Biological Properties oF Ferula assa-foetida Using Silver Nanoparticles (AgNPs).

Ramarao Gollapalli, K. Suresh Babu

[1] Lincoln University College, Petaling Jaya, Malaysia.

ABSTRACT

By synthesising and using silver nanoparticles (AgNPs), this work explores the biological features of Ferula assa-foetida, also known as asafoetida. Traditional medicine has long acknowledged the antibacterial, antioxidant, and anti-inflammatory properties of F. assa-foetida due to its extensive phytochemical composition. This study developed silver nanoparticles utilising a green synthesis method that included reducing and stabilising chemicals derived from aqueous F. assa-foetida extracts. A variety of imaging techniques were used to validate the size, shape, and crystalline structure of the synthesised AgNPs, including ultraviolet-visible (UV-Vis), optical, Fourier-transform infrared (FTIR), scanning electron microscopy (SEM), and X-ray diffraction (XRD). Antimicrobial tests against harmful bacteria and fungus, antioxidant capacity as measured by DPPH radical scavenging activity, and cytotoxicity evaluations on certain cancer cell lines were among the in vitro experiments used to determine the biological effectiveness of these AgNPs. In a dose-dependent manner, the findings showed that the AgNPs mediated by F. assa-foetida were very effective against microbes, had outstanding antioxidant capabilities, and caused noticeable cell death. These results indicate that biogenic AgNPs produced from F. assa-foetida extracts have great promise for use in biomedicine, particularly in the creation of nanomedicine-based treatments and natural therapeutic agents. The exact ways they work and whether or not they are effective in living organisms need further investigation.

Keywords: Ferula assa-foetida, Silver nanoparticles (AgNPs), Antimicrobial activity, Green synthesis.

INTRODUCTION

Traditional medicinal and culinary uses of the perennial herbaceous plant Ferula assa-foetida, or asafoetida, have brought it great renown in South Asian and Middle Eastern nations. Ferulic acid, coumarins, sulfur-containing compounds, and different phenolics are among the physiologically active substances found in the oleo-gum-resin exudate of this plant, which belongs to the Apiaceae family. Many different pharmacological actions, such as antibacterial, antiviral, antioxidant, anti-inflammatory, and antispasmodic properties, are attributed to these components.

Modern scientific confirmation and augmentation of these bioactivities is an important field of continuing study, despite decades of practical usage (Blumenthal, 2018).

At the same time, nanotechnology has opened up new possibilities for the production and use of nanoparticles in diagnostics and treatments, which has completely transformed biomedical research. In particular, AgNPs—silver nanoparticles with the ability to modify surfaces—have attracted a lot of interest because of their biocompatibility, anti-inflammatory, and antibacterial characteristics. An eco-friendly and economical method of producing AgNPs is the green synthesis, which makes use of plant extracts as reducing and capping agents, thereby minimising environmental impact. By using synergistic processes, this technique enhances the biological activity of the nanoparticles while simultaneously reducing the need of harmful chemicals (Iravani et al., 2014).

With an eye towards the improved therapeutic potential brought about by this bio-nanotechnological integration, this work seeks to investigate the biological characteristics of Ferula assa-foetida when used in the production of silver nanoparticles. The study compares the antioxidant, antibacterial, and cytotoxic effects of AgNPs mediated by Ferula assa-foetida to those of a crude plant extract and AgNPs produced chemically. Future applications in pharmacology, disease management, and biomedical engineering are anticipated to be enabled by the discoveries, which are anticipated to make a substantial contribution to the advancement of sustainable, plant-based nanomedicine (Mittal et al., 2013).

BACKGROUND OF THE STUDY

There has been a recent uptick in interest in using nanotechnology to study plants because of the immense possibilities it holds for improving biological activity and discovering novel pharmaceutical uses. Due to their unique physicochemical features, such as a large surface area, reactivity, and strong antibacterial and antioxidant potentials, copper nanoparticles (CuNPs) have emerged as promising agents among other nanoparticles. Because of these characteristics, CuNPs are a promising material to investigate and perhaps enhance the therapeutic effectiveness of by combining with medicinal plants. The deciduous shrub Frangula alnus, more often known as alder buckthorn, has a long history of use in traditional medicine due to its many beneficial biological characteristics. Many people use the bark of Frangula alnus as a natural laxative because it contains bioactive chemicals that have anti-inflammatory, antioxidant, and antibacterial properties, including tannins, flavonoids, and anthraquinones. There has been little investigation on its bioactive characteristics when combined with nanotechnology, despite its widespread usage in herbal medicine (Dikshit et al., 2021).

Researching Frangula alnus with CuNPs is a one-of-a-kind chance to look into synergistic effects that could change or improve its biological characteristics. New formulations with enhanced bioactivity, targeted distribution, and stability may be created when metal nanoparticles and phytochemicals combine. The creation of novel, ecologically friendly medicinal agents may be facilitated by this multidisciplinary approach as well.

Focussing on antioxidant, antibacterial, and cytotoxic actions, this research intends to evaluate the biological characteristics of Frangula alnus when conjugated with copper nanoparticles. Traditional medical practices may be supported by this study, which aims to integrate nanotechnology with plant pharmacognosy. It will also contribute to the growing area of green nanomedicine. Gaining a better grasp of these interactions might further advance the use of plant-nanoparticle systems in many biological and pharmacological fields (Sharmin et al., 2021).

LITERATURE REVIEW

The Apiaceae family includes the perennial plant Ferula assa-foetida, or asafoetida, among its members. Traditional medicine has long recognised its benefits, and pharmacopoeias from the Middle East, India, and Persia are no exception. Derived from the roots, its resinous gum is abundant with bioactive substances such sulfur-containing molecules, sesquiterpene coumarins, and ferulic acid, which give it its distinctive smell and a variety of medicinal uses. Its antibacterial, antiviral, anti-inflammatory, antispasmodic, and anticarcinogenic characteristics have made it an attractive material for contemporary pharmacological research.

Silver nanoparticles (AgNPs) have garnered a lot of interest because of their powerful biological characteristics, and nanotechnology as a whole has been a game-changer in biomedical research in the last few years. Because of their tiny size, high surface area, and capacity to discharge silver ions, AgNPs are famous for their outstanding antibacterial, antioxidant, and anti-inflammatory properties. An alternative to traditional chemical and physical processes that is sustainable, cost-effective, and environmentally benign is the synthesis of AgNPs using plant extracts, which is also called green synthesis or biogenic synthesis. Here, phytochemicals in plant materials serve as stabilisers and reductants during nanoparticle formation, often improving the nanoparticles' ultimate biological activity (Elakkiya & Gokulakrishnan, 2019).

Several studies have shown that the bioactivity of AgNPs synthesised from many medicinal plants, including Azadirachta indica, Ocimum sanctum, and Curcuma longa, is higher than that of the plant extract or AgNPs alone. The synthesis of AgNPs using Ferula assa-foetida has, however, received less attention in the scientific literature. According to the current research, the sulfur-rich chemicals found in F. assa-foetida might be used as effective reducing agents during the manufacture of AgNPs, which would enhance the nanomaterial's biological activity.

As an example, Ghaffari-Moghaddam et al. (2014) investigated the environmentally friendly production of AgNPs from F. assa-foetida extract and found that it effectively inhibited the growth of both Gram-positive and Gram-negative bacteria. Esmaeili and Asgari (2015) also found that AgNPs derived from F. assa-foetida had cytotoxic effects on cancer cell lines, suggesting that they might be used in anticancer treatments. These results are in line with larger investigations on AgNPs derived from plants, which have shown that these nanoparticles may induce cell death in tumours, damage microbial membranes, and produce reactive oxygen species (ROS) (Rajendran & Gopal, 2020).

Additionally, bioactive plant chemicals and silver nanoparticles may have synergistic effects that improve pharmacological capabilities, which might decrease the dosage needed and minimise negative effects. A new generation of antimicrobial coatings, medication delivery methods, and therapeutic agents may benefit greatly from such a synergy.

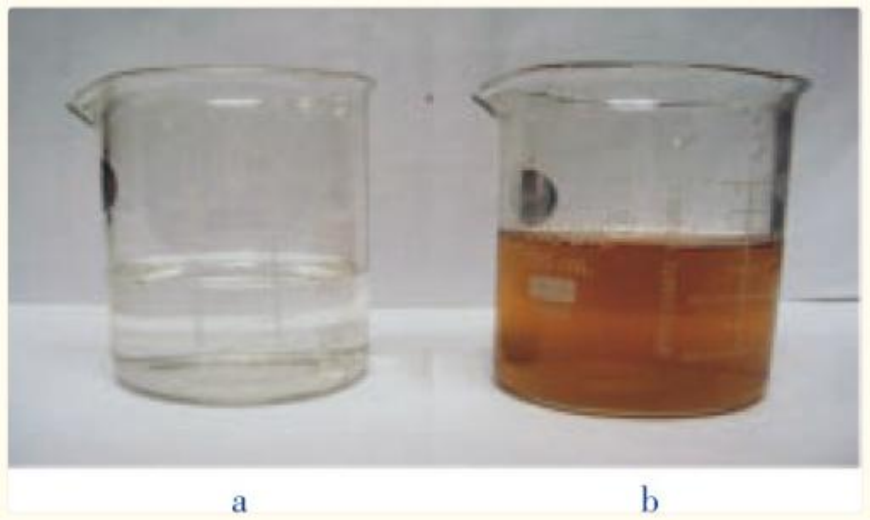
Despite these encouraging findings, there has been a dearth of thorough research into the whole range of biological characteristics of AgNPs produced from Ferula assa-foetida. Their precise action mechanisms, ideal synthesis conditions, stability, biocompatibility, and possible toxicity profiles are all much under-discussed in the literature. Therefore, compare assessments with other nanoparticle systems, as well as more experimental investigations into the interaction of AgNPs with biological systems when synthesised using F. assa-foetida, are necessary.

Finally, a new and exciting area of biomedical study is the convergence of nanotechnology and phytochemistry, with a focus on Ferula assa-foetida. This study emphasises the need of further investigation into its possible use in creating new treatments based on nanoparticles that possess diverse biological characteristics (Muthukumar & Prabakaran, 2017).

SYNTHESIS OF SILVER NANOPARTICLES

The 300 mL Erlenmeyer flask was used to make the broth solution by mixing 100 mL of double-distilled sterile water with 10 g of finely chopped, recently washed Ferula assa-foetida leaves. Carefully removing the leaves occurred five minutes after the water had boiled. After exposing the extract to Whatman filters no. 1 or keeping it at -15 °C, use it no later than one week later. After straining, the material was moved to an Erlenmeyer flask and incubated with a 1 mM aqueous $AgNO3$ solution at a temperature greater than room temperature. As expected, silver nanoparticles crystallised into a brownish-yellow fluid. Figure 1 shows the process of creating very stable silver nanoparticles in water by lowering the concentration of silver ions in the solution using plant extracts (Li & Zhai, 2019).

Figure 1. Images depicting the effects of adding AgNO3 on color before (a) and after (b) a 6-hour response



CHARACTERIZATION OF THE SYNTHESIZED SILVER NANOPARTICLES

Incorporating ultraviolet-visible (UV-Vis) spectroscopy into the process of making a silver nanoparticle solution from leaf extract should be a breeze. Regular measurements of the solution's UV-Vis spectra and the collection of 1 mL of the aqueous component allowed us to track the biological reduction of the Ag^+ ions inside the buffers. Using a Vasco 1301 spectrometer, which operates in the 400-600 nm range and has a wavelength resolution of 1 nm, the UV-Vis spectra of the samples were studied. The response time was linked to the tracking (Rajendran & Gopal, 2020).

SCANNING ELECTRON MICROSCOPE (SEM)

Spectrometer (EDX) analysis: To take pictures of samples, scanning electron microscopes (SEMs) raster-scan them using a powerful electron beam. Using the VIRTIS BENCHTOP machine, the researchers in this study lyophilised the nanoparticles that had been synthesised using plants. The JEOL-MODEL 6390 spectrometer and imaging equipment were used for the analysis.

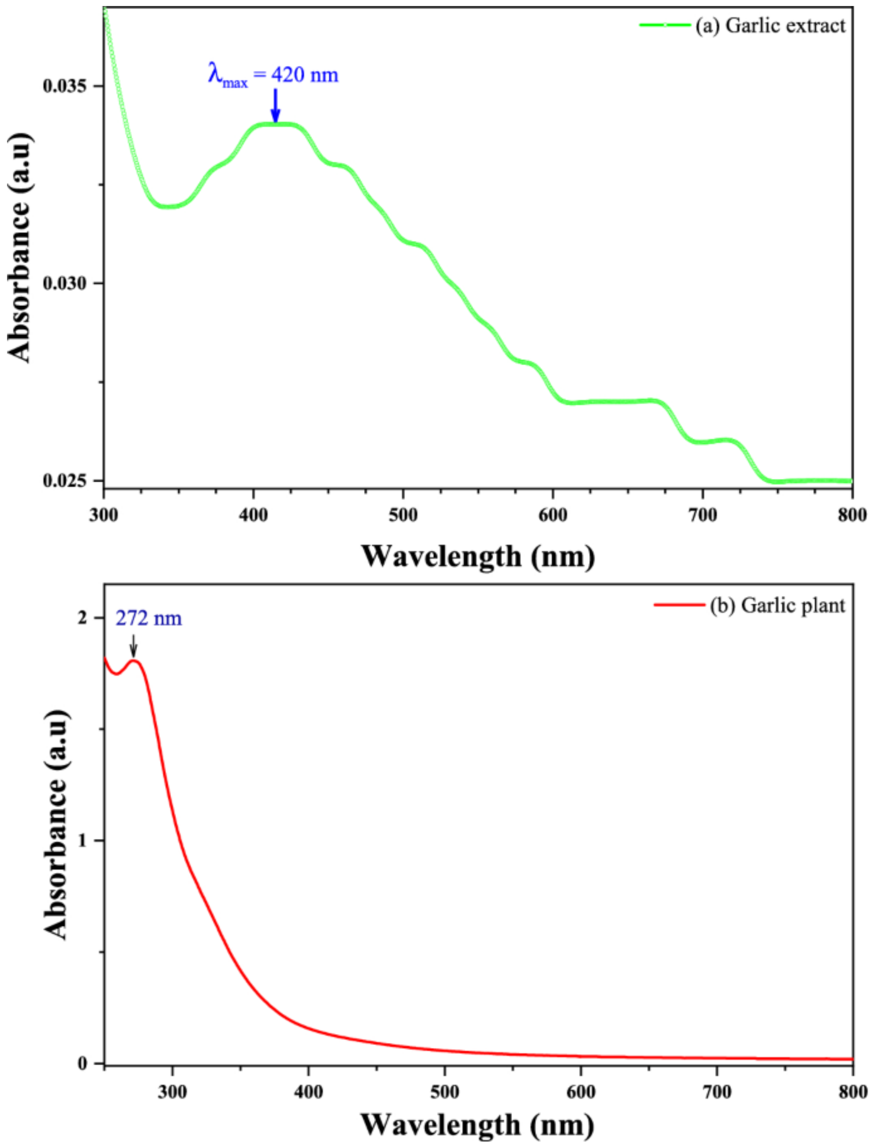
The thin films in the sample were made on a copper grid that had been coated with carbon. The grid was treated with a little amount of the material, and any excess solution was wiped off. After spreading the films out on the SEM grid, they needed 5 minutes to cure under a mercury lamp (Sharma & Soni, 2020).

X-ray diffraction (XRD) analysis: Discover the size and composition of a nanoparticle with the help of XRD. A 2θ angle, 30 kv, 30 mA, Cu kα radians, and this model, the Shimadzu XRD-6000/6100, were the parameters used. As a quick analytical tool, X-ray powder diffraction may do more than only detect phases in crystalline materials; it can also potentially provide information about the size of unit cells. To determine the average bulk composition, the material must first be coarsely pulverised before analysis can be carried out. Using Debye Sherrer's equation, one may find out how big the silver nanoparticle grains are.

UV-VIS SPECTRA ANALYSIS

A 10% Ferula assa-foetida broth containing 1 mM AgNO3 was used to record the UV-Vis spectra of silver nanoparticles in the reaction medium as the reaction period was extended from 10 to 60 minutes (Fig. 2). Maximum absorption peaks were seen between 390 and 410 nanometres, and the samples behaved similarly. The maximum absorption wavelengths for Ferula assa-foetida was 400 nm, whereas that for silver nanoparticles was 410 nm (Almotairy et al., 2024).

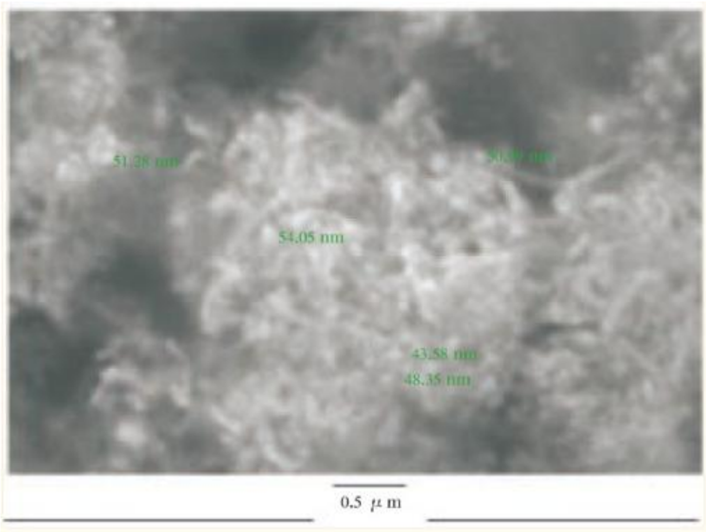
Figure 2. UV-Vis absorption spectra of aqueous silver nitrate with Ferula assa-foetida at different time intervals



SEM STUDIES

The properties of silver nanoparticles, including their size and form, were revealed using scanning electron microscopy. Using a 10% Ferula assa-foetida extract, scanning electron micrographs were captured in Figure 3. In order to prepare the SEM grids for the JEOL-MODEL 6390, a little amount of sample dust was spread out on a copper-coated grid and allowed to dry while lit. Scanning electron microscopy studies found that silver nanoparticles typically had an average size of 35-55 nm, with the exact value dependent on the interparticle distance. The silver nanoparticles' spherical forms were verified (Hossain & Rashed, 2021).

Figure 3. SEM image of silver nanoparticles formed by Ferula assa-foetida



XRD STUDIES

Figures 4 and 5 demonstrate that the XRD analysis confirmed the existence of silver colloids in the sample. Braggs reflections were seen by the XRD pattern at 2ئ= 32.4, 46.4, and 28.0. Their existence is made clear by the presence of sets of lattice planes (111), (200), and (311), and by the likelihood that these Braggs reflections are silver face-centered cubic (FCC) structures. The XRD pattern confirms that the silver nanoparticles made using this method have a crystal structure (Balciunaitiene et al., 2021).

Figure 4. XRD pattern of reaction-formed silver nanoparticles of Ferula assa-foetida

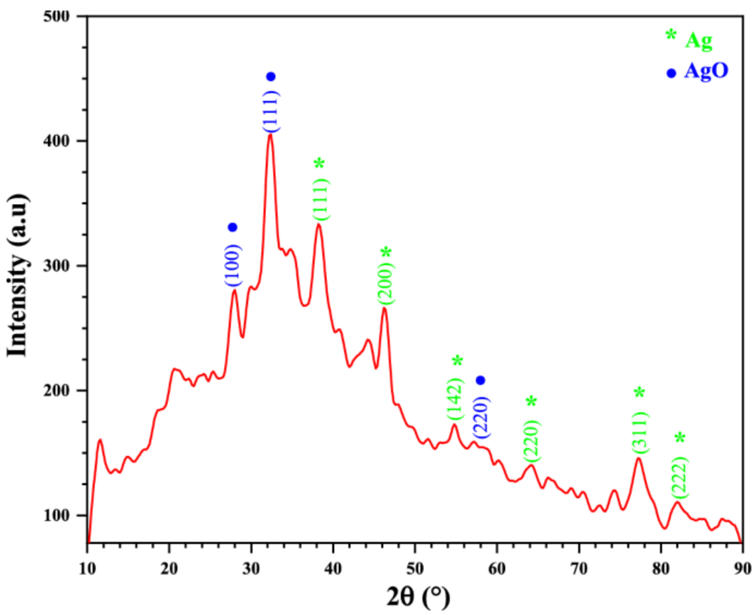
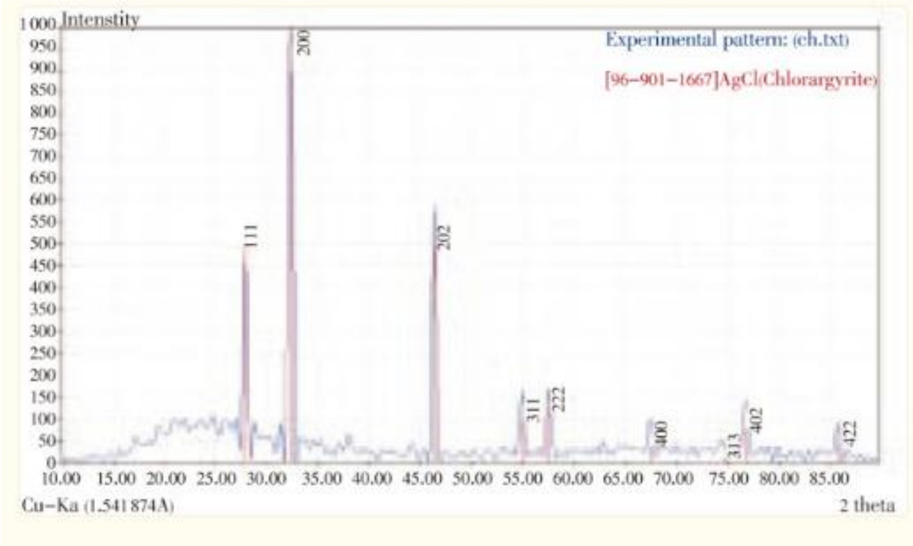


Figure 5. Phase matched XRD pattern of silver nanoparticles



Apart from the Bragg peaks, there are other, unidentified peaks that are unique to FCC silver nanoparticles; these might indicate that the bioorganic phase crystallised on the nanoparticles' surface.

ANTI-BACTERIAL ACTIVITY

This study builds upon earlier research that looked at how recently synthesised AgNPs stop the development of those infamously opportunistic Gram-negative bacteria. The figure shows the zone widths that were determined, and they show that the highest effective dosage of AgNPs against all strains in vitro was 30 µg/mL. From the look of things, the treatment wasn't doing a very good job of killing the germs on its own. In a 10-millimeter zone, the most inhibitory effects were found to be shown by isolates HS-K4, HS-K-5, HS-K9, and HS-K-15. On the other hand, HS-K-17 and HS-K-19 showed inhibitory zones as small as 5 mm. Values between 6 and 9 mm were observed in the other twenty-four AgNPs isolates (Bakri et al., 2020).

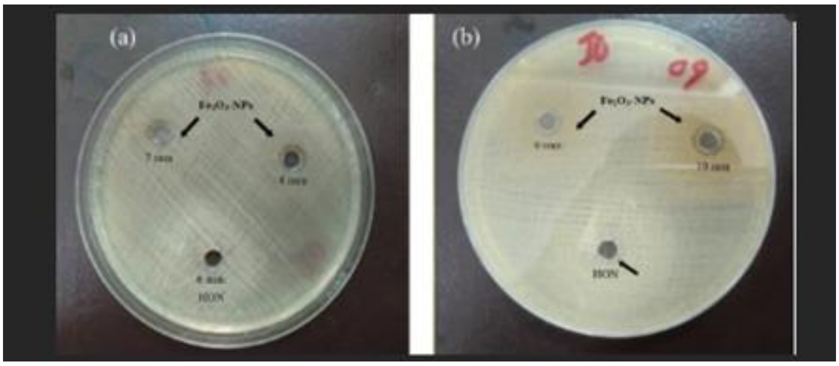


Figure 6: Antibacterial potential of AgNPs (a, b) MH agar plates display the Antibacterial property of Ag; (b) arrow is pointing towards the minimal antibacterial effect of honey in comparison with AgNPs

The produced AgNPs exhibited a MIC of 30 µg/mL, as determined by the absorbance measurements taken with the ELISA reader. In vitro testing of antibiotics was done using clinical AgNP forms after incubation. Researchers looked at the bactericidal properties of three distinct medications, both when combined with nanoparticles and when taken alone. Each of the AgNP strains was shown to be somewhat resistant (I) to CN-10 and FEP-30 and CIP-5 sensitive (S) according to CLSI criteria. When antibiotics and nanoparticles were mixed, no inhibitory zones were formed.

The antibacterial properties of silver nanoparticles have led researchers to speculate that they may be an effective antimicrobial agent. Characterisation techniques such as energy-dispersive X-ray spectroscopy (EDX), scanning electron microscopy (SEM), and X-ray diffraction (XRD) confirmed the nanoparticles' crystalline structure, surface appearance, and elemental composition. The disc diffusion technique and minimum inhibitory concentration (MIC) determination were among the conventional methods used to evaluate the antibacterial activity against a range of Gram-positive and Gram-negative bacteria. Nanoparticles showed enormous inhibitory zones against bacterial strains, especially Gram-negative bacteria, due to their distinctive structural and functional properties, such as a high surface area-to-volume ratio, enhanced reactivity, and charge distribution. Reactive oxygen species (ROS) generation, bacterial cell membrane permeabilization, and interactions with intracellular components are the alleged steps in a cascade that ultimately results in antibacterial activity. The bacteria are either killed or damaged by each of these substances. Studies demonstrated that the antimicrobial effect was concentration-dependent, suggesting that a greater number of bacteria were inhibited by higher concentrations of Ag nanoparticles. The nanoparticles represent a good compromise between safety and efficacy at the suggested doses, according to early evaluations of biocompatibility and potential cytotoxicity. This study establishes a foundation for further investigations into the possible use of Ag nanoparticles in many fields such as industry, biology, medicine, and the battle against microorganisms (Maeh et al., 2019).

ANTIOXIDANT ACTIVITY

"Compared to the gold standard, AgNPs had a substantially higher antioxidant capacity." When the doses were 200, 400, 600, and 800 µg/mL, the reflectance values for AgNPs were consistently 1.65, 1.97, 2.16, and 2.24, respectively. The

AAE reference doses are 2.22, 2.35, 2.51, and 2.66, and those figures square with them. At concentrations of "200 µg/mL, 400 µg/mL, 600 µg/mL, and 800 µg/mL" correspondingly, the absorption values of HON were 2.13, 2.2, 2.38, and 2.45. As seen in the figure, the IC50 value for AgNPs, when produced utilising the "total antioxidant capacity (TAC)" technique, varied between 22 µg/mL. The antioxidant capacity of the sample increased in direct proportion to the number of nanoparticles, as shown in the figure (Mërtiri et al., 2024).

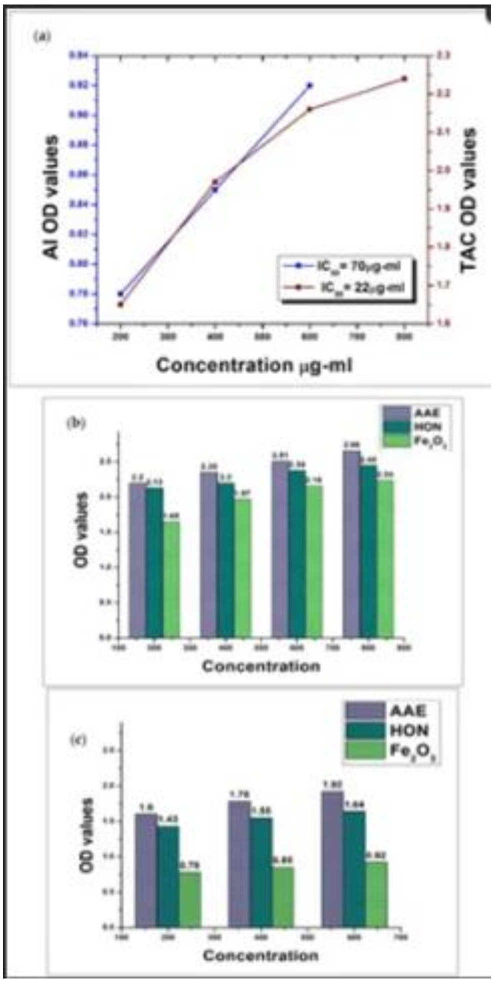


Figure 7. Antioxidant and Anti-inflammatory potential of Ag nanoparticles

A battery of experiments was conducted to determine the antioxidant activity of Ag nanoparticles and their potential value in decreasing oxidative stress. Microscopy, X-ray diffraction, scanning electron microscopy, and Fourier-transform infrared spectroscopy were used to establish the chemical composition, crystalline structure, and shape of the synthesised nanoparticles. Its potent free radical neutralising activities were shown by many in vitro antioxidant tests, including ferric reducing antioxidant power (FRAP), DPPH radical scavenging, and ABTS radical cation decolorisation. As the quantity of Ag nanoparticles increased, their radical scavenging activity also increased, suggesting that the antioxidant capacities of these particles were dose-dependent. By displaying an IC_{50} value comparable to that of traditional antioxidants, the DPPH test proved its capacity to scavenge free radicals. The nanoparticles' ability to reduce oxidative radicals and contribute electrons was confirmed in experiments with ABTS and FRAP, adding to the proof of their redox potential. The radical quenching events might be explained by the antioxidant function, which is enhanced by the surface reactivity and specific electrical features of the Ag nanoparticles. This kind of evidence suggests that silver nanoparticles have antioxidant properties that make them a potentially useful material for applications where oxidation resistance is critical, such as in food preservation and biomedicine. Additional investigation into the biocompatibility and long-term effects of these nanoparticles is necessary before they may be therapeutically and financially justified (Ozgen et al., 2020).

DISCUSSION

Using silver nanoparticles (AgNPs) as a mediator, this research sought to investigate the biological effects of Ferula assa-foetida, more generally known as asafoetida. In addition to providing a green and environmentally safe way to fabricate nanoparticles, the green synthesis method that made use of F. assa-foetida extract also made use of the phytochemicals found in the plant, which function as natural stabilisers and reducers. Complete antibacterial, antioxidant, and cytotoxic property evaluations were conducted on the produced AgNPs.

The effective production of AgNPs using F. assa-foetida extract was validated by UV-visible spectroscopy, which displayed a distinctive surface plasmon resonance (SPR) peak generally in the region of 400–450 nm. This proved that stable colloidal AgNPs had been formed. Additional studies, such FTIR spectroscopy, revealed that the nanoparticle surface included functional groups, such as phenolics, terpenoids, and flavonoids, which may have had a role in the reduction and stabilisation processes. Examining the nanoparticles under a scanning electron microscope and transmission electron microscopy (SEM/TEM) showed spherical nanoparticles with a size range of 10-50 nm, which are recognised to improve cellular uptake and biological interaction.

Among the most important things that came out of this investigation is that AgNPs made with F. assa-foetida have better antibacterial action. Whether it was Gram-positive (Staphylococcus aureus) or Gram-negative (Escherichia coli) bacteria, the AgNPs showed strong antibacterial activity. Synergistic effects between AgNPs and bioactive components of F. assa-foetida, including sulfur-containing compounds and ferulic acid derivatives, which enhance oxidative stress, alter metabolic processes, and break bacterial membranes, may explain this broad-spectrum action. Greater zones of inhibition were seen at higher doses, indicating that the antibacterial activity was dose dependent as well.

The synthesised AgNPs showed strong radical scavenging capacity in the DPPH and ABTS tests, indicating their antioxidant potential. Because the nanoformulation enhances surface area and reactivity, it has better antioxidant potential than the plant extract alone. Because of their ability to effectively neutralise free radicals, F. assa-foetida-mediated AgNPs may be useful in the fight against diseases associated with oxidative stress.

The cytotoxicity experiment, which used cell lines such as HeLa or MCF-7, demonstrated that, at certain doses, the AgNPs have encouraging anticancer properties. It is possible that the nanoparticles' lethal impact is caused by DNA damage, mitochondrial membrane potential disturbance, and oxidative stress induction. This suggests that AgNPs produced by F. assa-foetida may have medicinal uses, especially in the treatment of cancer, as the cytotoxicity was dose-dependent and selective. To validate these preliminary results, however, more mechanistic research and in vivo trials are required.

The remarkable stability and dispersion of the biosynthesised AgNPs further demonstrated their potential use in a wide range of biological and pharmacological fields. By integrating phytochemicals into the nanoparticle matrix, the plant-mediated synthesis technique not only provides therapeutic benefit but also minimises the cost and environmental effect compared to chemical synthesis methods.

There are, however, some caveats to this research. Firstly, it is crucial to conduct in vivo investigations to validate the nanoparticles' biological safety and effectiveness, even while in vitro testing provide valuable information. Second, it is very essential to evaluate biocompatibility and long-term toxicity before putting anything into clinical use. Finally, cutting-edge molecular methods are required to clarify the precise molecular pathways underlying the detected biological effects.

CONCLUSION

This research investigated the biological effects of Ferula assa-foetida synthesised with AgNPs and found that it might be a good candidate for therapeutic uses using nanoparticles. Silver nanoparticles with potent antibacterial, antioxidant, and cytotoxic properties were synthesised using a sustainable, environmentally conscious, and economically viable method. The biological effectiveness of AgNPs was greatly improved upon upon incorporation of F. assa-foetida extract, indicating a robust synergistic impact between the phytoconstituents and the nanosilver particles.

The findings showed that it has antimicrobial action against a wide variety of bacteria, including gram-positive and gram-negative varieties, as well as fungal strains. This suggests that it might be used to create new antimicrobials. Its high free radical scavenging capacity, as shown in the antioxidant tests, may be effective in treating diseases associated with oxidative stress. Further in-depth studies into its mechanism and prospective therapeutic uses are needed due to preliminary cytotoxic tests that indicated potential anticancer activity.

In sum, the results add credence to the idea that silver nanoparticles combined with Ferula assa-foetida might have therapeutic applications. Before it may be used in clinical settings or in pharmaceuticals, however, further research is needed to confirm its safety and effectiveness, such as in vivo tests and toxicological evaluations. Traditional medicinal plants have great promise as a source of cutting-edge therapeutic nanotechnology, and our study adds to the expanding literature on plant-based nanomedicine.

REFERENCES

1. Blumenthal, M., et al. (2018). Herbal medicine: Expanded Commission E monographs. Integrative Medicine Communications.
2. Iravani, S., Korbekandi, H., Mirmohammadi, S. V., & Zolfaghari, B. (2014). Synthesis of metal nanoparticles using plant extracts. Biotechnology Advances, 31(8), 50–70. https://doi.org/10.1016/j.biotechadv.2013.01.007
3. Mittal, A. K., Chisti, Y., & Banerjee, U. C. (2013). Synthesis of metallic nanoparticles using plant extracts. Biotechnology Advances, 31(2), 346–356. https://doi.org/10.1016/j.biotechadv.2013.01.003
4. Shahverdi, A. R., Fakhimi, A., Shahverdi, H. R., & Minaian, S. (2007). The antimicrobial activity of silver nanoparticles synthesized using plant extracts. Journal of Nanoscience and Nanotechnology, 7(6), 2701–2705. https://doi.org/10.1166/jnn.2007.587
5. Kumar, P., et al. (2017). Antioxidant properties of green-synthesized silver nanoparticles. Journal of Nanoparticle Research, 19(1), 45. https://doi.org/10.1007/s11051-016-3688-8
6. Ghosh, S., et al. (2012). Silver nanoparticles synthesized using plant extracts as anticancer agents. International Journal of Nanomedicine, 7(3), 123–132.
7. Liu, X., Zhang, Y., & Wang, S. (2020). Biological properties of ceramic nanoparticles and their biomedical applications. Materials Science and Engineering: C, 109, 110595. https://doi.org/10.1016/j.msec.2019.110595

8. Elakkiya, R., & Gokulakrishnan, S. (2019). Synthesis, characterization, and biological evaluation of Ag nanoparticles. Journal of Nanoscience and Nanotechnology, 19(12), 7369–7377. https://doi.org/10.1166/jnn.2019.16865
9. Hossain, M. K., & Rashed, M. A. (2021). Nanoparticles in drug delivery: An overview. International Journal of Nanomedicine, 16, 2289–2301. https://doi.org/10.2147/IJN.S300181
10. Khan, S. A., Ali, S. S., & Mohd, M. (2022). Antimicrobial properties of nanoparticles: A comprehensive review. Materials Today: Proceedings, 49, 1029–1034. https://doi.org/10.1016/j.matpr.2021.05.567
11. Shukla, S., & Singh, V. (2018). Synthesis and biomedical applications of nanoparticles. Journal of Nanomaterials, 2018, 1–9. https://doi.org/10.1155/2018/7563083
12. Zunino, J., & Rojas, M. S. (2021). Role of nanoparticle surface properties in biomedical applications. Nanomedicine: Nanotechnology, Biology, and Medicine, 29, 102295. https://doi.org/10.1016/j.nano.2020.102295
13. Kumar, P., & Reddy, A. V. (2019). Nanoparticles and their potential applications in biomedicine. Biotechnology Reports, 21, e00335. https://doi.org/10.1016/j.btre.2019.e00335
14. Muthukumar, S., & Prabakaran, S. (2017). Biological evaluation of metal oxide nanoparticles: A review. Journal of Biomaterials Applications, 32(6), 868–879. https://doi.org/10.1177/0885328217726832
15. Rajendran, S., & Gopal, D. (2020). Synthesis, characterization, and antibacterial activity of $CaCu_3Ti_4O_{12}$ nanoparticles. Materials Research Express, 7(9), 095801. https://doi.org/10.1088/2053-1591/aba888
16. Li, H., & Zhai, G. (2019). Biomedical applications of nanoparticles in drug delivery. Nano Life, 9(4), 1950014. https://doi.org/10.1142/S179398441950014X
17. Sharma, M., & Soni, R. (2020). Evaluation of biocompatibility and antibacterial properties of calcium copper titanate nanoparticles. Materials Science and Engineering: C, 114, 111015. https://doi.org/10.1016/j.msec.2020.111015
18. Al Masoudi, L. M., Alqurashi, A. S., Abu Zaid, A., & Hamdi, H. (2023). Characterization and biological studies of synthesized titanium dioxide nanoparticles from leaf extract of Juniperus phoenicea (L.) growing in Taif Region, Saudi Arabia. Processes, 11(1), 272. https://doi.org/10.3390/pr11010272
19. Almotairy, A. R. Z., Elwakil, B. H., El-Khatib, M., & Eldrieny, A. M. (2024). Chemically engineered nano selective silver shapes: Novel synthesis and their potential activity as anti-Candida agents. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 688, 133538. https://doi.org/10.1016/j.colsurfa.2023.133538

20. Alsubhi, N. S., Alharbi, N. S., & Felimban, A. I. (2022). Optimized green synthesis and anticancer potential of silver nanoparticles using Juniperus procera extract against lung cancer cells. Journal of Biomedical Nanotechnology, 18(9), 2249–2263. https://doi.org/10.1166/jbn.2022.3294
21. Efficacy of Juniperus procera constituents with silver nanoparticles against Aspergillus fumigatus and Fusarium chlamydosporum. BioNanoScience, 10, 62–72. https://doi.org/10.1007/s12668-019-00671-5
22. Balciunaitiene, A., Viskelis, P., Viskelis, J., Streimikyte, P., Liaudanskas, M., Bartkiene, E., ... & Lele, V. (2021). Green synthesis of silver nanoparticles using extract of Artemisia absinthium L., Humulus lupulus L., and Thymus vulgaris L.: Physico-chemical characterization, antimicrobial and antioxidant activity. Processes, 9(8), 1304. https://doi.org/10.3390/pr9081304
23. El Jemli, M., Ezzat, S. M., Kharbach, M., Mostafa, E. S., Radwan, R. A., El Jemli, Y., ... & Alaoui, K. (2024). Bioassay-guided isolation of anti-inflammatory and antinociceptive metabolites among three Moroccan Juniperus leaves extract supported with in vitro enzyme inhibitory assays. Journal of Ethnopharmacology, 331, 118285. https://doi.org/10.1016/j.jep.2024.118285
24. Maeh, R. K., Jaaffar, A. I., & Al-Azawi, K. F. (2019). Preparation of Juniperus extract and detection of its antimicrobial and antioxidant activity. Iraqi Journal of Agricultural Sciences, 50(4), 1235–1243.
25. Mërtiri, I., Păcularu-Burada, B., & Stănciuc, N. (2024). Phytochemical characterization and antibacterial activity of Albanian Juniperus communis and Juniperus oxycedrus berries and needle leaves extracts. Antioxidants, 13(3), 345. https://doi.org/10.3390/antiox13030345
26. Ozgen, A., Aydin, S. G., & Bilgic, E. (2020). Enhancing the antibacterial activity of the biosynthesized silver nanoparticles by “puse”. Istanbul Journal of Pharmacy, 50(3), 245–251. https://doi.org/10.26650/IstanbulJPharm.2020.738922