve this worldwide problem, nanomaterials with antimicrobial properties could be the key. Materials of silver nanoparticles (AgNPs) have been widely studied due to their antimicrobial effect in different organisms (Vazquez-Muñoz *et al*., 2019). Consequently, development of or modification in antimicrobial compounds to improve bactericidal potential is a priority area of research in modern era (Humberto *et al*., 2010). This paper describes the preparation, characterization

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and biomedical application of as-prepared silver nanoparticles.

Materials and methods

Chemicals

Hydrochloric acid (37%) (Sigma, Germany), Sodium Hydroxide (BDH, UK) and silver nitrate (BDH, UK) were used as described during experimentation.

Instruments

The following instruments were used in the study: Double-beam ultraviolet visible spectrophotometer Photo Lab 7600 (UV-VIS 7600) (WTW, Germany), Ultra-compact Fourier transform infrared spectroscopy (FTIR) Cary 630 (Agilent, US), pH meter (WTW, Germany), JSM-5610LV scanning electron microscope (SEM) (JEOL, Japan) which is a SEM designed for high-resolution imaging of samples and the PW 1710 X-ray diffractometer (Philips, Netherlands) is a laboratory X-ray diffraction instrument designed for the analysis of the atomic and molecular structure of materials.

Culture Media

Muller Hinton agar, sterile petri plates, sterile swabs were used during bacterial cultures; *Staphylococcus aureus* and *Escherichia coli*.

Antibiotic Disc

The antibiotics used during the experimentation in this study are provided in Table 1.

Table 1. Antibiotics used to assess antibacterial activity in this study.

No	Antibiotic	Code	Concentration/tablet
1	Amikacin	AK	30µg
2	Trimethoprim	SXT	25µg
3	Tetracycline	TE	30µg

Synthesis of silver nanoparticles

Preparation of plant extract

The Salvia officinalis (sage) leaves were collected from the garden in Al-Fornaj, Tripoli, washed thoroughly with water, distilled water and finally deionized water to remove any dust and contaminants. The leaves were air-dried and finely ground into a fine powder using a mortar and pestle; 5g of the leaf powder was immersed in 100ml of deionized water and heated at a temperature of 55°C for 35 minutes. The extract (60% yield) was cooled at room temperature, centrifuged and filtered to obtain a clear and homogeneous solution of S. officinalis leaf extract and stored at ~4°C for further use.

Salvia Officinalis commonly known as Sage, is an evergreen subshrub with greyish leaves and purple flowers (Figure 1). Its original habitat is the Mediterranean region belonging to the mint family (Lamiaceae). It encompasses diverse bio-active compounds such phenolic acids, proteins, vitamins and alkaloids that are involved in the reduction of silver ions and the stability of nanoparticles.

The leaves of sage are green on the upper side and nearly white underneath having a distinctive and aromatic scent. The antibactertial activity experiments were conducted in the laboratory of Al-Khadra hospital facilities in Tripoli.



Figure 1. Leaves of Salvia officinalis (sage) plant.

Biosynthesis of silver nanoparticles

Silver nanoparticles were synthesized according to the technique described by Albeladi et al. by applying few adjustments (Albeladi *et al*., 2020). After stirring the silver nitrate solution at 25°C and adding the aqueous extract of salvia officinalis dropwise for 15 minutes, the color of the solution changed from pale yellow to dark brown, indicating the formation of colloidal silver nanoparticles. The above procedure was repeated under different conditions: [Ag^+] (9.0-10.5mM), volume ratio, plant extract: [Ag^+] (0.5:5, 1:5, 2:5, 2.5:5 and 3:5), contact time (1-6 days), temperature (25, 40, 60 and 80 °C) and pH (4, 7, 9 and 10). The suspended particles were centrifuged at 6000rpm for 25 min and washed 3 times with deionized water, dried at 70°C and preserved in the dark.

Ethical approval

All experimental work in this study was conducted *in-vitro* and according to the good lab practices.

Results and discussion

Effect of pH

The synthesis process of silver nanoparticles was monitored at different pH values using the UV-VIS spectrophotometer, as shown in figure 2. The rate of silver nanoparticles increases with increasing pH, the absorbance of the surface plasmon resonance (SPR) band increased with time and showed a blue shift.

After implementing the synthesis of AgNPs process at various contact time and pH values. It was observed that pH 10 was the best condition since the absorbance of the SPR band increases with increase in reaction time (6 days) in addition to appearance of the SPR band in the blue shift compared to the pH values 4, 7 and 9, resulting in the formation of smaller size silver nanoparticles.

Effect of Temperature

The reaction temperature, particularly in the case of silver nanoparticles (Carbone *et al*., 2016), may significantly influence particle size and distribution. Temperature is another crucial parameter that must be taken into account while synthesizing silver nanoparticles. The experiment was conducted at different temperatures in the range of 25-80°C. Figure 3 shows that the absorbance increased between 20°C and 60°C and decreased between 60°C and 90°C, which is consistent with the results reported by Gou *et al*., 2015.

The best-achieved SPR appeared at 443nm at 60°C for the synthesized AgNPs.

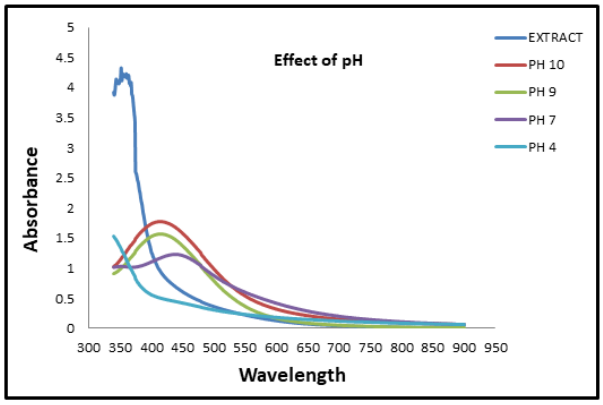


Figure 2. Effect of extract pH on AgNPs synthesis

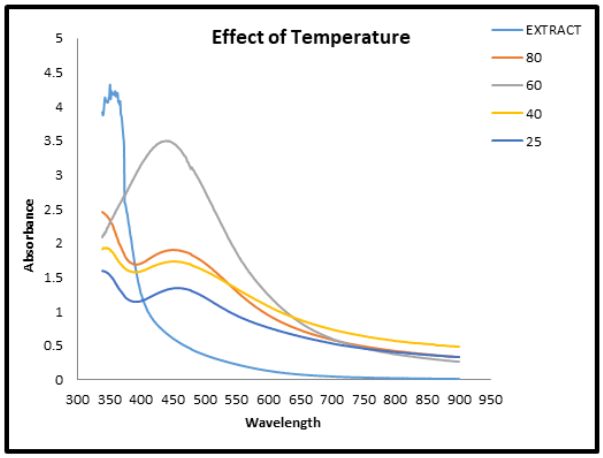


Figure 3. Effect of temperature on AgNPs synthesis.

Effect of volume ratio ($AgNO_3$: extract)

The volume ratio of silver ion solution to the S. officinalis extract plays a crucial role in the synthesis of silver nanoparticles, as reported previously (Dada *et al*., 2018). It was observed that as the concentration of the S. officinalis extract increased the SPR band shifted to longer wavelengths. This shift indicates that higher concentrations of phytochemicals in the extract, which serve as reducing and capping/stabilizing agents and promote the synthesis of silver nanoparticles. However, beyond 2ml of S. officinalis extract volume, precipitation occurs due to an excess of reducing agents, leading to a decrease in the SPR band intensity. This phenomenon was consistent with the findings reported by Albeladi *et al*., 2020. The optimum volume ratio of silver ion solution to S. officinalis extract was 2:5, which resulted in the best SPR as shown in figure 4.

Effect of silver ion concentration

The formation of silver nanoparticles is predominantly influenced by the concentration of silver ions. The experiment was conducted using different concentrations of silver nitrate solutions (9, 9.5, 10 and 10.5 mM) in order to determine the optimum concentration of silver ions on the synthesis of AgNPs, which was studied previously (Dada *et al*., 2018). The optimum concentration of silver nitrate was 10mM where the best SPR was observed at 441nm, as shown figure 5, which is consistent with results reported previously (Albeladi *et al*., 2020; Nicolescu, *et al*., 2017).

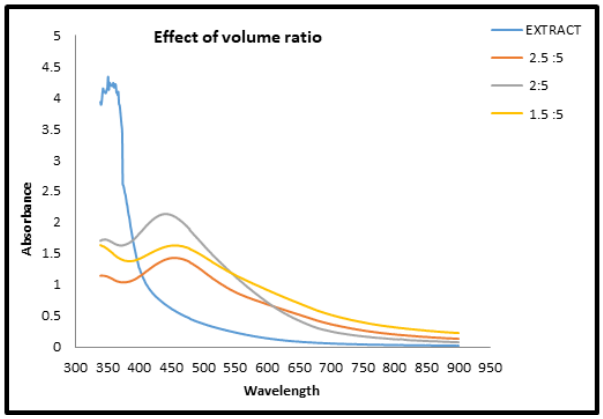


Figure 4. Effect of volume ratio on AgNPs synthesis.

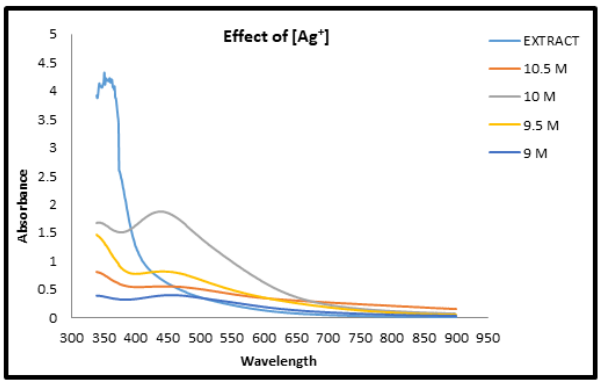


Figure 5. Effect of concentration of [Ag^+] on AgNPs synthesis.

On conclusion, the optimum working conditions for the synthesis of silver nanoparticles were found to be pH 10, a contact time of 5 days, volume ratio of 2:5 (plant extract: Ag^+ solution) and 60°C temperature, giving the maximum absorbance band at λmax 405nm, the λmax obtained indicates that the size of the nanoparticles will be smaller in size compared to other parameters. This behavior is supported by Bejanzadeh *et al*. 2012, who demonstrated that larger silver nanoparticles exhibit absorbance peaks (λmax) at longer wavelengths (Red shift).

Characterization

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectrum for AgNPs synthesized by S. officinalis aqueous extract is shown in figure 6. The FTIR analysis was used to identify the role of the phytochemicals that were responsible for capping and stabilizing silver nanoparticles. The FTIR spectrum of the biosynthesized silver nanoparticles show shifts that correspond to the stretching vibrations of carboxyl groups (–C=O) from 1634.43 to 1640 cm^{-1}, hydroxyl groups (–OH) from 3326.64 to 3339.69 cm^{-1} and amine groups from 2076.12 to 2117.12 cm^{-1} on comparison with the spectrum of the S. officinalis aqueous extract. A similar behavior was reported indicating the stabilization and reduction effect of leaf extracts during the production of stable silver nanoparticles demonstrated by

the FTIR analysis (Magudapathy *et al*., 2001, Muthukrishnan *et al*., 2015).

Scanning electron microscopy (SEM)

A scanning electron microscope was used to study the surface morphology and size of nanoparticles. The image obtained from the SEM (figure 7) clearly showing that the morphology of AgNPs is spherical and the particles are almost evenly distributed with an average particle size of 30.95nm. This is in good agreement with the shape of SPR band in the UV–Vis spectra (Singh *et al*., 2013; Dada *et al*., 2017; Dada *et al*., 2018).

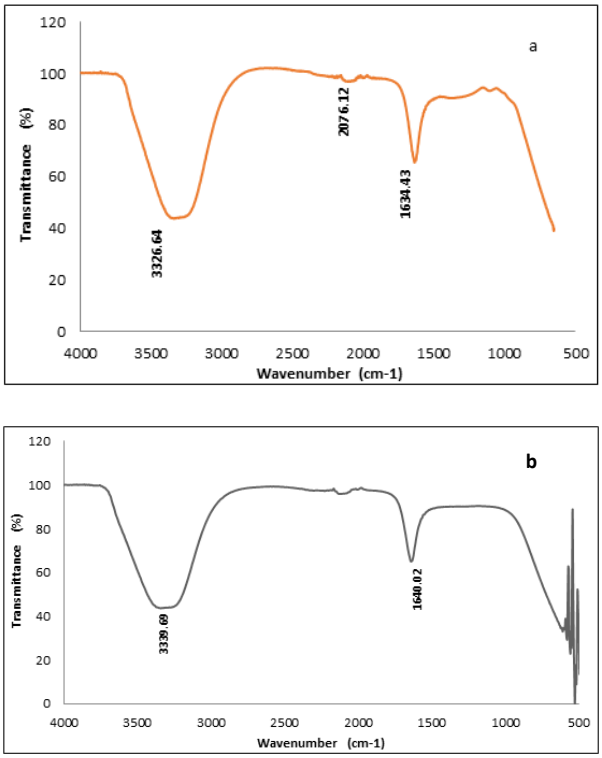


Figure 6. FTIR analysis of aqueous leaf extract of S. officinalis (a) and AgNPs (b).

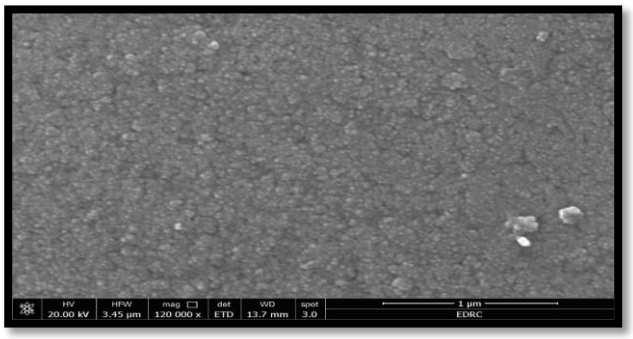


Figure 7: SEM image of silver nanoparticles.

X-ray diffraction (XRD)

The XRD analysis depicted in figure 8 confirmed the crystalline structure of silver nanoparticles. The XRD spectrum showed distinct peaks around 38°, 44°, 65°, and 77°, corresponding to the (111), (200), (220), and (311) planes, respectively. These peaks are characteristic of the face-centered cubic (FCC) crystalline structure of silver nanoparticles. Additionally, high-intensity peaks observed around 28° and 32° in the XRD spectra are likely attributed to the presence of phytochemical compounds from the aqueous extract of S. officinalis. These compounds may coat the surface of the synthesized AgNPs, contributing to their stabilization. These findings are consistent with previous studies by Okaiyeto *et al*. 2021 and Kamaraj *et al*., 2017. This pattern closely matches the standard powder diffraction data from the Joint Committee on Powder Diffraction Standards (JCPDS) file no. 04-0783, confirming the crystalline nature of the synthesized silver nanoparticles. This confirms that the synthesized silver nanoparticles exhibited a FCC crystalline structure typical of metallic silver, with additional peaks indicating the presence of organic coatings from the plant extract.

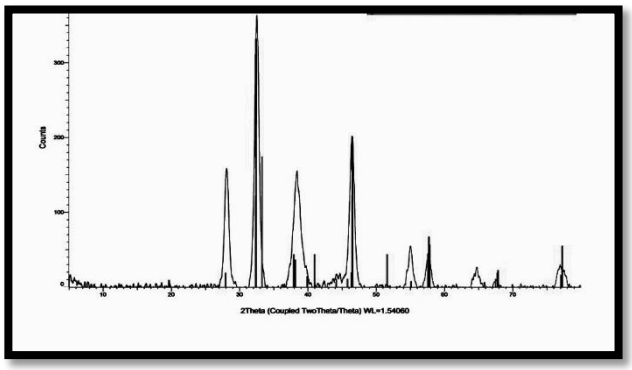


Figure 8: XRD pattern of AgNPs from aqueous extract of S. officinalis.

Antibacterial activity of silver nanoparticles coated on antibiotics

The antimicrobial efficacy of silver nanoparticles was assessed in relation to their synergistic effects with antibiotics against microbial populations. The additive antibacterial effect of combining antibiotics with AgNPs was evaluated using the disk diffusion method, showed all antibiotic effects against gram-negative and gram-positive bacteria in varying proportions. The inhibitory efficacy of all antibiotics was enhanced on combination with nanoparticles. The obtained results are shown in table 2 and figures 9 and 10 showing the synergistic action of silver nanoparticles with various antibiotics, which is better than antibacterial effects of individual nanoparticles or antibiotics alone (Li *et al*., 2014). It was found that the effect of combining antibiotics with silver nanoparticles is greater against bacteria than they were applied separately. Thus, the consumption of overall treatment doses and side effects can be significantly reduced, by directing the activity only in the area exposed to harm and without any higher doses needed (Chen *et al*., 2013). A study found that AgNPs significantly enhanced antibiotic activity against bacteria, and can be applied against resistant bacteria infection (Hussein *et al*., 2019).

Conclusion

An eco-friendly and facile method was established for the synthesis of silver nanoparticles from Salvia officinalis aqueous extract. Findings showed that the optimum conditions for the synthesis of silver nanoparticles with a uniform spherical morphology and average particle size of 30.9 nm was at; pH 10, silver ion

concentration 10mM, volume ratio (2:5), 60°C and 5 days reaction time. The prepared silver nanoparticles are promising in the medical field since they showed a significant anti-bacterial activity and enhanced the efficacy of some antibiotics.

Table 2. Antibacterial activity of silver nanoparticles coated on antibiotics.

Bacteria	Antibiotic used	Zone of inhibition (mm)	
		Control	Test
Escherichia coli	Tetracycline (30µg)	21	27
	Trimethoprim (25µg)	29	32
Staphylococcus aureus	Amikacin (30µg)	20	24
	Trimethoprim (25µg)	22	23

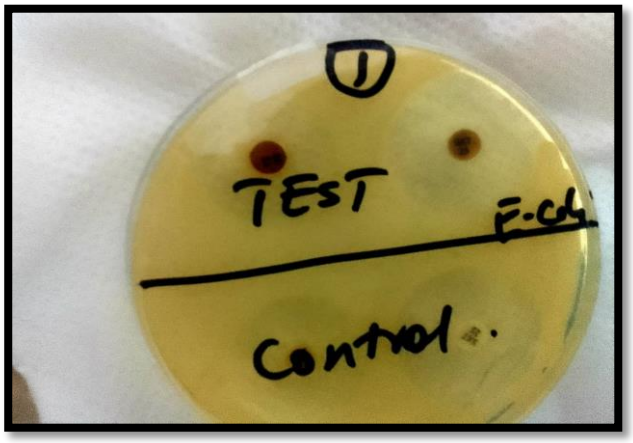


Figure 9. Antibacterial activity against *Escherichia coli* of biologically synthesized 1mM silver nanoparticles coated on antibiotic.

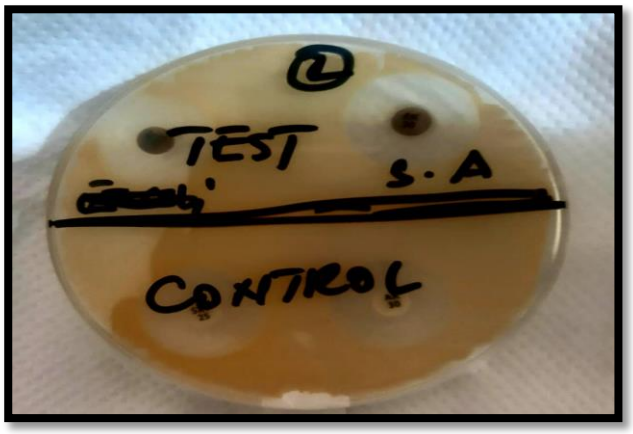


Figure 10. Antibacterial activity against *Staphylococcus aureus* of biologically synthesized 1mM silver nanoparticles coated on antibiotic.

Conflict of interest

The authors declare no conflicts of interest in relation to this work.

Authors' contribution

Nafa N.A. performed the experimental work. Alhanash H. B. designed the study, supervised the experimentation and drafted the manuscript. Issa R. A. M. collaborated in interpretation of results and critically read and approved the final manuscript.

Acknowledgment

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