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# Evaluation of the green synthesis, characterization and antibacterial activity of silver nanoparticles from corm extract of *Crocus sativus* var. Haussknechtii

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## ABSTRACT

**Purpose:** To prevent chemical toxicity, biosynthesis of silver nanoparticles has been proposed as a cost-effective and environmentally friendly reducing agent option by corm extract of *Crocus sativus* var. *Haussknechtii* and as well as, evaluate their effects against *Staphylococcus aureus* and *Escherichia coli.* **Research method:** Silver nanoparticles were produced in the presence of secondary metabolites of this plant. The nanoparticles were then identified using the technique ultraviolet-visible spectroscopy (UV–Vis), fourier transform infrared (FTIR), field emission scanning electron microscope (FESEM). The antibacterial properties were used against two microorganisms, *S. aureus* (Gram-positive) and *E. coli* (Gram-negative), using the agar well propagation method. **Findings:** The observation of the peak at 450 nm in the UV-Vis spectra for corm synthesized silver nanoparticles reveals the reduction of silver metal ions into silver nanoparticles. The optimum pH and AgNO3 concentrations were 9 and 4 mM, respectively. FESEM images detected the spherical Ag-NPs shape with diverse sizes ranged within 70.70–192.02 nm. Additionally, based on the antibacterial test that has been done for nanoparticles, the mean diameter of the inhibition zone after exposure to *S. aureus* and *E. coli* was 22.67±0.58 and 20.00±00 mm, respectively. **Limitations:** There was no significant limitation to report. **Originality/Value:** The corm extract of *C. haussknechtii* is a promising agent for the biosynthesis of almost spherical silver nanoparticles. The synthesized nanoparticles show good Inhibition activity in different concentrations. The AgNPs synthesized by corm extract in high concentrations are found to be high antibacterial activity against two bacterial organisms. This indicates that the increase in nanoparticle diameter is directly related to antimicrobial properties.

## INTRODUCTION

In the past few decades, tremendous interest and substantial research efforts were directed toward the biomedical evaluation and revaluation of metallic nanoparticles derived from noble metals, such as silver and copper, thanks to their specific and natural chemical, biological, and physical properties (Anandalakshmi et al., 2016; Bagherzade et al., 2017; Khani et al., 2018). There is a growing need to develop environmentally benign nanoparticle synthesis processes that do not use toxic chemicals in the synthesis protocol (Shankar et al., 2004). In particular, impressive attention was oriented toward the biomedicine-related assessment of silver nanoparticles (AgNPs), which first attracted worldwide attention as unconventional antimicrobial agents (Burdușel et al., 2018). The increasing tendency of microbial infections, the rapid emergence of drug-resistance to recent antibiotics, and quick evolution through mutation necessitate the development or modification of antimicrobial compounds and alternative treatments. Advanced research in nanotechnology recently comes with developing nano-scale objects with prominent antimicrobial actions against multidrug-resistant pathogens suggesting a platform to fight against bacterial mutation arch. Among the most advanced nanotechnological applications, metal nanoparticles provide the most effective results with their unique mode of action (Roy et al., 2019). Silver is well known for possessing an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes (Jiang et al., 2004). Nanoscale silver particles with a high surface-area-to-volume ratio (size below 100 nm) are of prime interest due to high antimicrobial actions against Gram-positive and Gram-negative bacteria, viruses, and other eukaryotic microorganisms as compared to other metals in their nano form (Roy et al., 2019). *S. aureus* and *E. coli* are two pathogens of concern to the healthcare and pharmaceutical industry as they are highly resistant to antibiotics (Chambers & DeLeo, 2009; Lister et al., 2009). Many studies have shown antimicrobial effects of single metallic nanoparticle on these pathogens and therefore, by integrating different combinations of known antimicrobial metallic nanoparticle (such as aluminum oxide, zinc oxide, titanium dioxide, nickel oxide, and silver) within devices at specific concentrations could prevent contamination by these pathogens (Bankier et al., 2019; Gour & Jain, 2019). Different parts of plants such as roots, stems, shoots, leaves, flowers, seeds, and their metabolites have been successfully used for the biosynthesis of nanoparticles (Husen, 2017). In this biosynthesis, Extract of corms of *C. haussknechtii* was used as a reducing and stabilizing agent. This plant is known as the Jo-ghasem saffron (*C. palasii* subsp. *Haussknechtii* (Boiss and Reut. Ex Maw) B. Mathew Syn. *C. sativus var*. *Haussknechtii* Boiss and Reut. Ex Maw *C. haussknechtii* (Boiss and Reut. Ex Maw) Boiss) and the commonly called pešuk, it is native to western Iran and belongs to the family Iris (Dada et al., 2019). Few studies of the chemical and biochemical constituents of this plant have been published. The *hausknechtii* subspecies have a corm up to 30 mm diameter with a fibrous neck up to 10 cm (Fig. 1). The flowering time is October to November. It has a unique resemblance to agronomic saffron (Saxena, 2010). Joe Ghasem is the descendent of wild saffron domesticated in the Zagros slopes and cultivated, and its final product may be edible saffron. The endemic people in that area used to call it Karkam and Karkamice from the 10 to 18th centuries. Its Persian name was Karkam, and Karkimas means Zaffran. *C. haussknechtii* has shown several biological activities, including cytotoxic, anti-angiogenesis, and anti-proliferative properties. The corm (the nut and the surrounding fibers) and aerial parts of the species have shown anti-angiogenesis properties in HUV-EC-C cells (Hamzeloo-Moghadam et al., 2019).

**Fig. 1.** Photograph of *C. haussknechtii* corm.

The size, morphology, and stability of nanoparticles depend on the method of preparation, nature of the solvent, concentration, and the reducing agent (Siddiqi et al., 2018). Because of these effects of three operational parameters, which are Silver nitrate concentration, extract volume ratio, and pH value on the formation of AgNPs, were investigated, and the study was monitored using a UV-Vis spectrophotometer (Dada et al., 2019).

In previous study, silver nanoparticles with the help of corm *C. Haussknechtii* extract were synthesized by reducing $Ag^+$ to $Ag^0$ from silver nitrate solution, and the antibacterial activity of nanoparticles against *S. aureus* and *E. coli* was investigated. Characterization of synthesized AgNPs is necessary before evaluating their toxicity (Rajeshkumar & Bharath, 2017). Several analytical techniques have been used for the characterization of nanoparticles, such as Ultraviolet-visible spectroscopy (UV-Vis), field emission scanning electron microscope (FESEM), Fourier transform infrared spectroscopy (FTIR). Such studies could prove to have an enormous impact in the immediate future if plant tissue culture and downstream processing procedures are applied to synthesize metallic as well as oxide nanoparticles on an industrial scale (Jha & Prasad, 2010).

## MATERIALS AND METHODS

### Plants and chemicals

Silver nitrate ($AgNO_3$) and methanol were purchased from Merck Company. Glasswares were washed with deionized water and were put in the oven at 160 ˚C for two h, plastic equipment was autoclaved for antibacterial assays before using. The corm part of *C. haussknechtii* was collected in spring from Lorestan province (33° 29' 25.6" N 48° 03' 10.0" E) in western, Iran. The plant was identified by our research team botanist and then; were stored in a refrigerated at 4 ˚C for further studies. Two experiments were designed to test the antibacterial properties of nanoparticles and compare them with conventional plant extracts.

### Preparation of plant extract and silver nitrate solution for antibacterial assays

To prepare the methanol extract of corms of *C. haussknechtii*, the sample was cut into small pieces, and 20 g was weighed, then into a 250 mL flask. Methanol (100 mL) was added. The solution was placed on a by dynamic maceration in a multipoint magnetic stirrer with thermostatic and a dark place at the room temperature for 72 h. The solution was separated by filtration with Whatman No.1 filter paper and then centrifuged at 5500 rpm for 10 min. The upper phase was retained for the synthesis of AgNPs. To prepare the plant extract solution and silver nitrate for antibacterial measurement all methods for preparing extract are the same as in the previous section, but to remove germs, the centrifuged extract was filtered through a

0.45 mm filter and then used for further experiment. For more precautions, the solution of silver nitrate was filtered through a 0.45 mm filter. Antimicrobial activity of crude extract and biosynthesized AgNPs was also studied, and antibiotics (gentamicin) were used as positive controls.

**Synthesis of AgNPs**

The methanol extract of corm *C. haussknechtii* was used for the synthesis of AgNPs. To this end, the effect of the amount of extract and the concentration of $AgNO_3$ solution was investigated to obtain the optimum values. The best concentration of the extract was obtained by changing the amount of extract. Then 1 ml of the extract was added to 20 ml of 4 mM solution of $AgNO_3$ drop by drop. The ultrasonic irradiation (20 to 25°C and 20 kHz) was applied to the mixed solution for 3 h. The AgNPs mixture obtained was subsequently purified by centrifugation at 9000 rpm for 30 minutes. The dried AgNPs were kept in a microtube for further study.

**Optimization pH of the synthesis medium**

To optimize the pH value, 6 series of solutions containing 5 ml extract and 2 ml of silver nitrate solution with a concentration of 2 mM were prepared. After adjusting the pH to the values (4, 5, 6, 7, 8, and 9), the absorption spectra of the solutions were read by the UV–Vis spectrophotometer in the range of 200-800 nm. pH changes were performed using 0.1 M solutions of hydrochloric acid (HCl) and sodium hydroxide (NaOH).

**Optimization of *C.* haussknechtii corm extract**

To obtain the optimal amount of *C. haussknechtii* extract, 1 to 5 ml of corm extract was added to 2 ml of 2 mM $AgNO_3$ solution. The reaction pH was set to the optimal pH. The adsorption of each of the prepared solutions in the range of 200-800 nm was taken by the UV–Vis spectrophotometer, and finally, the optimum volume of extract was selected.

**Optimization of silver nitrate concentration**

To investigate the effect of $AgNO_3$ solution concentration, the optimized amount of the extract was added to 4 ml of different concentrations of $AgNO_3$ (0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 mM) after adjusting the optimal adsorption pH. Solutions were taken in the range of 200-800 nm, and the optimal concentration was selected.

**Characterization of AgNPs**

To identify the extract composition responsible for the reduction of $Ag^+$ ions to produce the AgNPs and their capping, infrared spectra were obtained for the extract alone and the AgNPs. The spectra were acquired using an FT-IR spectrophotometer (Spectrum Two models by PerkinElmer). The shape of the AgNPs is examined by FESEM experiments using (SIGMA VP) Field Emission Scanning Electron Microscope. Furthermore, UV–Vis spectra (wavelength range from 200-800 nm) of the AgNPs were obtained using a UV/Vis Win 5.0 spectrophotometer. These techniques are useful for the determining of size, shape, surface modification, crystallinity, and dispersity of nanoparticles.

**The antibacterial potentiality of AgNPs**

The synthesized AgNPs using *C. haussknechtii* corm extract were tested for antimicrobial activity using the agar well diffusion method. The bacterial test cultures included *E. coli* and *S. aureus*. Experiments were performed with fresh bacteria previously incubated on Nutrient

Agar plates at 37 $^{\circ}$C for 24 hours. Agar plates were provided by dissolving agar powder (15.0 g $L^{-1}$), tryptone (5.0 g $L^{-1}$), yeast extract (2.5 g $L^{-1}$), glucose (1.0 g $L^{-1}$) in deionized water. The above mixture was heated and stirred until the agar dissolved. In the following, the pH of the solution was adjusted to 7.0 ± 0.1 with NaOH or HCl, and the plates were sterilized by autoclaving. The antibacterial activity of biosynthesized AgNPs with four different concentrations (25, 50, 75, and 100% V/V), methanolic extract of *C. haussknechtii* and $AgNO_3$ solution, and gentamicin was used as the positive control are evaluated. The inhibition zones were measured in mm and compared with the inhibition zone of the gentamycin as a standard positive control.

## RESULTS AND DISCUSSION

### Effect of pH on the synthesis medium

This study justifies the research that AgNPs can be synthesized using the medicinal plant extract of *C. haussknechtii*. It was also showed that the synthesis of *C. haussknechtii* silver nanoparticles depends on various experimental operational parameters. UV-vis spectrophotometry is one of the most commonly used techniques for the characterization of synthesized nanoparticles, which is also used to monitor the stability and synthesis of AgNPs (Rajeshkumar & Bharath, 2017). The bio-reduction of the AgNO3 solution was observed primarily with reddish-brown color formation and then determined by UV–Vis spectrophotometer. The Reddish-brown color of AgNPs was assessed by UV-Vis spectra (Fig. 2). At pH 4 to 9, various reddish-brown (Fig. 3), indicating the optimum pH at 9 suggests the synthesis of silver nanoparticles. The reduction of Ag ions by the *C. haussknechtii* corm extract was easily analyzed by UV-Vis spectroscopy (Fig. 6). In support of the present results, reported that at pH 6.7 and 7.7 a large number of functional groups are available for silver binding, facilitated a higher number of Ag ions to bind and subsequently form a large number of nanoparticles with smaller diameters (Hamouda et al., 2019). AgNPs spectra showed unique optical properties, depending upon size, shape, and distribution. pH value greatly influences the size and morphology of the biologically synthesized nanoparticles, where pH strongly altered the electrical charges of biomolecules and capping agents resulting in changing their ability to bind and reduce metal ions.

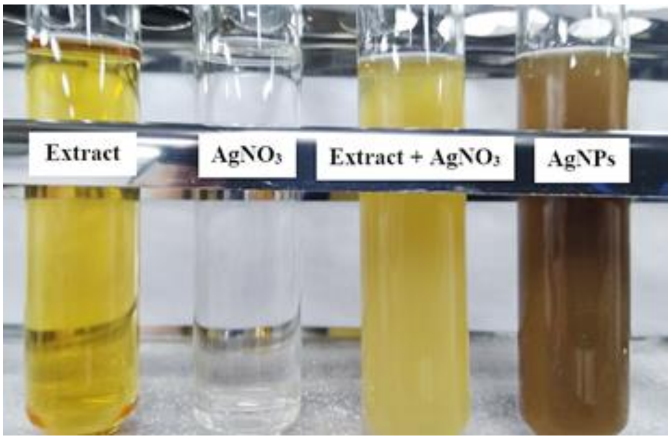


**Fig. 2.** Changes in color of *C. haussknechtii* extract by silver ions: extract, silver nitrate solution, extract and silver nitrate solution, and color changes of silver nitrate solution with extract after some time of incubation (left to right).

### Effect of corm *C.* haussknechtii extract

The effect of the extract *C. haussknechtii* was evaluated with different volumes (1, 2, 3, 4, and 5 ml) (Fig. 4). It has been shown that with decreasing the amount of the extract, nanoparticle synthesis's efficiency increases. Here the most appropriate amount of extract is 1 ml (Fig. 4). Figure 7 shows that increasing the amount of extract from 1 to 5 ml, respectively, significantly reduces the adsorption of the resulting peaks in the range of 400-500 nm and shows low production of AgNP. In contrast, the adsorption is high in the range of 250-300 nm, because the excess of the extract that did not participate in the reaction shows higher adsorption. The amount of extract is one of the significant parameters that affect the characteristics of NPs by controlling their size and shape. Figure 4, shows that an increasing amount of *C. haussknechtii* induced an apparent decrease in the absorbance of the resulted peaks, respectively indicating the low production of AgNPs.

### Effect of silver nitrate concentration

By investigating the effect of $AgNO_3$ concentration, with different concentrations (0.5 to 4 mM) with increasing concentration up to 4 mM, a positive effect was observed on the biosynthetic efficiency of silver nanoparticles as it increases with the concentration of 4 mM (Fig. 5 and 8). The amount of synthesized nanoparticles increases, but increasing concentrations of a certain amount do not significantly affect the size of the nanoparticles. Therefore, varying the concentration of $Ag^+$ solution affects the size and shape of the AgNPs. The UV-Vis absorption spectra of the samples synthesized by various $AgNO_3$ concentrations are shown in Figure 5. The absorption intensity increases quasilinearly with an increase of $AgNO_3$ concentration during the reaction (Fig. 8). All the solutions exhibited the characteristic of the AgNPs surface Plasmon resonance (SPR) (Chouhan, 2018; Noah, 2019).

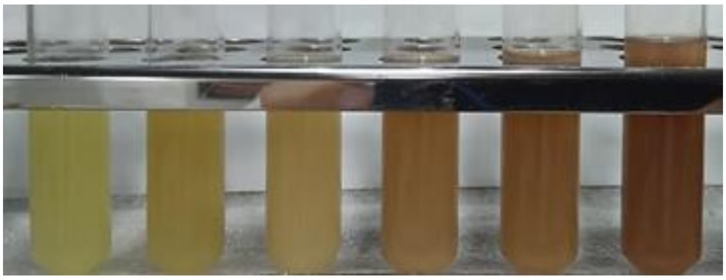

**Fig. 3.** Color change due to increased pH (left to right) in the extract solution and silver nitrate.

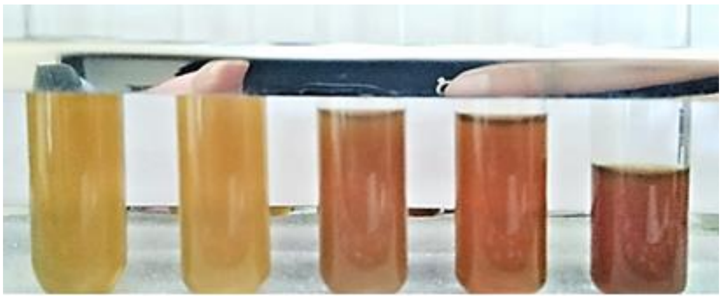

**Fig. 4.** Color change by reducing the amount of extract from 5 to 1 ml (left to right).

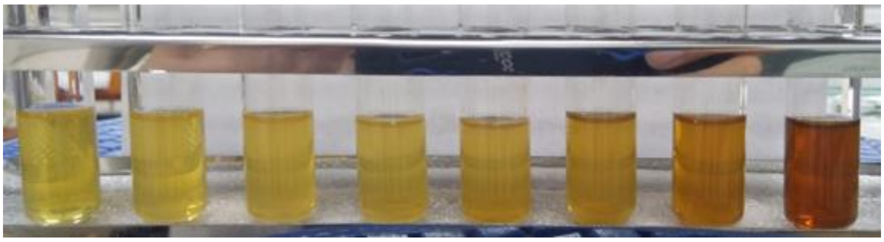

**Fig. 5.** Color change with increasing concentration of silver nitrate salt from 0.5 to 4 mM (left to right).

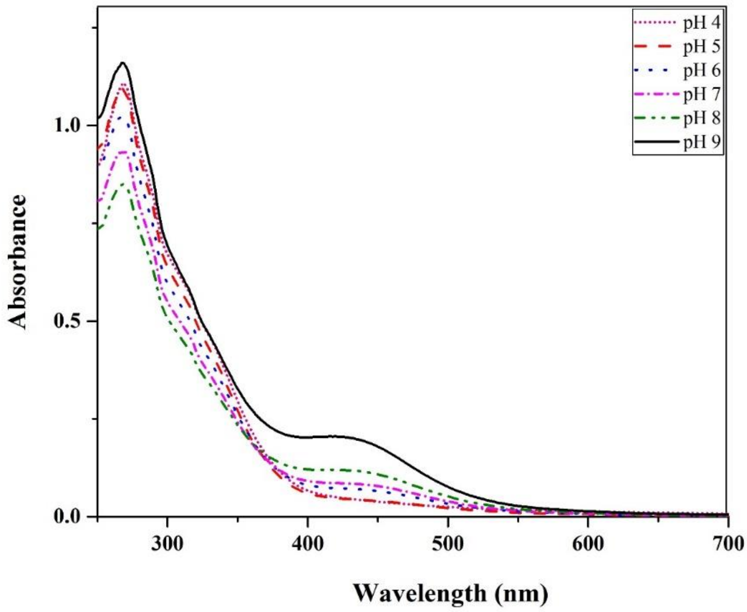


**Fig. 6.** Absorption spectrum in the UV/Vis region of biosynthetic silver nanoparticles at different pH values (4, 5, 6, 7, 8 and 9).

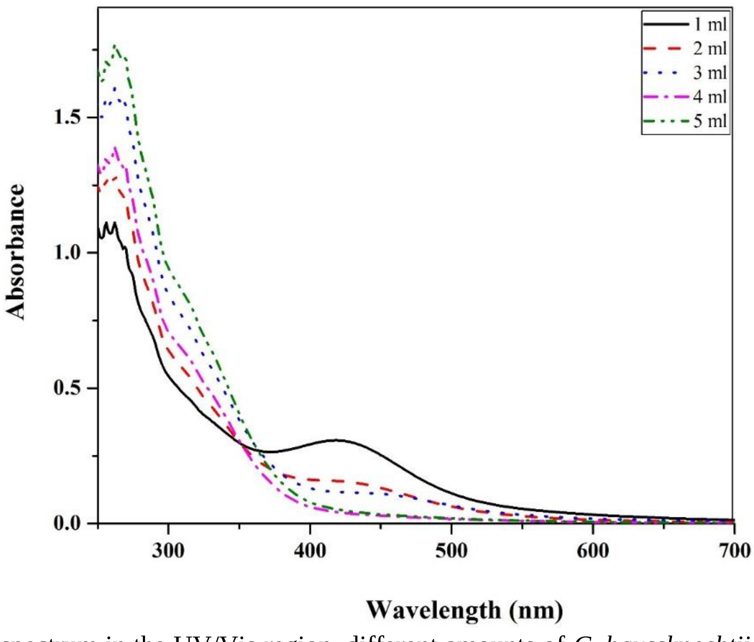


**Fig. 7.** Absorption spectrum in the UV/Vis region, different amounts of *C. haussknechtii* plant extract (1, 2, 3, 4 and 5 ml) in the same solution of silver nanoparticles.

**Fourier-transform infrared spectroscopy analysis**

FT-IR measurements were conducted to reveal the possible potential biomolecules that participated in the bioreduction of silver and stabilization of AgNPs. The silver nanoparticle sample shows peaks at 3349, 2973, 2837, 1642, 1387, 879, and 631 $cm^{-1}$ (Fig. 9). Silver nanoparticles peaks were observed at 3349 $cm^{-1}$ (O-H and N-H groups or from aromatic rings), 2973 and 2873 $cm^{-1}$ (aliphatic C-H stretching of hydrocarbon chains and N-H bending), 1642 $cm^{-1}$ (could be attributed to amides (N-H) stretching in addition to peptide bond and C=C stretching) adsorbed on the surface of metal nanoparticles (Khani et al., 2018; Shankar et al., 2004). The presence of reducing sugars in the broth could be responsible for the reduction of metal ions and the formation of the corresponding metal nanoparticles. It is also possible that the terpenoids play a role in the reduction of metal ions by oxidation of aldehydic groups in the molecules to carboxylic acids (Shankar et al., 2004). It is seen that the same phytochemicals and functional groups are present in both the corm extract and AgNP. So, it is proved that the corm extract of *C. haussknechtii* is a powerful reducing agent in the synthesis of the silver nanoparticles (Francis et al., 2018).

It is observed that most of the IR bands in AgNP have undergone a slight shift in wavenumber from that of plant extract because of its surface modification. The exact mechanism of the reduction process needs separation, purification, and identification of phytocomponents present in the methanolic corm extract of *C. haussknechtii* (Francis et al., 2018). FTIR result obtained confirmed the phytochemical screening result of some biomolecules. It implies that the biomolecules functioned as reducing, capping, and stabilizing agents. Analysis of the FTIR result indicates that the AgNPs were surrounded by terpenoids, alcohols, lactone, and carbonyl group from amine serving as a strong binding site for AgNPs (Dada et al., 2018). Also, it was observed that flavonoids and terpenoids were the main compounds in the FT-IR spectrum of the plant extract, which could be responsible for the reduction, capping, and stabilization of AgNPs (Kumar et al., 2018).

**FE-SEM analysis**

The results of the biosynthesis of AgNPs by FTIR and FESEM analysis clearly showed the ability to reduce $Ag^+$ ions to $Ag^0$ by the biomolecules of corm extract. Many different plant extracts have been used to synthesize silver nanoparticles to produce AgNPs presenting different morphologies. The surface features of synthesized AgNPs were monitored using the field emission scanning electron microscope (FESEM) (SIGMA VP). The FESEM images of bio-synthesized AgNPs are shown in (Fig. 10). Entirely, the synthesized silver nanoparticles are spherical, and some undefined shapes are also observed with slight aggregation. The silver nanoparticle sizes were found to be in a range of 70.70–192.02 nm. FESEM images Figure 10, illustrated the contour of the synthesized AgNPs and the excellent distribution of the precipitated silver nanoparticles. FESEM studies have shown that the reducing agent's presence in a plant-mediated synthesis of AgNPs, where the plant extract acts as reducing agents, shapes the nanoparticle during its growth. The use of medicinal plants in the synthesis of AgNPs is not only used for size and shape control, but also for providing plant antimicrobial properties to AgNPs (Tippayawat et al., 2016). These findings confirm that such bio-compatible nanoparticles with biological activity may find their applications in the field of nanomedicine. Moreover, research in pharmacological studies, especially in vivo, is needed to develop and design future antimicrobial therapeutic agents.

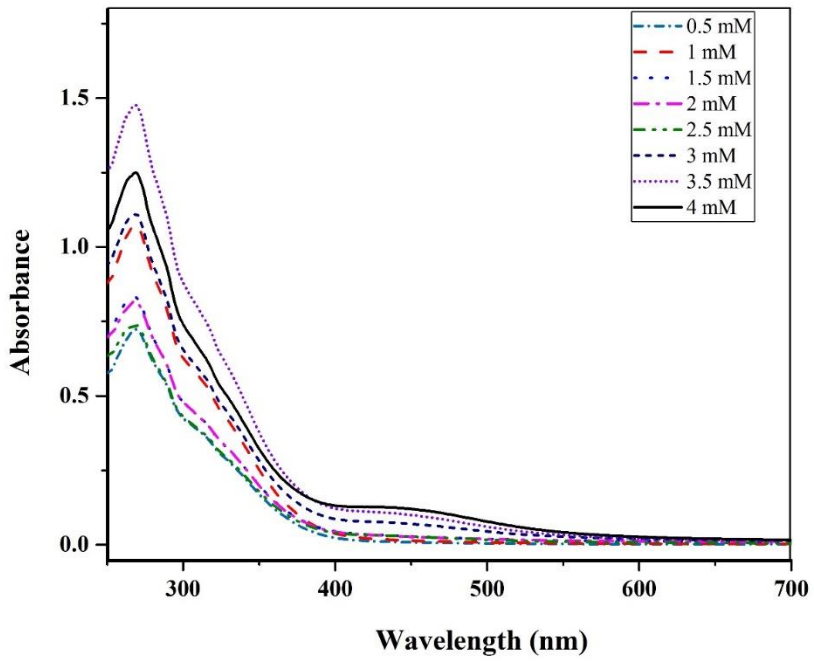


**Fig. 8.** Absorption spectrum in the UV/Vis region of different concentrations of silver nanoparticle solution (mM).

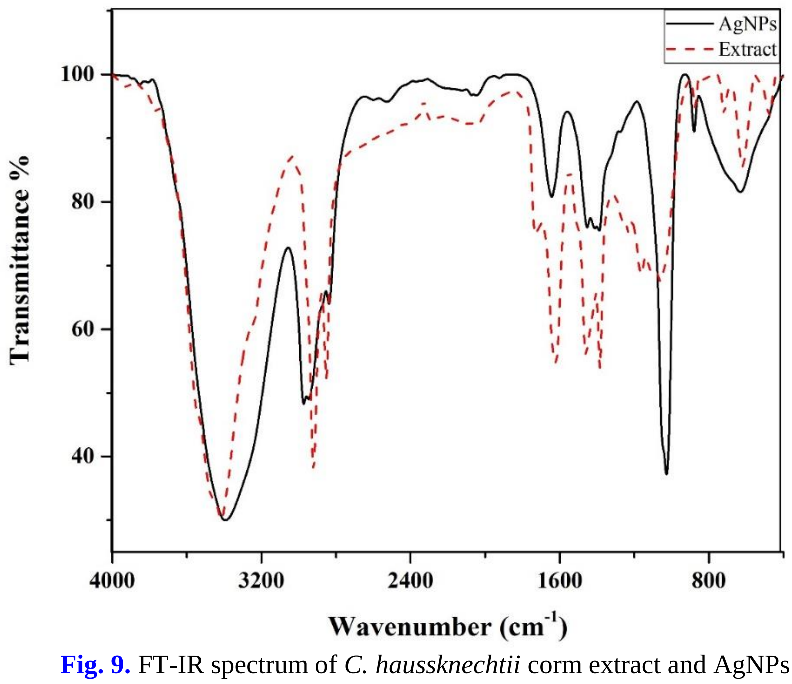


**Fig. 9.** FT-IR spectrum of *C. haussknechtii* corm extract and AgNPs.

However, there is a dire need to engineer nanoparticles on a pilot scale. In summary, in this context, many researchers for reasons, i.e. (1) bioactive compounds of natural plant origin, environmentally friendly and renewable, (2) the nanoparticle synthesis process is easy and scalable, (3) stable material with adjustable sizes and shapes, (4) less use of chemical compounds and (5) less toxic contaminants/by-products, etc. have directed or redirected their interest to explore new in nanotechnology in particular.

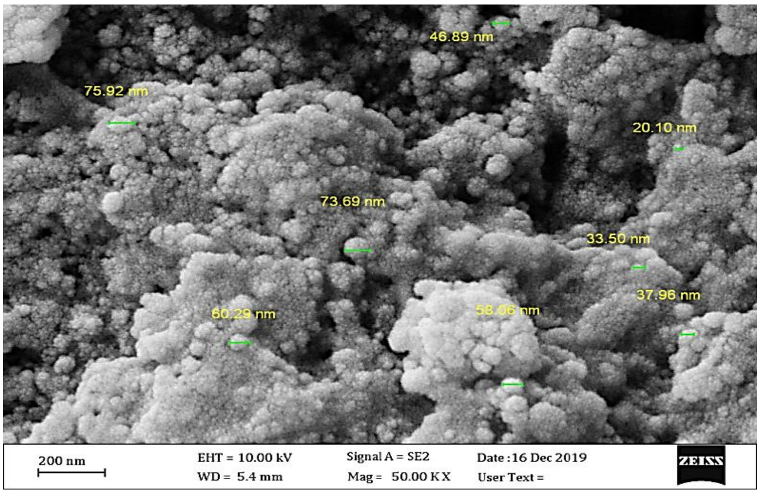


**Fig. 10.** FESEM image of biosynthesized AgNPs.

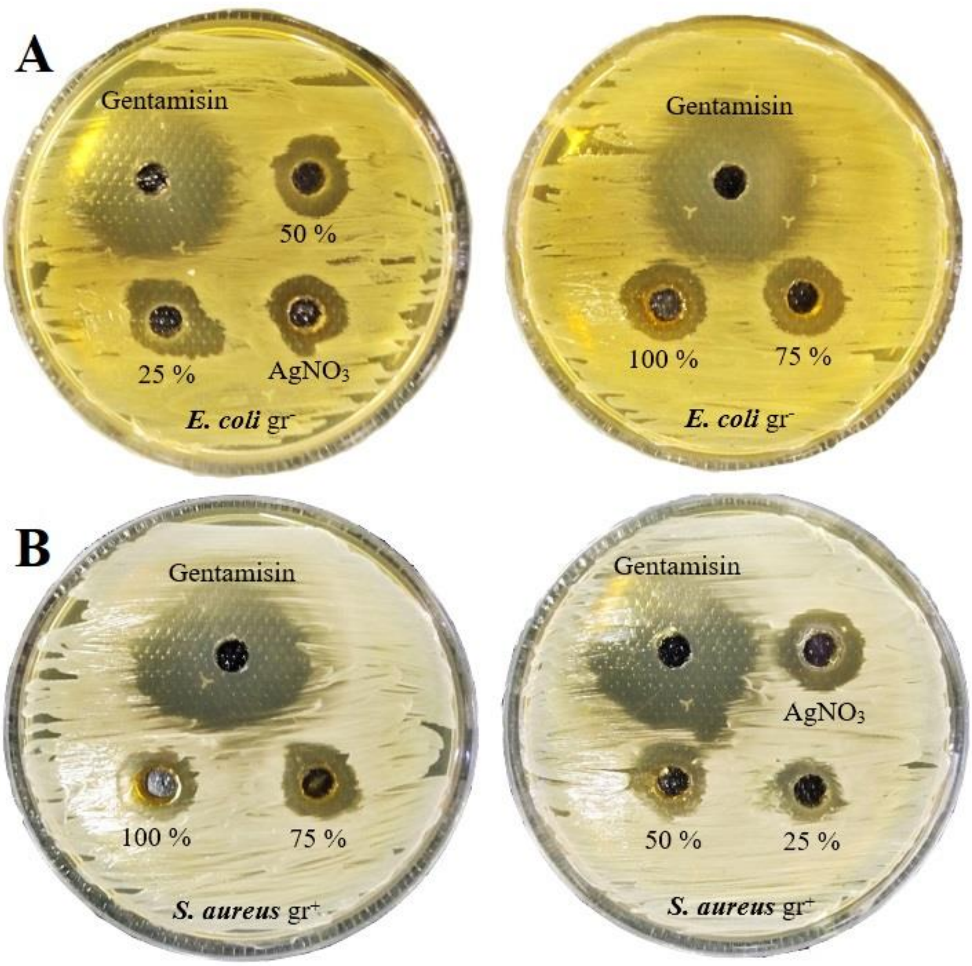


**Fig. 11.** Antibacterial activity (zone of inhibition) of AgNPs against two human pathogenic bacteria, A) *E. coli* and B) *S. aureus* AgNPs with four different concentrations (25, 50, 75 and 100% V/V), Alone AgNPs synthesized by corm extract of *C. haussknechtii*, and gentamicin was used as the positive control.

## Antibacterial activity of AgNPs

In addition, it was demonstrated AgNPs are used as antimicrobial agents; they have great bactericidal potential against both gram-positive and gram-negative pathogens. The use of the extract, obtained from the corm of *C. haussknechtii*, as a reducing and stabilizing agent represents a suitable alternative to produce antimicrobial metal nanoparticles. Agar wells method test revealed that the best means in the bacteria Inhibition was *S. aureus* 22.67±0.58 mm and the mean *E. coli* 20.00±00 mm Table 1. From the result obtained (Table 1 and Fig. 11), it is obvious that AgNPs exhibited significant antimicrobial activities against the bacteria it was tested. The diameter of the zone of inhibition measured varied from 15 mm to 22 mm as presented in Figure 11.

The extract of corm *C. haussknechtii* contains phenolic compounds such as flavonoids (e.g., kaempferol and Apigenin) and phenolic acids (e.g., Chicoric acid, Chlorogenic acid, and Syringic acid). According to other studies, it has been stated that the key mechanism behind the plant-mediated synthesis of AgNPs is a plant assisted reduction due to secondary metabolites. The secondary metabolites such as ketones, terpenoids, amides, flavones, carboxylic acids, and aldehydes (Rajeshkumar & Bharath, 2017). Biosynthesis of silver nanoparticles extracted from living systems Biological and their antimicrobial activities against representative microorganisms, are summarized in Table 2. The hydroxyl groups of Polyphenolic compounds have a stronger ability to bind silver ions and involve in the biosynthesis of silver nanoparticles and act as a reducing agent for the reduction of silver ions ($Ag^{+}$) to silver nanoparticles ($Ag^{0}$) (Solgi, 2014). The exact antimicrobial action of silver is not completely understood; several possible mechanisms have been proposed, which involve the interaction of silver with biological macromolecules, such as enzymes and DNA (Jiang et al., 2004; Le Ouay & Stellacci, 2015). The antibacterial effects of AgNPs correlate with the particle dimension and shapes. The size of the nanoparticle implies that it has a large surface area to come in contact with the bacterial cells, and hence, it will have a higher percentage of interaction than bigger particles (Rai et al., 2009). The bigger the diameter is, the weaker the impact. According to Pal et al spherical nanoparticles show bacterial inhibition with a silver content of 12.5 µg is needed (Pal et al., 2007). It can be concluded that AgNPs can be initially absorbed on the surface of the cell and then undermine the cell membrane. after that, the particles may be transported into the cytoplasm and imposed on a variety of macromolecules, either directly or indirectly, followed by DNA aggregation, protein degradation, or intracellular substance release and eventually, cell death (Liu et al., 2020; Yun'an Qing et al., 2018). Nevertheless, further research on this subject is required to elucidate completely the interaction mechanism between AgNPs and bacteria (Escárcega-González et al., 2018).

**Table 1.** Comparison of antibacterial activity of crude extract, AgNPs synthesized at four different concentrations (25, 50, 75 and 100% V/V) by corm extract of *C. haussknechtii*, and antibiotic gentamicin

| | Zone of inhibition (mm) | |
|---|---|---|
| | *S. aureus* | *E. coli* |
| Solution $AgNO_3$ | 18.00±00 | 18.00±00 |
| Gentamicin | 35.00±0.58 | 35.00±0.58 |
| AgNPs 25% | 15.33±0.58 | 15.33±0.58 |
| AgNPs 50% | 18.67±0.58 | 16.00±1 |
| AgNps 75% | 20.67±0.58 | 18.33±0.58 |
| AgNPs 100% | 22.67±0.58 | 20.00±00 |

## CONCLUSION

This study justifies the research that silver nanoparticles can be synthesized using the medicinal plant extract of C. *haussknechtii*. This study showed that the synthesis of C. *haussknechtii* silver nanoparticles depends on various experimental operational parameters. The AgNPs were characterized by UV–Vis, FTIR, and FESEM analysis. The maceration-assisted synthetic strategies of nanoparticle preparation were exploited in the case of AgNP. The biosynthesis of AgNP benign *C. haussknechtii* plant extract was successfully carried out. In conclusion, was demonstrated AgNPs are used as antimicrobial agents; they have great bactericidal potential against both gram-positive and gram-negative pathogens. Experimental optimization studies were also carried out on various parameters. The operational parameters were monitored using the UV–Vis spectrophotometer. The use of the extract, obtained from the corm of *C. haussknechtii*, as a reducing and stabilizing agent represents a suitable alternative to produce antimicrobial metal nanoparticles. The results of the biosynthesis of silver nanoparticles by FTIR and FESEM analysis clearly showed the ability to reduce $Ag^{+}$ ions to $Ag^{0}$ by the biomolecules of corm extract. These findings confirm that such bio-compatible nanoparticles with biological activity may find their applications in the field of nanomedicine. Also, silver nanoparticles can be used effectively in pharmaceutical, biotechnology, and biomedical applications. Moreover, research in pharmacological studies, especially in vivo, is needed to develop and design future antimicrobial therapeutic agents.

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## REFERENCES

Anandalakshmi, K., Venugobal, J., & Ramasamy, V. (2016). Characterization of silver nanoparticles by green synthesis method using *Pedalium murex* leaf extract and their antibacterial activity. *Applied Nanoscience, 6*(3), 399-408. https://doi.org/10.1007/s13204-015-0449-z

Bagherzade, G., Tavakoli, M. M., & Namaei, M. H. (2017). Green synthesis of silver nanoparticles using aqueous extract of saffron (*Crocus sativus* L.) wastages and its antibacterial activity against six bacteria. *Asian Pacific Journal of Tropical Biomedicine*, *7*(3), 227-233. https://doi.org/10.1016/j.apjtb.2016.12.014

Bankier, C., Matharu, R., Cheong, Y. K., Ren, G., Cloutman-Green, E., & Ciric, L. (2019). Synergistic antibacterial effects of metallic nanoparticle combinations. *Scientific Reports, 9*(1), 1-8. https://doi.org/10.1038/s41598-019-52473-2

Burdușel, A. C., Gherasim, O., Grumezescu, A. M., Mogoantă, L., Ficai, A., & Andronescu, E. (2018). Biomedical applications of silver nanoparticles: An up-to-date overview. *Nanomaterials, 8*(9), 681. https://doi.org/10.3390/nano8090681

Chambers, H. F., & DeLeo, F. R. (2009). Waves of resistance: Staphylococcus aureus in the antibiotic era. *Nature Reviews Microbiology*, *7*(9), 629-641. https://doi.org/10.1038/nrmicro2200

Chouhan, N. (2018). Silver nanoparticles: synthesis, characterization and applications. *Silver Nanoparticles-Fabrication, Characterization and Applications*, 21-56. https://doi.org/10.5772/intechopen.75611

Dada, A. O., Adekola, F. A., Dada, F. E., Adelani-Akande, A. T., Bello, M. O., Okonkwo, C. R., & Ajanaku, K. O. (2019). Silver nanoparticle synthesis by *Acalypha wilkesiana* extract: phytochemical screening, characterization, influence of operational parameters, and preliminary antibacterial testing. *Heliyon, 5*(10), e02517. https://doi.org/10.1016/j.heliyon.2019.e02517

Dada, A. O., Inyinbor, A. A., Idu, E. I., Bello, O. M., Oluyori, A. P., Adelani-Akande, T. A., & Dada, O. (2018). Effect of operational parameters, characterization and antibacterial studies of green synthesis of silver nanoparticles using Tithonia diversifolia. *Peer Journal 6*, e5865.

https://doi.org/10.7717/peerj.5865

Escárcega-González, C. E., Garza-Cervantes, J. A., Vazquez-Rodríguez, A., Montelongo-Peralta, L. Z., Treviño-Gonzalez, M. T., Castro, E. D. B., & González, F. T. (2018). In vivo antimicrobial activity of silver nanoparticles produced via a green chemistry synthesis using *Acacia rigidula* as a reducing and capping agent. *International Journal of Nanomedicine, 13*, 2349. https://doi.org/10.2147/IJN.S160605

Francis, S., Joseph, S., Koshy, E. P., & Mathew, B. (2018). Microwave assisted green synthesis of silver nanoparticles using leaf extract of elephantopus scaber and its environmental and biological applications. *Artificial Cells, Nanomedicine, and Biotechnology*, *46*(4), 795-804. https://doi.org/10.1080/21691401.2017.1345921

Gour, A., & Jain, N. K. (2019). Advances in green synthesis of nanoparticles. *Artificial Cells, Nanomedicine, and Biotechnology*, *47*(1), 844-851. https://doi.org/10.1080/21691401.2019.1577878

Hamouda, R. A., Hussein, M. H., Abo-elmagd, R. A., & Bawazir, S. S. (2019). Synthesis and biological characterization of silver nanoparticles derived from the cyanobacterium *Oscillatoria limnetica*. *Scientific Reports*, *9*(1), 1-17. https://doi.org/10.1038/s41598-019-49444-y

Hamzeloo-Moghadam, M., Shahrestani, R., Keramatian, B., & Mohebby, S. (2019). cytotoxic activity of *Crocus pallasii* subsp. haussknechth in human cancer cell lines. *International Journal of Pharmaceutical Sciences and Research*, *10*(1), 117-120. https://doi.org/10.13040/IJPSR.0975-8232.10(1).117-20

Husen, A. (2017). Gold nanoparticles from plant system: synthesis, characterization and their application. *Nanoscience and plant–soil systems* (pp. 455-479): Springer. https://doi.org/10.1007/978-3-319-46835-8_17

Jha, A. K., & Prasad, K. (2010). Green synthesis of silver nanoparticles using Cycas leaf. *International Journal of Green Nanotechnology: Physics and Chemistry, 1*(2), P110-P117. https://doi.org/10.1080/19430871003684572

Jiang, H., Manolache, S., Wong, A. C. L., & Denes, F. S. (2004). Plasma-enhanced deposition of silver nanoparticles onto polymer and metal surfaces for the generation of antimicrobial characteristics. *Journal of Applied Polymer Science, 93*(3), 1411-1422. https://doi.org/10.1002/app.20561

Khani, R., Roostaei, B., Bagherzade, G., & Moudi, M. (2018). Green synthesis of copper nanoparticles by fruit extract of *Ziziphus spina-christi* (L.) Willd.: Application for adsorption of triphenylmethane dye and antibacterial assay. *Journal of Molecular Liquids*, *255*, 541-549. https://doi.org/10.1016/j.molliq.2018.02.010

Kumar, D., Kumar, G., & Agrawal, V. (2018). Green synthesis of silver nanoparticles using *Holarrhena antidysenterica* (L.) Wall. bark extract and their larvicidal activity against dengue and filariasis vectors. *Parasitology Research, 117*(2), 377-389. https://doi.org/ 10.1007/s00436-017-5711-8

Le Ouay, B., & Stellacci, F. (2015). Antibacterial activity of silver nanoparticles: a surface science insight. *Nano Today, 10*(3), 339-354. https://doi.org/10.1016/j.nantod.2015.04.002

Lister, P. D., Wolter, D. J., & Hanson, N. D. (2009). Antibacterial-resistant *Pseudomonas aeruginosa*: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clinical Microbiology Reviews, 22*(4), 582-610. https://doi.org/10.1128/CMR.00040-09

Liu, X., Cai, J., Chen, H., Zhong, Q., Hou, Y., Chen, W., & Chen, W. (2020). Antibacterial activity and mechanism of linalool against *Pseudomonas aeruginosa*. *Microbial Pathogenesis*, *141*, 103980. https://doi.org/10.1016/j.micpath.2020.103980

Noah, N. (2019). Green synthesis: Characterization and application of silver and gold nanoparticles *Green Synthesis, Characterization and Applications of Nanoparticles* (pp. 111-135): Elsevier. https://doi.org/10.1016/B978-0-08-102579-6.00006-X

Pal, S., Tak, Y. K., & Song, J. M. (2007). Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. *Applied and Environmental Microbiology*, *73*(6), 1712-1720. https://doi.org/10.1128/AEM.02218-06

Rai, M., Yadav, A., & Gade, A. (2009). Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances*, *27*(1), 76-83. https://doi.org/10.1016/j.biotechadv.2008.09.002

Rajeshkumar, S., & Bharath, L. (2017). Mechanism of plant-mediated synthesis of silver nanoparticles–a review on biomolecules involved, characterisation and antibacterial activity. *Chemico-Biological Interactions, 273,* 219-227. https://doi.org/10.1016/j.cbi.2017.06.019

Roy, A., Bulut, O., Some, S., Mandal, A. K., & Yilmaz, M. D. (2019). Green synthesis of silver nanoparticles: biomolecule-nanoparticle organizations targeting antimicrobial activity. *RSC Advances*, *9*(5), 2673-2702. https://doi.org/10.1039/c8ra08982e

Saxena, R. (2010). Botany, taxonomy and cytology of *Crocus sativus* series. *Ayu*, *31*(3), 374-381. https://doi.org/10.4103/0974-8520.77153

Shankar, S. S., Rai, A., Ahmad, A., & Sastry, M. (2004). Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. *Journal of Colloid and Interface Science, 275*(2), 496-502. https://doi.org/10.1016/j.jcis.2004.03.003

Siddiqi, K. S., Husen, A., & Rao, R. A. (2018). A review on biosynthesis of silver nanoparticles and their biocidal properties. *Journal of Nanobiotechnology, 16*(1), 14. https://doi.org/10.1186/s12951-018-0334-5

Solgi, M. (2014). Evaluation of plant-mediated silver nanoparticles synthesis and its application in postharvest physiology of cut flowers. *Physiology and Molecular Biology of Plants, 20*(3), 279-285. https://doi.org/10.1007/s12298-014-0237-3

Tippayawat, P., Phromviyo, N., Boueroy, P., & Chompoosor, A. (2016). Green synthesis of silver nanoparticles in aloe vera plant extract prepared by a hydrothermal method and their synergistic antibacterial activity. *Peer Journal*, *4*, e2589. https://doi.org/10.7717/peerj.2589

Yun'an Qing, L. C., Li, R., Liu, G., Zhang, Y., Tang, X., Wang, J., & Qin, Y. (2018). Potential antibacterial mechanism of silver nanoparticles and the optimization of orthopedic implants by advanced modification technologies. *International Journal of Nanomedicine*, *13*, 3311-3327. https://doi.org/10.2147/IJN.S165125