EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 12
Issue: 3
Year: 2025

ANTIMYCOBACTERIAL POTENTIAL OF SILVER NANOPARTICLE AND FORMULATION OF EXTERNAL APPLICATION FOR CUTANEOUS TUBERCULOSIS FROM *FICUS HISPIDA*

Nikhila V.S.* and Dr. Sapna Shrikumar

Student M. Pharm, Department of Pharmacognosy, Nehru College of Pharmacy, Thrisssur, Kerala.
Principal, Ahalia School of Pharmcy, Palakkad, Kerala.

***Corresponding Author: Nikhila V. S.**
Student M. Pharm, Department of Pharmacognosy, Nehru College of Pharmacy, Thrisssur, Kerala.

Article Received on 09/01/2025 **Article Revised on 30/01/2025** **Article Published on 20/02/2025**

ABSTRACT
The green synthesis of silver nanoparticles utilizing various plant extracts has gained considerable attention in recent years due to its simplicity, affordability, and eco-friendliness. The present study investigates the anti-mycobacterial properties of green-synthesized silver nanoparticles (AgNPs) for the treatment of cutaneous tuberculosis. Also referred to as dermal tuberculosis or tuberculosis cutis (extrapulmonary tuberculosis) this condition can manifest in individuals of all ages, regardless of their pulmonary tuberculosis status. The existing treatment for the disease involves the administration of oral anti-tubercular medications, which are associated with numerous side effects, including hepatotoxicity, headache, anxiety, euphoria, insomnia, eosinophilia, and hepatitis. In order to reduce these side effects and improve the effectiveness of the existing therapy, a herbal topical gel has been formulated using the plant *Ficus hispida*. Furthermore, silver nanoparticles were synthesized from this biofraction, and both the biofraction and the silver nanoparticles demonstrated significant antimycobacterial activity. Overall, the findings suggest that the AgNP loaded antimycobacterial gel could offer a new therapeutic approach with potential anti-mycobacterial activity and can overcome the limitations of existing CTB therapies.

KEYWORDS: *Ficus hispida*, Cutaneous tuberculosis, Silver nanoparticle, Antimycobacterial activity, Antimycobacterial gel.

INTRODUCTION
The use of herbal medicines to treat a variety of illnesses is still expanding quickly on a global scale. Humanity's acceptance and interest in natural pharmaceuticals has significantlyincreased, both in developed and developing nations; apothecaries and food stores can stock these herbal remedies.[1] Phytochemicals and their derivations present in the shops are able to enhance remedial efficacy in cases with colorful affections.[2] Nanotechnology refers to the exploration of materials and systems that exhibit functional organization at the nanoscale, defined as one billionth of a meter in at least one dimension. This interdisciplinary field integrates principles from both science and engineering.[3] To enhance patient adherence and minimize the frequency of administration, a scientific approach is necessary for the sustained delivery of phototherapeutics.[4] Due to their unique physical and chemical properties, Silver nanoparticles (AgNPs) are increasingly being applied in diverse sectors, including consumer goods, industrial applications, food safety, medical practices, and healthcare.[5] The foundational concepts of environmentally friendly chemistry advocate for the biogenic synthesis of silver nanoparticles to enhance their stability.[6]

Tuberculosis (TB) continues to pose a huge worldwide health concern, contributing to large morbidity and mortality rates. It is estimated that 2–3 billion people, or about one-third of the world's population, are infected with Mycobacterium tuberculosis (M. tuberculosis), and that 5–15% of these people will eventually get ill with active TB disease. It is important the therapy for TB normally entails a course of medicines, and without prompt intervention, the disease can be fatal ant to note that those who are infected but asymptomatic do not pose a risk of transmission.[7] CTB, or cutaneous tuberculosis, is mainly due to Mycobacterium tuberculosis, an infectious disease. Although it can also be attributed to other mycobacterial species such as *Mycobacterium bovis* and, on rare occasions, the Calmette-Guerin bacillus. This infection predominantly affects the pulmonary system; however, when it manifests in the skin, it is referred to as cutaneous tuberculosis. This form

of the disease is considered uncommon.[8] Compared to pulmonary tuberculosis, cutaneous tuberculosis is relatively rare, occurring in approximately 1% to 2% of patients with extra pulmonary tuberculosis.[9] Its incidence is notably higher in areas with a high prevalence of HIV or among individuals with compromised immune systems. The treatmentregimen for cutaneous tuberculosis mirrors that of systemic tuberculosis, typically involving multidrug therapy. Commonly prescribed medications include streptomycin or ethambutol, pyrazinamide, rifampicin, and isoniazid.[10] Recent advancements in herbal drug technology have incorporated nanotechnology, with studies indicating that silver nanoparticles synthesized from *Ficus hispida* leaves exhibit significant antimicrobial and antioxidant properties.[11] The study started with the pharmacognosticla and phytochemical screening, isolation of the active constituents from the plant *Ficus hispida*.[12][13][14] So the present study investigated the Antimycobacterial potential of synthesized silver nanoparticle from *Ficus hispida* leaves and tried out an idea herbal based external application for Cutaneous Tuberculosis.

MATERIALS AND METHODS

Synthesis of silver nanoparticles

In the process of synthesizing silver nanoparticles, 10ml of biofraction from *Ficus hispida* Linn. were introduced into 100 mL of a 4 mM AgNO3 solution contained within a 250 mL Erlenmeyer flask. The mixture was stirred in a water bath for 60 minutes at a temperature of 90 °C. The pH was subsequently adjusted to 9 using 0.1 N NaOH and 0.1 N H3PO4. The transition of the reaction mixture to a reddish-brown hue indicated the successful formation of silver nanoparticles. This procedure was replicated with variations in the concentrations of AgNO3, the volume of plant extract, pH, and temperature.[15]

Characterization of the Prepared Silver Nanoparticle[16]

Characterizing silver nanoparticles synthesized from Ficus hispida is essential for understanding their functional characteristics. This process involves various analytical techniques, including Scanning Electron Microscopy (SEM), particle size assessment, zeta potential analysis for stability, and drug entrapment efficiency evaluation for elemental analysis.

Visual Examination

The primary method for confirming the synthesis of Ficus hispida silver nanoparticlesis through visual inspection. The alteration in color of the reaction mixture, which includes silver nitrate solution and biofraction, is observed over time.

Determination of Entrapment Efficiency

The entrapment efficiency of the nanoparticles is assessed by introducing 10 ml of phosphatebuffer at pH 7.4, followed by sonication in a bath sonicator and subsequent filtration. A 1 mlaliquot of the filtrate is then diluted to 10 ml with phosphate buffer and analyzed using a UV-visible spectrophotometer at 375 nm, with the phosphate buffer serving as a blank. The drugentrapment percentage is calculated using the formula:

$$\text{Drug entrapment (\%)} = (\text{Entrapped drug}/\text{Total drug}) \times 100.$$

Zeta Potential

The zeta potential, which indicates the overall charge of the silver nanoparticle in emulsions, can be negative, positive, or neutral, depending on the silver nanoparticle composition. This measurement is indicative of the stability of the silver nanoparticle in a given medium and is determined using a zeta analyzer. The procedure involves diluting 1 ml of the sample to 10 mlwith water, transferring 5 ml of this diluted sample to a cuvette, and measuring the zeta potential.

Particle Size

The particle size of the silver nanoparticle is quantified using a particle size analyser.

In vitro antimycobacterial activity[17,18,19]

Procedure for Microplate Alamar Blue Assay (MABA)

The Microplate Alamar Blue Assay was performed in a sterile environment. A 96- well plate was utilized for the experiment, with 100 µL of the test substance, either in 10% (v/v) DMSO or sterile water (typically at a stock concentration of 10 mg/mL), added to the first row. Then, 50 µL of normal saline was dispensed into each of the remaining wells. Serial dilutions were carried out by using a multichannel pipette, ensuring that each well contained 50 µL of the test substance at reduced concentrations. Thereafter, 10 µL of resazurin indicator solution was added into each well. To get a uniform final volume of nutrient broth, 30 µL of 3.3× strength isosensitised broth was added to the each well, followed by the addition of 10 µL of bacterial suspension. The plates were then loosely covered with a cling film to mitigate dehydration of the bacterial cultures. Control columns were developped, including one containing three antitubercular agents (Isoniazid, Pyrazinamide, and Streptomycin) as positive controls, another column with all solutions except the test compound, and a third column with all solutions except the bacterial suspension, which received 10 µL of nutrient broth instead. The plates were incubated at 37°C for 24 hours. A control well was treated with AlamarBlue dye and monitored for the emergence of pink coloration. If pink coloration was detected in the control well, the test samples and the standard drugs were subsequently applied to the experimental wells at varying concentrations (0.8, 1.6, 3.12, 6.25, 12.5,25,50,100 µg/ml). The plates were incubated for an additional 24 hours, after which Alamar Blue dye was added to all experimental wells, followed by further incubation to enhance the development of pink coloration. Results

were evaluated through visual inspection and absorbance measurements at 600 nm.

Formulation of silver nanoparticle loaded antimycobacterial gel[20]

Table 1: Ingredients for the formulation of silver nanoparticle loaded Anti-Mycobacterial Gel.

Ingredient	AgNP1	AgNP2	AgNP3
Silver nanoparticle	0.156mg	0.32mg	0.60mg
Carbopol 940	0.5g	0.5g	0.05g
Propylene Glycol 400	1ml	1ml	1ml
Triethanolamine (TEA)	0.1ml	0.1ml	0.1ml
Distilled Water	25ml	25ml	25ml

Dispersion method

Initially, Carbopol 940 was dispersed in 25 ml of distilled water, with constant stirring, and allowed to rest for half an hour to promote the swelling of the Carbopol. Afterward, silver nanoparticles were dispersed in Propylene Glycol 400 and mixed into the gel base. To fine-tune the skin pH and attain the desired gel consistency, Triethanolamine was addeddropwise to the mixture..

Evaluation of Silver nanoparticle Loaded Anti-Inflammatory Gel

The evaluation of the herbal formulation's quality is based on several physicochemical parameters, including color, odor, irritancypreliminary assessment of the formulation wasconducted as follows:

a) Physical Evaluation

Visual examination was employed to assess physical parameters such as color and overallappearance.

b) pH Measurement

A digital pH meter was utilized to measure the gel's pH, which should ideally be close to the normal pH of the skin to minimize irritation.

c) Homogeneity

The homogeneity of the developed gel was visually inspected after it was set in its container, focusing on its appearance. Studies on Green Synthesized Silver Nanoparticle for Antimycobacterial Activity from Ficus Hispida. Linn Leaves and Its Formulation.

d) Grittiness

A microscopic analysis was performed to detect any particulate substances present within the formulation.

e) Viscosity

The viscosity of the gel was assessed utilizing a Brookfield viscometer equipped with spindle number 64. The gel was subjected to rotation at varying RPMs, and the corresponding dial readings were meticulously documented.

f) Stability Study

Stability assessments were carried out in accordance with the guidelines established by the International Council for Harmonisation (ICH). The formulated gel was maintained at ambient temperature (25ºC) and systematically evaluated for its appearance, pH, viscosity, and spreadability at specified intervals (0, 15, and 30 days) to determine its stability.

g) Spreadability

This parameter measures the extent to which the gel can be spread upon application to the skin or the affected area. Two glass slides, each measuring 20 cm × 20 cm, were utilized, with a small quantity of the sample placed between them. A weight of 100 g was applied tothe upper slide to ensure the gel was evenly pressed into a thin layer. Following the removalof the weight, the upper slide was secured to a stand, allowing it to slide off freely. The duration required for the upper slide to detach from the lower slide was recorded using a stopwatch. The spreadability was then calculated using the formula:

$$S = M.L/T$$

Where S represents spreadability, M denotes the weight applied to the upper slide, L indicates the distance moved by the glass slide, and T signifies the time (in seconds) taken for the slides to completely separate.

RESULT AND DISCUSSION

Characterization of silver nanoparticle

Fig 1: Aqeous AgNO3 solution + Ficus hispida biofraction for synthesis of AgNPs before heating.

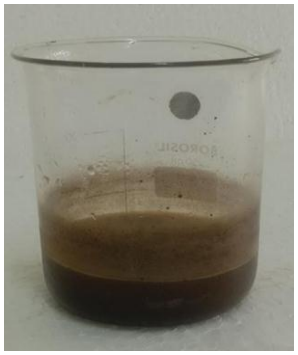

Fig 2: AgNPs formed after heating 60min.

Entrapment efficiency (%) by centrifugation

Table 2: Entrapment efficiency of synthesized silver nanoparticles.

Sl.No.	Formulationcode	Ratio of drug: AgNo3solution	Entrapment efficiency (%)
1	AgNP1	1:1	81.26%
2	AgNP2	1:2	84.38%
3	AgNP3	1:3	91.25%

Particle size

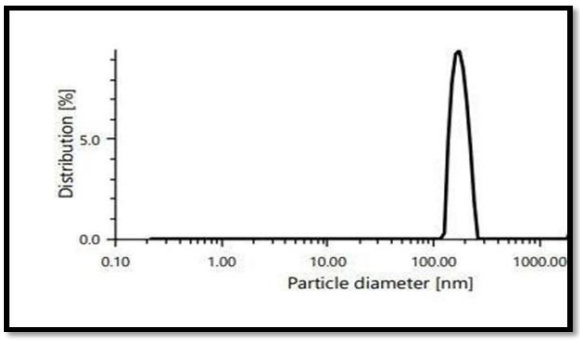

Fig. 3: particle size graph of prepared Silver nanoparticle.

The AgNPs exhibited relatively narrow particle size distribution. The particle size of the optimized silver nanoparticle was found to be 160 nm. This was in accordance with the particle size range of silvernanopaticles.

Zeta potential (ZP)

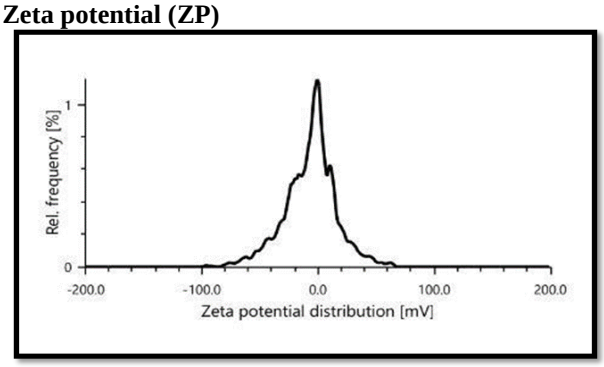

Fig. 4: Zeta potential graph of prepared Silver nanoparticle.

Zeta potential of the optimized silver nanoparticle was found to be -15.2 mv. This indicates that the sample is highly stable and do not form aggregates.

***IN VITRO* ANTIMYCOBACTERIAL ACTIVITY BY MABA METHOD**

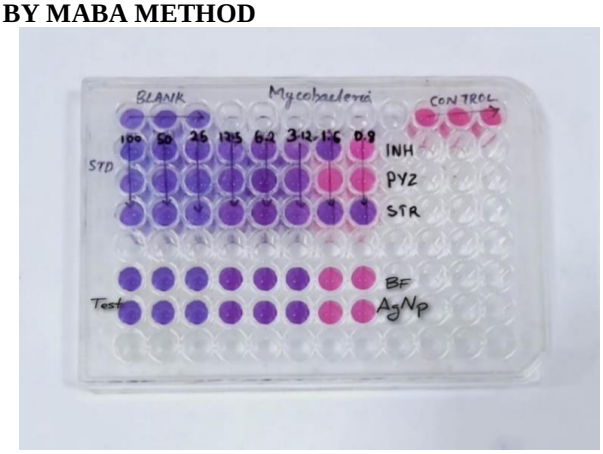

Fig. 5: MABA Assay of standard drugs and test samples.

FORMULATION OF SILVER NANOPARTICLE LOADED ANTIMYCOBACTERIAL GEL

Figure 6: Silver nanoparticle loaded 3 gel formulations.

Evaluation of Silver nanoparticle loaded Antimycobacterial Gel

Table 3: Evaluation of three (F1, F2, F3) Antimycobacterial Gel formulation.

Parameters	Observations		
	F1	F2	F3
Color	Light brown	Brown	Dark brown
Appearance	Smooth, homogenous	Smooth, homogenous	Smooth homogenous
Homogeneity	Good	Good	Good
Grittiness	Free from foreign particles andgrittiness	Free from foreign particles andgrittiness	Free from foreign particles andgrittiness
pH	6.98	6.58	7.02
Viscosity	2548 cp	2345 cp	2253 cp
Spreadability	8.89 gcm/sec	7.68 gcm/sec	6.52 gcm/sec

The silver nanoparticle loaded anti-mycobacterial gel was evaluated for colour, appearance, homogeneity, grittiness, pH, spreadability and Viscosity. The F2 gel formulation was found to be good in all above desirable properties.

Stability studies of silver nanoparticle loaded Antimycobacterial gel

Table 4: Stability studies of three (F1, F2, F3) Antimycobacterial Gel formulation.

Parameter	Monitoring	Observation		
		F1	F2	F3
Appearance	0th Day	Light brown, smooth, homogenous	Brown, smooth, homogenous	Dark brown, smooth, homogenous
	15th Day	Light brown, smooth, homogenous	Brown, smooth, homogenous	Dark brown, smooth, homogenous
	30th Day	Light brown, smooth, homogenous	Brown, smooth, homogenous	Dark brown, smooth, homogenous
pH	0th Day	6.98	6.58	7.02
	15th Day	6.98	6.58	7.02
	30th Day	6.98	6.58	7.02
Viscosity	0th Day	2548 cP	2345 cP	2253 cP
	15th Day	2548 cP	2345 cP	2253 cP
	30th Day	2548 cP	2345 cP	2253 cP

The stability studies of the Silver nanoparticle loaded anti-mycobacterial gel wasperformed and it was found to be stable.

As the concentration of the sample increases, F3 shows better, however, F2 is chosen as the preferred option because it serves as the intermediate formulation.

CONCLUSION

The oral administration of anti-tubercular medications for the treatment of cutaneous tuberculosis faces challenges due to the insufficient amount of the drug reaching the peripheralsite. The lack of existing literature on herbal topical therapies for this condition highlights the urgent need for the creation of a plant-based topical formulation aimed at enhancing the efficacy of current treatment protocols. This research introduces an alternative therapeutic approach for cutaneous tuberculosis, utilizing the evergreen plant *Ficus hispida*, which is prevalent globally, in conjunction with silver nanotechnology. The extract from this plant has been identified to possess a substantial quantity of terpenoids and flavonoids, both of which have been previously documented to exhibit anti-mycobacterial properties. In light of absence of herbal formulations for cutaneous tuberculosis, a gel formulation incorporating silver nanoparticles has been developed. This gel is designed to mitigate the side effects associated with oral therapies while also providing enhanced skin penetration and exhibiting greater therapeutic efficacy.

REFERENCES

1. Ramesh S. Chaughule• Rajesh S. Barve. Role of herbal medicines in the treatment of infectious diseases. Vegetos, 2024; 37: 41–51. https://doi.org/10.1007/s42535-022- 00549-2.
2. Mohammad Ali, Shahid Ud Din Wani, Md Salahuddin, Manjula S.N, Mruthunjaya K, Tathagata Dey, Mohammed Iqbal Zargar et.al., Recent advance of herbal medicines in cancer- a molecular approach
3. Rajiv Saini, Santosh Saini and Sugandha Sharma. Nanotechnology: The Future Medicine. J Cutan Aesthet Surg, Jan-Apr., 2010; 3(1): 32–33.
4. 26. S. H. Ansari, Farha Islam, and Mohd. Sameem. Influence of nanotechnology on herbal drugs: A Review. Journal of Advanced Pharmaceutical Technology & Research, Jul-Sep. 2012; 3(3): 142–146.
5. Xi-Feng Zhang, Zhi-Guo Liu, Wei Shen, and Sangiliyandi Gurunathan. Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. International journal of molecular sciences, Sep. 2016; 17(9): 1534.
6. Mohanpuria, P., Rana, N. K. & Yadav, S. K. Biosynthesis of nanoparticles: technologicalconcepts and future applications. *J Nanopart Res.*, 2008; **10:** 507–517.
7. Dye C, Floyd K. Tuberculosis. In: Jamison DT, Breman JG, Measham AR, et al. editors. Disease Control Priorities in Developing Countries, 2nd ed. New York: Oxford University Press, 2006.
8. Ahmad Charifa; Rupinder Mangat; Amanda M. Oakley. Cutaneous Tuberculosis. August 14, 2023. Anais brasileiros de dermatologia, Mar-Apr., 2014; 89(2): 219–229.
9. Michael K. Hill[1], Charles V. Sanders. Cutaneous Tuberculosis. Microbiology spectrum, 27 February 2017; 5(1): 10.1128.
10. Arival Cardoso de Brito, Clivia Maria Moraes de Oliveira, Deborah Aben-Athar Unger, Maraya de Jesus Semblano Bittencourt. Cutaneous tuberculosis: epidemiological, clinical, diagnostic and therapeutic update. Anais Brasileiros de Dermatologia, March–April, 2022; 97(2): 129-144. http://creativecommons.org/licenses/by/4.0.
11. AV Ramesha, Dharmasoth Rama Devi, GangaRao Battub, K Basavaiaha, A Facile plant mediated synthesis of silver nanoparticles using an aqueous leaf extract of Ficus hispida Linn. For catalytic,

antioxidant and antibacterial applications, *South African Journal of Chemical Engineering*, December 2018; 26: 25-34.
12. Sapna Shrikumar, Nikhila V S, Archana S, Akshaya Unni K C. The pharmacognostic studies, phytochemical screening and tlc studies of the leaves of ficushispida.linn. Novyi mir research journal, 2024; 9(10): 23-35.
13. Nikhila V S, Dr. Sapna Shrikumar, Archana S, Akshaya Unni K C, Isolation Of Terpenoidal Biofraction And Ftir Interpretation Of Ficus Hispida Leaves**,** novyi mir research journal, 2024; 9(10): 135-143.
14. Dr. Sapna Shrikumar, Nikhila V S, Dr. Akhila S., Sangeetha Sukumaran, Shani Dominic, Amina Ali. A review on advance herbal research and new opportunities of ficus hispida.linn, African Journal of BiologicalScience, Sep 2024; 6(14): 12618-12630.
15. AV Ramesha, Dharmasoth Rama Devi, GangaRao Battub , K Basavaiaha, A Facile plant mediated synthesis of silver nanoparticles using an aqueous leaf extract of Ficus hispida Linn. For catalytic, antioxidant and antibacterial applications, South African Journal of Chemical Engineering, December 2018; 26: 25-34.
16. Vadapally Pranitha, Gudikandula Krishna and Maringanti Alha Singara charya (2017), Silver Nanoparticles Biosynthesized Using Ficus hispida Aqueous Extract And Their Antibacterial Studies, International Journal of Pharmaceutical Science Invention, July 2017; 6(7): 44-47.
17. Ankita Panda, Ujjwal Manna, Deokrishna Kumar Choudhary. Evaluating the Antimicrobial Potential of Silver Nanoparticles against Two Prominent Mycobacterium.
18. Tuberculosis Strains. International Journal of Research Publication and Reviews, September 2023; 4(9): 173-178.
19. Rao Monica, Kadam Manali, Rao Shivani. Formulation and Evaluation of Topical Formulation for Cutaneous Tuberculosis. Journal of Drug Delivery & Therapeutics, 2018; 8(4): 102-116.
20. Carla Rosero, Lucy Baldeón, Valeria Alulema, Adriana Rueda, Lorena Vaca, Daniela Galarza, Cesar Procel. Cutaneous tuberculosis, different clinical spectrum of the same disease: the importance of pre-test probability. Dermatology Reports, 2024; 16: 9770.