



BIOPHYSICAL CHARACTERIZATION AND BIOLOGICAL APPLICATIONS OF SILVER NANOPARTICLES DERIVED FROM *FICUS CARICA*

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Abstract

Silver nanoparticles (AgNPs) have gained significant attention due to their unique physicochemical properties and diverse biological activities. In the study, exploration was conducted into the biophysical characterization and biological application of AgNPs derived from *Ficus carica*. The synthesized nanoparticles were characterized using various techniques, including UV-visible spectroscopy, scanning electron microscopy, Fourier-transform infrared spectroscopy, and X-ray diffraction. The biological activity of AgNPs derived from *F. carica* was assessed using different microbes. Their antimicrobial potential against bacteria and fungi was evaluated, along with cytotoxicity and antileishmanial activity. The results of this study contribute to the expanding knowledge based on the synthesis and application of silver nanoparticles, specifically those derived from *Ficus carica*, and highlight their potential for diverse biomedical applications.

Keywords: silver nanoparticles, *Ficus carica*, biophysical characterization, biological applications

1. INTRODUCTION

Nanotechnology has emerged as a promising field in the realm of materials science and biomedical research due to its potential for novel applications. Among various nanomaterials, AgNPs have gained significant attention due to their distinctive properties and diverse biological activities. AgNPs possess inherent antimicrobial, anti-inflammatory, and antifungal properties, making them highly sought after in various biomedical applications.

In recent years, there has been a growing interest in exploring natural sources for the synthesis of nanoparticles, owing to their eco-friendly and sustainable attributes. One such natural source is the *F. carica*, commonly known as the fig tree [1]. *F. carica* has an extensively studied plant species renowned for its medicinal properties and rich bioactive constituents. However, investigations into its potential for synthesizing silver nanoparticles and their subsequent applications remain limited [2, 3]. Various bioactive compounds such as steroidal saponins, flavonoids, tannins, glycosides, amide derivatives, terpenoids, phytosterols, amino acids and proteins have been isolated from *Ficus carica* leaves [4-6]. Among these steroidal saponins are the most abundant, while flavonoids are considered to be the most important bioactive agent [7]. *F. carica* leaves also contain a variety of secondary metabolites, including polyphenols such as proanthocyanins, act as reducing agents for the production of nanoparticles [8].

Nanoparticles have been found great interest to researchers due to their size-dependent properties, which enable them to be used in a wide range of applications such as electronics, biomedical, and environmental. One of the most popular approaches to synthesizing nanoparticles is the chemical method, however, this approach has many drawbacks such as the use of toxic chemicals and the production of large amounts of waste. In recent years, an alternative approach to synthesizing nanoparticles has emerged—green synthesis. Green synthesis utilizes natural materials as the source of materials and is a much more sustainable approach to synthesizing nanoparticles. In particular, the use of leaves of *F. carica* to synthesize silver and gold nanoparticles has attracted much attention due to its low cost and the potential of the nanoparticles produced to be used in a variety of applications. In this paper, the biophysical characterization and biological application of silver nanoparticles synthesized from leaves of *F. carica* were discussed [9].

***Ficus carica* leaves**

The leaves of *F. carica*, a member of the Moraceae family, have been used in traditional medicine for centuries. In recent years, the application of these leaves has been extended to the production of silver and gold nanoparticles. Silver and gold nanoparticles are of great interest due to their unique physical and chemical properties. Their small size, high surface area, and tunable optical and electrical properties make them suitable for a wide range of applications, including medical diagnostics, drug delivery, and photocatalysis. This review article will highlight the biophysical characterization and biological applications of silver and gold nanoparticles derived from *F. carica* leaves, as well as their potential for further development in the field of nanotechnology [10].

Medicinal importance

F. carica, also known as common fig, is a species of flowering plant in the family Moraceae. It is native to the Mediterranean region, western Asia and North Africa. *F. carica* has been used medicinally for centuries. It is believed to have anti-inflammatory, antioxidant, antifungal, antibacterial and antidiarrheal properties. *F. carica* has been used to treat a range of ailments, including digestive problems, skin diseases, respiratory infections, diabetes and even cancer. Studies have also shown that *F. carica* can reduce blood pressure, improve circulation and boost the immune system. Additionally, its high fiber content makes it a great food choice for those looking to regulate their blood sugar levels and manage their weight. *F. carica* is a highly important crop cultivated worldwide. Its fruit is consumed in both its fresh and dry forms, and is a reliable source of minerals and vitamins. In some old Mediterranean cultures, the sap of the fig plant was used to alleviate calluses, prevent parasites, and remove moles. The root and leaves of the fig tree are also used in traditional medicine to treat respiratory issues, gastrointestinal problems, and cardiovascular ailments. Moreover, figs are a great source of fiber and polyphenols, which are organic compounds with known antioxidant and anti-inflammatory properties. Prominently in many cultures around the world and are widely used due to their medicinal properties. Recently, silver nanoparticles derived from *F. carica* leaves have been explored for their potential applications in biomedicine. Studies have demonstrated that silver nanoparticles exhibit a wide range of biological activities, including antimicrobial and anticancer properties. This has led to the development of various novel strategies for their use in the diagnosis and treatment of various diseases [11]. Therefore, biophysical characterization and biological application of silver nanoparticles derived from *F. carica* leaves have gained immense interest in recent years. This research aims to discuss the biophysical characterization and biological application of silver nanoparticles derived from *F. carica* leaves [12]. Additionally, the synthesis strategies and their potential applications in drug delivery and tissue engineering are also discussed. The synthesis of AgNPs using plant extracts offers a green and cost-effective approach, eliminating the need for hazardous chemicals and energy-intensive processes. Furthermore, the biocompatible nature of plant-mediated AgNPs holds immense potential for various biomedical applications, including drug delivery systems, diagnostic tools, and wound healing agents [13].

The field of nanotechnology has witnessed significant advancements in recent years, offering exciting possibilities for various biomedical applications. AgNPs have demonstrated inherent antimicrobial, anti-inflammatory, and anticancer properties, making them highly promising candidates for a wide range of biomedical applications, including drug delivery systems, diagnostic tools, and wound healing agents. In the pursuit of eco-friendly and sustainable approaches to nanoparticle synthesis, researchers have increasingly turned to natural sources. *F. carica*, commonly known as the fig tree, has gained recognition for its medicinal properties and abundant bioactive constituents [14]. However, the potential of *F. carica* for synthesizing AgNPs and their subsequent biomedical applications remain relatively unexplored. This research article aims to investigate the biophysical characterization and biological activity of AgNPs derived from *F. carica*. The comprehensive analysis of the physicochemical properties of the synthesized AgNPs, such as size, shape, surface charge, and stability, will be conducted to gain insights into their structural and chemical attributes. Techniques including UV-visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and X-ray diffraction (XRD) will be employed to evaluate these properties [15]. In addition to characterization, the biological activity of AgNPs derived from *Ficus carica* will be conducted. The antimicrobial potential of the nanoparticles will be evaluated against a diverse range of pathogens, encompassing bacteria and fungi. Established microbiological techniques will be employed to determine the inhibitory effects of AgNPs. These investigations will shed light on the effectiveness of AgNPs in combating microbial growth [16,17].

Additionally, literature review shows, apoptosis assays, cell cycle analysis and migration/invasion assays will provide insights into the anticancer potential of these nanoparticles [18]. These studies hold the potential to uncover AgNPs derived from *F. carica* as a viable alternative therapy for malignant diseases. By comprehensively characterizing AgNPs derived from *F. carica* and evaluating their biological activities, this research article aims to contribute to the expanding knowledge base surrounding the synthesis and application of these silver nanoparticles [19, 20]. The findings will highlight the potential of AgNPs derived from *Ficus carica* in diverse biomedical fields, presenting opportunities for the development of innovative and sustainable nanotechnology-based solutions. Overall, this research article aims to contribute to the expanding knowledge base surrounding the synthesis and application of silver nanoparticles derived from *F. carica*. The biophysical characterization and biological evaluation of these nanoparticles hold tremendous promise for their utilization in various biomedical fields, paving the way for innovative and sustainable nanotechnology-based solutions [21-24].

2 MATERIAL METHODS

In this study, high-quality chemical reagents obtained from reputable suppliers such as Merck and Oxoid were utilized. Strict purification procedures were done in order to maintain the purity of the reagents. The following chemicals were employed: nutrient agar, nutrient broth, Muller Hinton, ethanol, HCl, ethyl acetate, methanol, and a salt solution of AgNO₃. These reagents were used for the cultivation and maintenance of bacterial and fungal cultures throughout the experimental process.

2.1 Plant Material Collection and Processing

In March 2020, leaves of *F. carica* were collected from different locations in Khyber Pakhtunkhwa Peshawar. To ensure cleanliness, the plant material was thoroughly washed with tap water to remove any sand and dust particles. Subsequently, it was rinsed with distilled water and air-dried under shade to retain the integrity of the plant components. Once completely dried, the plant parts were finely ground into a powder and stored in airtight containers to maintain their quality and prevent contamination from airborne factors [25].

2.2 Preparation of plant extracts

A predetermined quantity of dried and powdered plant material was accurately measured and transferred to a conical flask. Distilled water was added in a ratio of 1:10 (w/w) with respect to the dry mass of the plant material. The flask was then securely covered with aluminum foil. Subsequently,

the flasks were placed in a shaking incubator at 45°C for a duration of two hours. Following incubation, the filtrate was collected in conical flasks by passing it through Whatman Filter paper No.1. To maintain its integrity, the obtained filtrate was stored in amber bottles at a temperature of 4°C. Throughout the experimental process, high-quality analytical grade chemicals obtained from reputable suppliers such as Merck and Oxoid were used. The utilized chemicals included nutrient agar, nutrient broth, Muller Hinton, ethanol, HCl, ethyl acetate, methanol, and a salt solution of AgNO₃[26].

2.3 Silver nanoparticles synthesis

Silver nanoparticles were synthesized utilizing a bio reduction approach employing aqueous extracts derived from *F. carica*. To prepare a stock solution, 5 mg of powdered plant parts were dissolved in 100 ml of distilled water, resulting in a concentration of 0.5% (w/v). The solution was then subjected to filtration and centrifugation to obtain a clear supernatant. Subsequently, an aqueous solution of AgNO₃ with a concentration of 1 mM was prepared and mixed with the 0.5% plant extract at a temperature of 25°C. The successful synthesis was indicated by straw yellow color, which was further confirmed by UV-vis spectrophotometry at a wavelength of 430 nm. To ensure reproducibility and efficient synthesis of silver nanoparticles, various reaction parameters such as pH, temperature, and reaction time were meticulously optimized [27,28].

2.3 Nanoparticles Characterization

The synthesized silver nanoparticles underwent comprehensive characterization employing various microscopic and spectroscopic techniques. The optical properties of the nanoparticles were assessed within the wavelength range of 300-700 nm through Surface Plasmon Resonance (SPR) analysis using a dual-beam spectrophotometer. Ultraviolet-visible (UV-vis) spectra were obtained spanning 250-800 nm. Fourier Transform Infrared (FTIR) spectra were acquired utilizing IR Solution software, covering wavenumbers from 4000 to 400 cm⁻¹.

Furthermore, X-ray diffraction (XRD) analysis was conducted using a JDX-3532 JEOL (Japan) diffractometer, employing a glass plate immersed in a solution of silver nitrate. The XRD instrument parameters were appropriately configured to measure the diffraction intensity employing 20-40 kV and θ to 2 θ configurations. The scanning range spanned from 10° to 80°, with a scan rate of 0.5°/min and a step size of 0.02°.

To assess the crystallinity of the silver nanoparticles, the peak positions and widths obtained from the XRD data were meticulously analyzed. The crystal size of the nanoparticles was estimated using the Debye-Scherrer equation, which is expressed as $D = 0.94\lambda / \beta \cos\theta$. In this equation, D represents the crystal size, θ corresponds to the diffraction angle, λ denotes the X-ray wavelength, and β signifies the full width at half maximum (FWHM) [29]. Scanning electron microscopy (SEM) was conducted by coating a thin film of silver nanoparticles mixed with distilled water onto a copper grid for 120 seconds using a sputter coater (SPI, USA). The sample was then dried at a temperature of 50-60°C for 5 minutes, and SEM images were captured using a JEOL-JSM 5910 instrument [30,31].

2.4 Antibacterial bioassay

The antibacterial bioassay was conducted by culturing and maintaining test microorganisms on nutrient agar plates. The bacterial strain was inoculated into nutrient broth media and incubated at 37°C for 24 hours to facilitate growth. Autoclaved nutrient agar media was poured into petri plates and allowed to solidify. These plates underwent a 24-hour incubation at 37°C to ensure sterility [32,33].

For the macro dilution method, each tube was filled with nutrient broth (2-3 mL) and a colony of hvKp bacteria from a fresh 24-hour culture was added to each tube [34]. The mixture in each tube was then compared to a 0.5 McFarland standard to ensure consistency in bacterial cell density. Bacterial growth was monitored in each plate to assess the efficacy of the antibacterial agents [35-37].

2.5 Antifungal assay

A stock solution of the synthesized nanoparticles was prepared by dissolving them in analytical grade DMSO solvent at a concentration of 24 mg/ml. To evaluate the antifungal activity, 24 mg of the silver nitrate solution was added to 5 mL of SDA medium in a test tube, which was then placed at an inclined position at 25°C for 12 hours to allow the nanoparticles to diffuse into the medium. After 24 hours the SDA slants had solidified, a fresh culture of the fungal strain was streaked on the surface of the test tubes [38,39].

DMSO was used as the negative control, while Fluconazole served as the standard antifungal agent. The test tubes were subsequently incubated at room temperature for a week to allow the growth of the microorganism. After incubation, the inhibitory zone (in millimeters) formed around each test tube was measured to determine the effectiveness of the nanoparticles against the fungal strain [40]. The obtained results were then interpreted for further analysis and discussion.

2.6 Anti- Leishmanial activity

Leishmania tropica, a protozoan parasite responsible for causing leishmaniasis, was cultured in RPMI media supplemented with 10% Fetal Bovine Serum (FBS) at a temperature of 25°C. The parasite concentration was adjusted to 1×10^5 /mL using a hemocytometer [41,42]

To assess the anti-Leishmanial activity, stock solutions of synthesized nanoparticles were prepared in DMSO solvent at a concentration of 500 µg/mL. In sterile 96-well plates, varying concentrations of the nanoparticles (ranging from 1.125 to 250 µL) were added to each well along with 10% FBS. Subsequently, 67.6×10^5 /mL of viable *L. tropica* cells were seeded into each well. The plates were then placed in an incubator at 25°C for a duration of 72 hours [43]

Following the incubation period, the number of live and dead cells was microscopically counted using a hemocytometer under a high-resolution microscope. The experiment was conducted in triplicate, and the results were expressed as the percentage of mortality (% inhibition) based on the count of live and dead parasites [44,45]

3. RESULTS:

In this study, silver nanoparticles were produced by utilizing *F. carica* plant extracts as reducing agents. The physical and chemical characteristics of the synthesized nanoparticles were examined employing a range of biophysical techniques. The absorbance of the reaction mixture was measured at 430 nm, under different conditions of temperature (pH 7, 4 h), pH values (25°C, 4 h), and reaction times (25°C, pH 7), as shown in the top, middle, and bottom sections of the graph, respectively. Optimal conditions for nanoparticle synthesis were found to be at 75°C, pH 7, and a reaction time of 4 hours. Confirmation of nanoparticle synthesis was achieved by observing a distinct straw yellow colour at a wavelength of 430 nm using a UV-visible spectrophotometer. This colour was compared to the control group consisting of plant extract only, which exhibited a straw yellow colour with an absorbance spectrum below 300 nm.

Scanning electron microscopy (SEM) images of the synthesized silver nanoparticles revealed cylindrical and uniformly sized particles with a diameter of less than 100 nm.

FTIR spectroscopy was used to study the functional groups involved in bonding, stabilizing, and capping the synthesized silver nanoparticles. The spectrum of the synthesized nanoparticles showed characteristic peaks corresponding to different functional groups, indicating the successful synthesis of silver nanoparticles. FTIR spectroscopy provided information about the the functional groups of *Ficus carica* which were OH, alkanes, phenols and amide.

X-ray diffraction (XRD) analysis confirmed the crystalline structure of the synthesized silver nanoparticles. The XRD spectrum exhibited peaks corresponding to the standard Bragg reflections of silver metal, confirming the successful synthesis of silver nanoparticles with a face-centered cubic (FCC) crystalline structure.

The antibacterial bioassay showed significant inhibitory effects of the synthesized silver nanoparticles against Gram-negative bacteria, while no antibacterial activity was observed against Gram-positive

bacteria. The nanoparticles also demonstrated non-cytotoxicity and did not induce hemagglutination in blood cell types.

The antifungal activity of the silver nanoparticles was tested against various fungal pathogens. The nanoparticles exhibited significant antifungal activity against *Candida albicans*, moderate activity against *P. notatum* and *Aspergillus niger*, and the lowest activity against *A. parasiticus*. No antifungal activity was observed against *V. longisporum*. The anti-Leishmanial activity of the silver nanoparticles derived from *Ficus carica* was evaluated. The nanoparticles showed significant inhibitory effects against *Leishmania* parasites, indicating their potential as therapeutic agents for the treatment of Leishmaniasis

4. DISCUSSION:

The results of this study demonstrate the successful synthesis of silver nanoparticles using *F. carica* plant extracts as reducing agents. The presence of phytochemical components in the plant extracts played a vital role in reducing and stabilizing the silver ions during the synthesis process. The characterization results using various biophysical techniques confirmed the formation of silver nanoparticles with desired physical and chemical properties. The UV-visible spectrophotometer analysis showed the characteristic absorption peak at 430 nm, indicating the presence of silver nanoparticles. SEM images revealed the morphology and size of the nanoparticles, while FTIR analysis provided information about the functional groups involved in the synthesis and stabilization of the nanoparticles. By systematically monitoring bacterial growth on agar plates containing silver nanoparticles, researchers can evaluate the impact of the nanoparticles on inhibiting or killing bacteria. The effectiveness is often demonstrated by comparing the growth on plates with silver nanoparticles to that on control plates without them. The overall goal is to provide a comprehensive understanding of the antibacterial properties of the synthesized silver nanoparticles. If the antibacterial activity involves creating a zone of inhibition around the area with silver nanoparticles, measure and document the diameter of these clear zones. The absence of bacterial growth in this zone indicates the inhibitory effect of the silver nanoparticles. The agar well diffusion method gives a visual and quantitative evaluation of the antifungal activity of synthesized silver nanoparticles, and the percentage of antifungal activity provides a standardized measure for comparison. Properly labelling the experimental setup is essential for transparent reporting.

The detail of performance is; first agar plates are prepared; a standardized amount of fungal strain is inoculated onto the agar plates to create a uniform lawn of fungal growth. Wells are carefully made in the agar, A known concentration of the synthesized *F. carica* silver nanoparticles are added to the wells. then incubated. After incubation, zones of inhibition (clear areas around the wells) indicate the antifungal activity. The larger the zone, the more significant the antifungal effect. After incubation, zones of inhibition (clear areas around the wells) indicate the antifungal activity. The larger the zone, the more significant the antifungal effect. The diameter of each zone of inhibition is measured using a ruler or calipers. The percentage of antifungal activity can be calculated using the following formula:
$$\text{Percentage of Antifungal Activity} = \left(\frac{\text{Diameter of Control Zone} - \text{Diameter of Treatment Zone}}{\text{Diameter of Control Zone}} \right) \times 100$$

The antibacterial and antifungal assays demonstrated the potential of the synthesized silver nanoparticles as antimicrobial agents. The nanoparticles showed significant inhibitory effects against Gram-negative bacteria and exhibited antifungal activity against *Candida albicans*, suggesting their potential application in combating bacterial and fungal infections.

Furthermore, the silver nanoparticles displayed promising anti-Leishmanial activity, inhibiting the growth and viability of *Leishmania* parasites. These findings suggest that the nanoparticles could be explored as a therapeutic option for the treatment of Leishmaniasis.



Figure 1. *Ficus carica* leaves

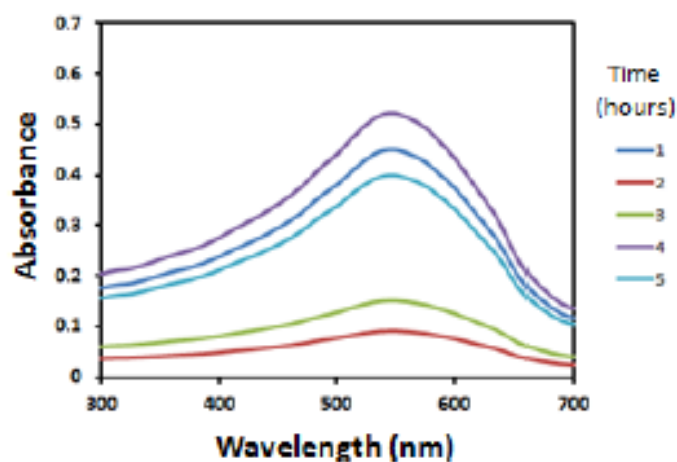


Figure 2: Optimization conditions for synthesized silver nanoparticles

Confirmation of nanoparticle synthesis was achieved by observing a distinct straw yellow colour at a wavelength of 430 nm using a UV-visible spectrophotometer. This colour was compared to the control group consisting of plant extract only, which exhibited a straw yellow colour with an absorbance spectrum below 300 nm

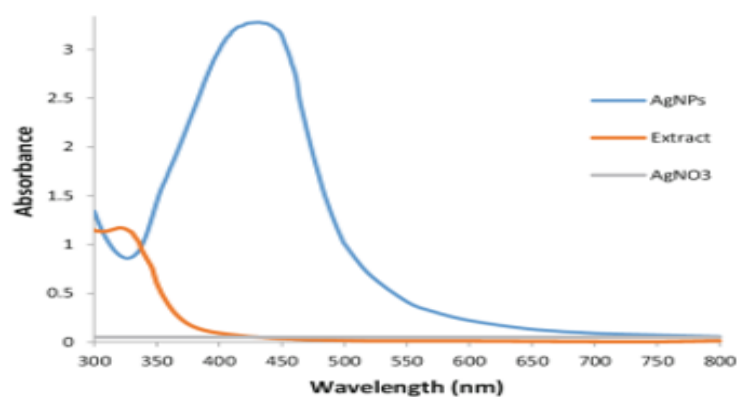


Figure 3: UV-vis spectrum of synthesized AgNPs derived from *Ficus carica*, plant extract and AgNO₃ solution

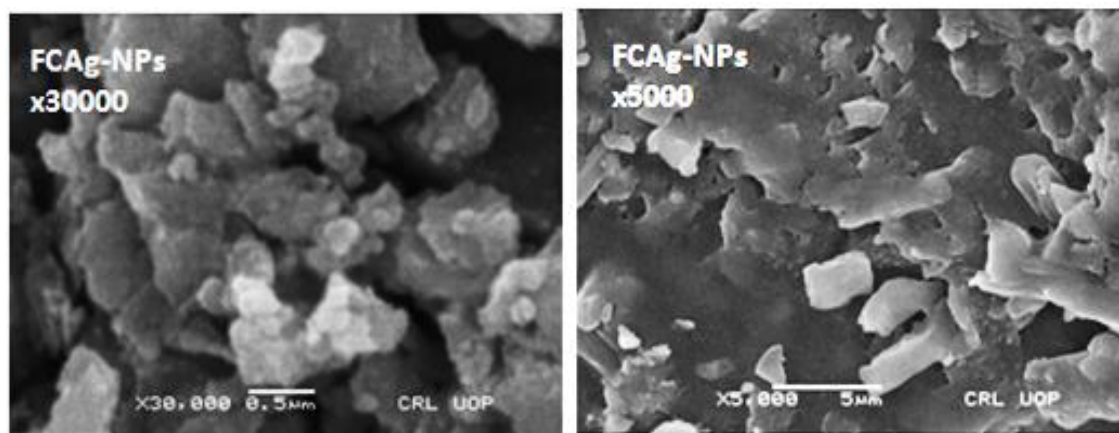


Figure 4: synthesized silver nanoparticles SEM images. At x5000 and x30000 magnification of *Ficus carica*

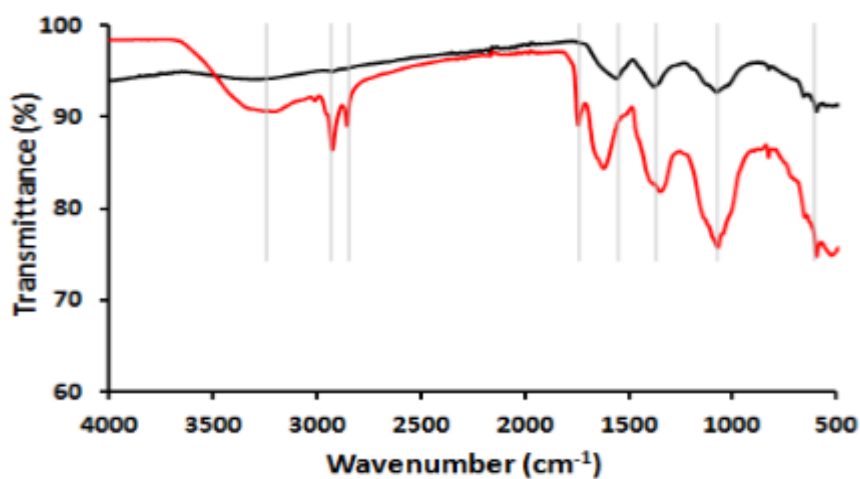


Figure 5: synthesized silver nanoparticles FTIR analysis. Spectra of synthesized AgNPs (red) *F. carica* plant extract (black)

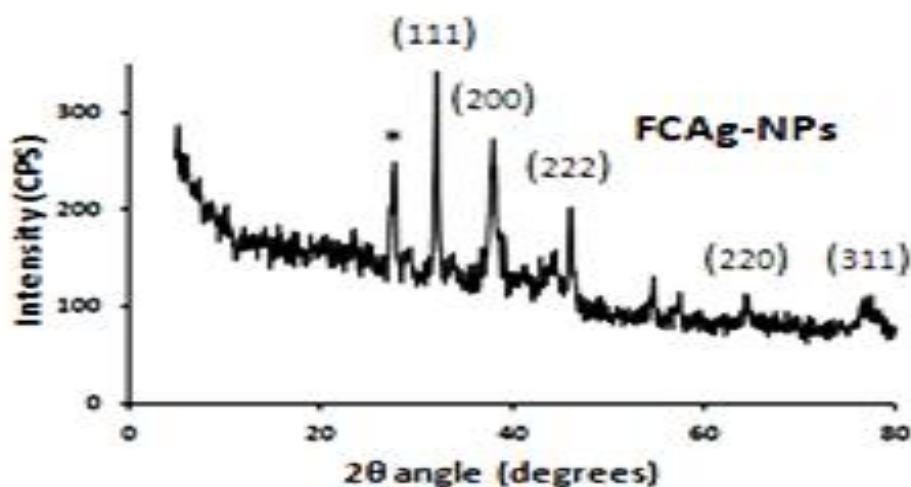


Figure 6. XRD spectra of synthesized *Ficus carica* silver nanoparticles

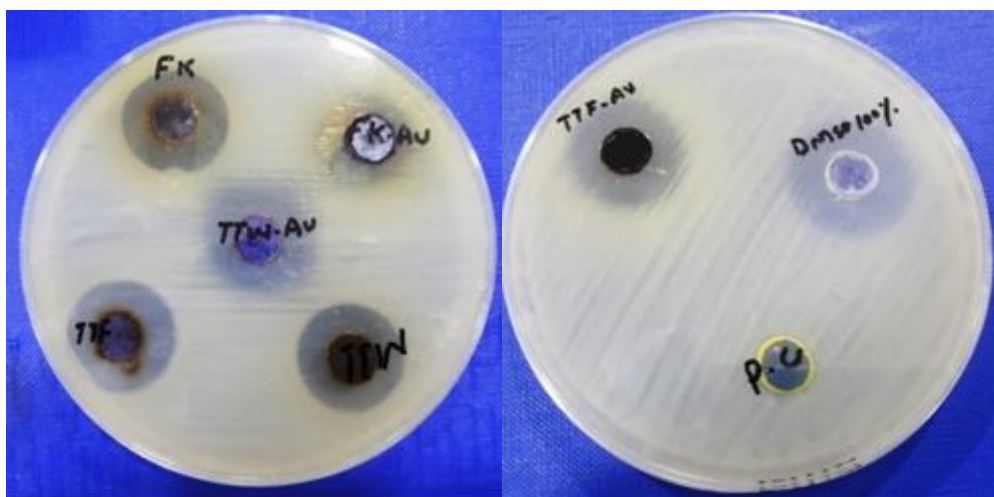


Figure 7. Antifungal activity of synthesized *Ficus carica* silver nanoparticles by agar well diffusion method.

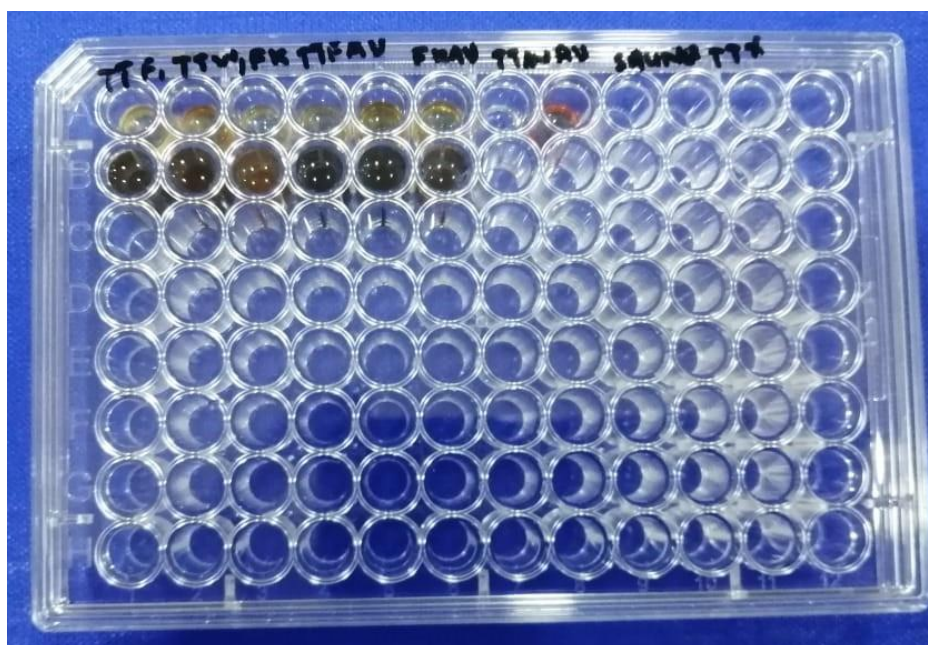


Figure 8. Antileishmanial test for synthesized silver nanoparticles derived from *F. carica* leaves and its plant extract.

Table 1: Antibacterial effects by broth micro dilution assay of the synthesized silver

Nanoparticles sample	<i>E. coli</i>	<i>E. faecalis</i>
FC Ag-NPs	Growth Inhibited	Growth Inhibited
Positive control	No growth occurred	Less growth
Negative control	Visible growth occurred	Visible growth occurred

Table 2: Antifungal activity of silver nano-particles

Fungi Name	linear growth Percent		
	+ive Control	-ive Control	Ag
<i>A.niger</i>	100	0	40
<i>candida albicans</i>	100	0	60
<i>A .parasiticus</i>	100	0	30
<i>P.notatum</i>	100	0	40
<i>V.longisporum</i>	100	0	0

Table 3: Anti-Leishmanial activity of Ag nanoparticles derived from *Ficus carica*

S. No	Samples	% inhibition of parasites at different concentration($\mu\text{g/mL}$)							
		1.25	2.25	5.62	22.5	25.51	62.5	125	250
1	FcAg-Nps	6.90	20	27.50	34.85	40.74	53.2	62.87	76.53
2	Standard	25	39.13	51.16	63.89	69.76	80.11	95.45	102

5. Conclusion and Future Prospects

Overall, this study successfully synthesized silver nanoparticles using *F. carica* plant extracts and demonstrated their physical, chemical, and biological properties. The results support the potential application of these nanoparticles in various fields, including medicine and biotechnology. Future research should focus on developing a new procedure to prepare *F. carica* derived silver nanoparticles with antibacterial properties. This involves monitoring reaction rates and nanoparticle synthesis success under varying conditions such as reactant concentrations, pH levels, and temperatures. The synthesized nanoparticles should be tested on a wider range of Gram-negative and Gram-positive bacteria to explore their antibacterial effects. It is worth investigating whether smaller nanoparticles, with a larger surface area, exhibit enhanced antibacterial activity. Additionally, their potential applications in medicine, medical implants, dentistry, cosmetics, biosensors, and agriculture should be explored. Furthermore, the optimized nanoparticles should be investigated for their antifungal, antioxidant, antiviral, anti-inflammatory, anticancer, and antidiabetic properties. Further research is needed to understand the underlying mechanisms of action and to optimize the condition used for the synthesis of silver nanoparticles and dosage of the nanoparticles for specific applications. In vivo studies are also required to evaluate the therapeutic effects of the nanoparticles.

Data Availability Statement:

The data such as the source file associated with this finding are available from the corresponding author upon request.

Conflicts of Interest:

The authors declare that they have no conflict of interest

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