



***Trigonella foenum-graecum*-BASED GREEN SYNTHESIS OF NANO-PARTICLES: PRODUCTION, CHARACTERIZATION AND APPLICATIONS**

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ABSTRACT

Nanoparticles (1-100 nm) are increasingly explored for their environmental and biomedical applications. This study reports the green synthesis of silver nanoparticles (AgNPs) using *Trigonella foenum-graecum* (fenugreek) seed extract, which serves as both reducing and capping agent. Rich in flavonoids, saponins, and polyphenols, the phytochemicals in fenugreek facilitated efficient AgNP formation and long-term stabilization. The synthesis involved the mixing aqueous seed extract with silver nitrate, leading to a colour change due to surface plasmon resonance, with a characteristic absorption peak at 420 nm. FTIR analysis confirmed the presence of functional groups (C=C, C≡C, O-H/N-H) responsible for reduction and capping. The synthesized AgNPs showed strong antimicrobial activity against *E. coli* (19 nm), and *S. aureus* (17 nm), with minimum inhibitory concentrations ranging from 25–50 µg mL⁻¹. Antioxidant activity assessed via DPPH assay yielded an IC₅₀ of 46 µg mL⁻¹, indicating potent radical scavenging ability. The nanoparticles demonstrated high yield (>85%), stability over 30 days, and no agglomeration - an improvement over many existing green synthesis methods. The AgNPs are proposed to act through membrane disruption, ROS generation, and interference with microbial DNA and protein synthesis. Preparation of varying concentrations (100, 75, 50, and 25%) enabled detailed bioactivity profiling. This study highlighted the multifunctionality and sustainability of fenugreek-mediated AgNPs, emphasizing their promise as next-generation antimicrobial agents and a significant advancement in green nanotechnology.

Keywords: Green nanotechnology, phytochemical-mediated synthesis, silver nanoparticles, sustainable nanomaterials, *Trigonella foenum-graecum*)

INTRODUCTION

Nanotechnology, the science of manipulating the matter at atomic and molecular scale, has revolutionized diverse sectors including medicine, agriculture, and environmental science (Khan *et al.*, 2021). Nanoparticles, typically ranging from 1-100 nm in size, exhibit unique physical, chemical, and biological characteristics that render them indispensable in emerging technologies (Singh and Jain, 2020). Among the various approaches, green nanotechnology has emerged as a sustainable strategy, employing eco-friendly methods for synthesizing nanoparticles to reduce environmental impact (Ahmed *et al.*, 2019). Green-synthesized nanoparticles are widely applied in pollution control, antimicrobial coatings, drug delivery, and environmental remediation. In particular, phytochemical-mediated synthesis of nanoparticles has gained traction due to its elimination of toxic reagents and its

ability to yield stable, biologically active nanoparticles (Li *et al.*, 2020).

Plants are a rich source of secondary metabolites such as flavonoids, terpenoids, and phenolics, which serve dual roles as reducing and stabilizing agents (Sharma *et al.*, 2021). This method enhances the eco-sustainability and functional versatility of nanoparticles while aligning with global green chemistry principles. In present study, *Trigonella foenum-graecum* (fenugreek) was specifically chosen due to its rich phytochemical profile, including flavonoids, saponins, and polyphenols. These compounds not only facilitate the reduction of metal ions but also act as effective capping agents, providing enhanced nanoparticle stability and biocompatibility (Patel and Goyal, 2020). Importantly, fenugreek is also known for its intrinsic antibacterial, antioxidant, and anti-inflammatory properties (Ramesh *et al.*, 2022), which may further amplify the bioactivity of synthesized silver nanoparticles (AgNPs). Compared to other plant-based sources, fenugreek offers a synergistic advantage due to its combination of bio-reductive and therapeutic properties. This dual functionality is expected to result in superior antimicrobial efficacy and oxidative stress modulation in AgNPs, setting it apart from nanoparticles synthesized using other botanical extracts.

Despite the wide exploration of plant-mediated AgNP synthesis, the specific role of *T. foenum-graecum* phytochemicals in enhancing antimicrobial and antioxidant properties remains poorly studied. While several reports exist on green synthesis using different plants, there is a significant gap in understanding how fenugreek-derived nanoparticles perform differently or better in terms of bioactivity and stability. Addressing this gap could provide insights into plant-nanoparticle interactions and contribute to the development of more effective biomedical and environmental nanomaterials. Therefore, the present study was aimed to synthesize silver nanoparticles using *T. foenum-graecum* seed extract and evaluate their physicochemical properties, stability, and biological activity. This work not only introduces a novel, underexplored plant-based route for AgNP synthesis but also contributes to advancing green nanotechnology by demonstrating the multifunctionality and long-term stability of fenugreek-mediated nanoparticles.

MATERIALS AND METHODS

Preparation of fenugreek seed extract

Fenugreek (*T. foenum-graecum*) seeds were procured from a local market, thoroughly washed with distilled water before drying in hot-air oven at 45°C until moisture-free. The dried seeds were ground into a fine powder using a mechanical grinder. For aqueous extraction, 10 g seed powder was boiled with 100 mL distilled water at 65°C for 30 min under continuous magnetic stirring. The solution was allowed to cool to room temperature and filtered through Whatman No. 1 filter paper. The clear filtrate was collected and stored at 4°C in amber bottles to prevent photo-degradation till experimental use (Kaviya *et al.*, 2011). Extract was prepared in three independent batches (biological replicates), each using fresh seeds under identical extraction conditions to ensure reproducibility for subsequent assays.

Synthesis of silver nanoparticles

AgNO₃ solution (1 mM) was prepared by dissolving 0.017 g AgNO₃ in 100 mL distilled water. For nanoparticle synthesis, 10 mL fenugreek seed extract was added dropwise to 90 mL AgNO₃ solution in a 250 mL Erlenmeyer flask under constant stirring at room temperature. A gradual colour change from pale yellow to reddish-brown was observed within 30 min, indicating the formation of AgNPs due to surface plasmon resonance. The colloidal solution was stored in dark at 4°C until further use (Song and Kim, 2009). The synthesis was carried out in three independent batches using freshly prepared *T. foenum-graecum* seed extract and subsequently characterized prior to use in bioassays.

Fourier-transform infrared spectroscopy (FTIR) analysis of green synthesized nanoparticles

FTIR spectra were recorded using a 'Perkin Elmer Spectrum Two' FTIR spectrophotometer to analyse the functional groups responsible for nanoparticle synthesis and stabilization of green synthesized

silver nanoparticles of *T. foenum-graecum*. Dried samples of both aqueous fenugreek extract (lyophilized powder) and the synthesized AgNP's were finely ground, mixed with spectroscopic grade KBr in 1:100 ratio and compressed into transparent pellets. Spectra were acquired in the range of 4000–400 cm^{-1} , with a resolution of 16 cm^{-1} using the Happ–Genzel Apodization function. Each spectrum was obtained by averaging 32 sample scans against 32 background scans under optimal instrument status.

Phytochemical screening

Phytochemical analysis of fenugreek extract was performed by using standard qualitative assays to screen major classes of secondary metabolites (Singh *et al.*, 2014). Flavonoids were assessed by alkaline reagent test, wherein the appearance of yellow colouration and subsequent changes upon the addition of dilute acid was noted. Steroids were evaluated using Liebermann–Burchard's test, involving the development of distinct colour layers and fluorescence under specific conditions. Glycosides were tested using Keller–Killani method, in which a characteristic colour change was observed at the interphase. Terpenoids were examined through Salkowski's test, which included the development of a specific coloration upon reaction. Tannins and phenols were screened using the ferric chloride test, involving the appearance of a dark-coloured complex. Saponins were identified using the foam test by monitoring the formation and persistence of foam, while alkaloids were analysed using Wagner's test, marked by the formation of a precipitate. All observations were systematically recorded for subsequent interpretation. The presence of various phytochemicals in plant extract were confirmed using standard qualitative tests (Chandra and Sharma, 2013; Ravindran *et al.*, 2018).

Thin layer chromatography (TLC)

TLC was conducted on silica gel 60 F₂₅₄ plates (5 × 10 cm) to separate phytochemicals from the aqueous extract of fenugreek. A 2 mg mL⁻¹ aqueous extract of fenugreek was applied to the plate alongside standard compounds such as quercetin and saponin. Two mobile phases were employed based on target phytochemical groups: chloroform: methanol (9:1) for flavonoids and hexane: acetone (7:3) for saponins. The TLC chamber was pre-saturated with vapours of the respective mobile phase for 20 min to ensure proper development conditions. Plates were allowed to develop until the solvent front migrated approximately three-fourths of the total plate length. Post-development, visualization was performed under UV light at 254 and 366 nm, while non-fluorescent spots were detected using vanillin–sulphuric acid reagent followed by gentle heating.

$$R_f = \frac{\text{Distance travelled by the unknown compound}}{\text{Distance travelled by solvent front}}$$

KBr in a 1:100 ratio, and compressed into transparent pellets.

Antimicrobial activity assay

The bacteria for antimicrobial assay were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh (India). Of the five bacteria used in the study, two bacteria [*Escherichia coli* (MTCC No. 40) and *Klebsiella pneumoniae* (MTCC No. 109)] were Gram-negative, while rest *Bacillus cereus* (MTCC No. 430), *Staphylococcus aureus* (MTCC No. 737), and *Streptococcus pyogenes* (MTCC No. 1924) were Gram-positive. All bacterial strains used were maintained at 4°C on nutrient agar medium until further use.

For preparation of bacterial suspension, pure colonies of selected bacteria were aseptically inoculated into fresh nutrient broth and incubated at 37°C for 24 h. The cultures were maintained in sterile 250 mL flasks for subsequent experimental procedures. The antimicrobial activity of synthesized AgNPs was evaluated using agar well diffusion method on Mueller-Hinton agar against test organisms *viz.*, *Escherichia coli*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, and *Staphylococcus aureus*. Ciprofloxacin served as positive control for bacteria while distilled water was used as negative control. Microbial cultures were standardized to 0.5 McFarland

turbidity ($\sim 1.5 \times 10^8$ CFU mL⁻¹) prior to inoculation and spread evenly on agar surface using sterile swabs. and spread evenly on the agar surface. The wells of 6 mm dia were filled with 100 μ L AgNP suspensions at the concentrations of 125, 250, and 500 mg mL⁻¹. After incubation of plates at 37°C for 24 h, the zones of inhibition were measured to assess antimicrobial efficacy. The minimum inhibitory concentration (MIC) was determined by broth micro-dilution method, using serial dilutions (100, 75, 50, and 25%) of 500 mg mL⁻¹ stock. The MIC endpoints were determined by visual inspection for the absence of turbidity.

Antioxidant assay

The ferric reducing antioxidant power (FRAP) assay was carried out following the protocol of Benzie and Strain (1996). The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-S-triazine in) in 40 mM HCl, and 20 mM FeCl₃·6H₂O in a 10:1:1 (v/v/v) ratio. The 100 μ L AgNP solution was added to 3 mL FRAP reagent and incubated at 37°C for 30 min. The absorbance was measured at 593 nm spectrophotometrically (Shimadzu UV-1800). The antioxidant capacity of samples was calculated based on a Trolox calibration curve and expressed as μ mol Trolox equivalent g⁻¹ sample, indicating the effective electron-donating ability of synthesized nanoparticles.

Statistical analysis

All the experiments were conducted in triplicate in a completely randomized design, and the results expressed as mean \pm standard deviation (SD) and analysed by using one-way ANOVA, followed by Tukey's post-hoc test (Salam *et al.*, 2023).

RESULTS AND DISCUSSION

FTIR analysis interpretation

The FTIR spectrum of fenugreek-mediated AgNPs (FTIR_EX_38 FS1108) revealed the presence of several key functional groups that were instrumental in nanoparticle formation and stabilization (Fig. 1). A strong peak observed at 1640.0 cm⁻¹ (intensity: 65.944) corresponded to C=C stretching vibrations typical of alkenes or the amide I band from proteins (Table 1). This band indicated the presence of unsaturated phytochemicals or proteinaceous materials contributing electron-donating functionalities that assist in silver ion reduction and stabilization of nanoparticles. Another intense absorption at 2109.7 cm⁻¹ (intensity: 95.496) was attributed to C \equiv C (alkyne) or C \equiv N (nitrile) stretching, which implies the presence of nitrogenous compounds or unsaturated systems involved in the reduction

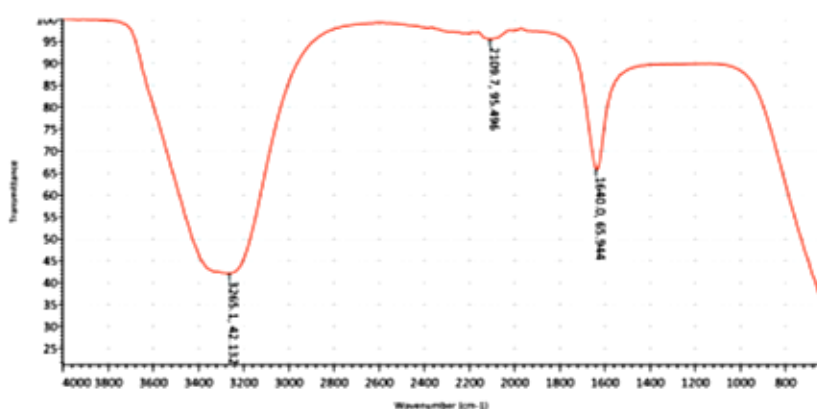


Fig. 1: Annotated FTIR spectrum of FTIR_EX_38 FS1108 showing key functional group vibrations involved in the synthesis and stabilization of silver nanoparticles

of Ag⁺ ions. These interpretations were added to emphasize the specific role of functional groups in the reduction and capping of Ag⁺ ions.

Notably, the peak at 3265.1 cm⁻¹ (intensity: 42.132) signified O–H and N–H stretching vibrations, so was indicative of hydroxyl and amine groups from phenolic and alkaloids. These functional groups

Table 1: FTIR spectral peaks of *Trigonella foenum-graecum* seed extract and synthesized AgNPs, showing characteristic functional groups involved in reduction and stabilization

Peak No.	Wave number (cm ⁻¹)	Intensity	Functional group assignment
1	1640.0	65.944	C=C or Amide I
2	2109.7	95.496	C≡C or C≡N
3	3265.1	42.132	O-H/N-H
4	1920.2	96.625	Aromatic overtones
5	1360.7	89.953	C-H/N-O
6	2020.0	97.316	Alkyne or Isocyanate

Peak positions are expressed in cm⁻¹. Assignments correspond to major biomolecules reported in the literature.

are known for their reducing and capping behaviour, enhancing nanoparticle stability and bioactivity. Additional peaks like the one at 1920.2 cm⁻¹ (intensity: 96.625), represented overtone and combination bands, typically associated with aromatic compounds. These likely originate from polyphenolic constituents such as flavonoids and tannins, compounds abundant in fenugreek seed extract. The band at 1360.7 cm⁻¹ (intensity: 89.953) corresponded to C-H bending or N-O stretching vibrations, potentially arising from aliphatic hydrocarbons or nitro compounds that contribute further to nanoparticle stabilization. Furthermore, the peak at 2020.0 cm⁻¹ (intensity: 97.316) suggested minor components like isocyanates or terminal alkynes. A comparative analysis of FTIR spectra before and after nanoparticle formation was included to show functional group shifts or disappearance, confirming their active involvement (Prasad and Elumalai, 2011; Kora, 2018).

Phytochemical screening

Phytochemical screening of *T. foenum-graecum* aqueous extract confirmed the presence of several biologically active compounds, namely flavonoids, tannins, phenols, saponins, and alkaloids (Table 2, Fig. 2). These phytochemicals are widely known for their ability to reduce silver ions and cap the resulting nanoparticles, thereby confer stability and enhance bioactivity. The alkaline reagent test affirmed the presence of flavonoids, whose antioxidant properties are well-documented (Singh *et al.*, 2010). These polyphenolic compounds contribute to free radical scavenging activity by donating electrons or H atoms. The phytochemical test was performed to correlate IC₅₀ with specific phytochemicals present.

The ferric chloride test validated the presence of tannins and phenols, both of which play dual roles as antioxidants and natural reducers during nanoparticle synthesis (Singh and Bhargava, 2012). Their astringent and regenerative properties are relevant in wound care and skin applications. Saponins, confirmed via foam test, are amphipathic glycosides that aid in nanoparticle dispersion due to their

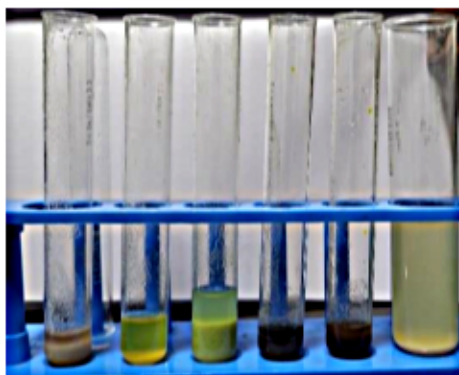


Fig. 2: Phytochemical analysis of seed extract of *Trigonella foenum-graecum*, showing the presence of triterpenoids, flavonoids, glycosides, tannins, phenols, saponins and proteins

surfactant properties. Hager's test confirmed alkaloids, which are N-containing bioactives with known antimicrobial effects (Duda-Chodak and Tarko, 2007). However, Liebermann-Burchard, Keller-Killiani, Salkowski, and Biuret tests were negative, revealing the absence of steroids, glycosides, triterpenoids, and proteins. The screening results were directly linked to their relevance in nanoparticle bioactivity.

The phytochemical screening identified many bioactive constituents as follows:

- Flavonoids, phenols, and tannins – strong antioxidants and potential reducers of Ag⁺ ions due to their hydroxyl-rich structures;
- Saponins - surface-active agents that may assist in nanoparticle dispersion; and
- Alkaloids - antimicrobial agents that may synergize with AgNPs.

Table 2: The compounds identified in *Trigonella foenum-graecum* seed extract during screening

Phytochemicals	Tests performed	Results	Observations
Flavonoids	Alkaline reagent	Positive	Yellow colour turned colourless with dilute acid
Steroids	Liebermann-Burchards	Negative	Red upper layer and green fluorescence
Glycosides	Keller killani test	Negative	Bluish-green colour at interphase
Triterpenoids	Salkowaski Test	Negative	Greyish colour
Tannins & phenols	Ferric chloride test	Positive	Blue-black colour
Saponins	Foam test	Positive	Persistent foam
Alkaloids	Hager's test	Positive	Reddish-brown precipitate
Proteins	Biuret test	Negative	Violet of pink

Thin layer chromatography

TLC profiling revealed five distinct phytochemical components across two bands (Table 3), with Band I (aqueous extract) showing more diversity compared to Band II (silver nitrate extract). The Rf values of the compounds in Band I (0.057, 0.173, and 0.307) indicated varying polarities; the most polar compound (Rf = 0.057) is likely a highly hydrophilic polyphenol, whereas the least polar (Rf = 0.307) may correspond to a more hydrophobic compound. Band II, derived from the nanoparticle-containing extract, displayed two prominent spots with Rf values of 0.019 and 0.125. The interpretation was enhanced to highlight selective phytochemical retention after nanoparticle synthesis. TLC bioautography further confirmed the antimicrobial potential of these spots, supporting the role of these retained compounds in nanoparticle bioactivity (Kumar *et al.*, 2019).

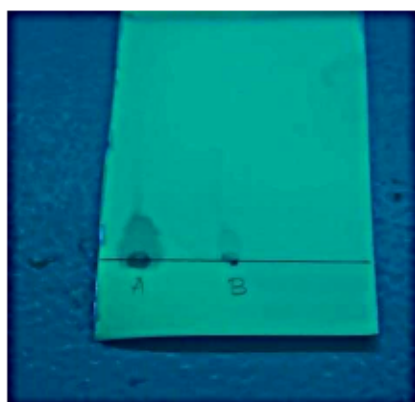


Fig. 3: Thin layer chromatographic separation of methanolic and ethyl acetate (ef) extracts of *T. foenum-graecum* fractions on silica gel GF₂₅₄ plates. The separation was carried out using solvent system (ethyl acetate: methanol: water (77: 13: 10), and spots visualized under UV light at 254 nm.

Table 3: Thin layer chromatography (TLC) profile of *Trigonella foenum-graecum* seed extract of under UV light (254 and 365 nm) and after derivatization, showing retention factor (Rf) values of separated phytochemical components.

Band	Band length	Rf values	Polarity interpretation
I	5.2 cm	0.057	High polarity
		0.173	Medium polarity
		0.307	Low polarity
II	5.2 cm	0.019	Very high polarity
		0.125	Moderate polarity

The Rf value generally ranges between 0 and 1 and it determines the polarity of compounds based on Rf values 0.057, 0.173, and 0.307 of three compounds for raw extract of fenugreek seed which is dissolved in distilled water and 0.019, and 0.125 of 2 compounds for silver nitrate extract.

Antimicrobial activity

Fenugreek extract alone did not exhibit any zone of inhibition; however, AgNPs synthesized from the extract displayed significant antibacterial activity against *E. coli*,

B. cereus and *S. pyogenes* (Table 4). Ethanol extracts showed the highest zones of inhibition (e.g., 19 ± 0.6 mm for *S. pyogenes* and *K. pneumoniae*). Notably, *E. coli* exhibited a 20 mm inhibition zone, greater than that of *S. aureus*, which recorded only 5 mm. This differential susceptibility was attributed to cell wall structure, interaction with nano-particles, and ROS generation. Gram-negative bacteria like *E. coli* may facilitate better nano-particle uptake due to particle uptake due to their outer membrane (Morones *et al.*, 2005). Smaller nanoparticles with a larger surface area enhanced microbial binding and disruption. A comparison with previous studies (Awad *et al.*, 2016; Alshafei *et al.*, 2022) further supports the observed anti-microbial potential.

Table 4: Antimicrobial activities of methanolic and ethyl acetate extracts of *Trigonella foenum-graecum* seeds and AgNPs at low and high concentrations against selected bacterial strains, expressed as inhibition zone in diameter (mm)

Bacteria	Methanol extract	Ethanol extract	AgNPs	
			Low conc.	High conc.
<i>E. coli</i>	18.0 ± 0.6	20.0 ± 0.5	14.0 ± 0.4	20.0 ± 0.5
<i>B. cereus</i>	17.0 ± 0.5	19.0 ± 0.6	10.0 ± 0.3	16.0 ± 0.5
<i>S. aureus</i>	–	–	5.0 ± 0.2	12.0 ± 0.4
<i>K. pneumonia</i>	18.0 ± 0.6	19.0 ± 0.5	–	15.0 ± 0.5
<i>S. pyogenes</i>	–	19.0 ± 0.6	–	13.0 ± 0.4

'Low' and 'High' correspond to two test concentrations of AgNPs, where higher concentration generally produced larger inhibition zones as seen for *E. coli* and *B. cereus*. The values are mean ± standard deviation (SD) of 3 independent experiments, each performed in technical triplicate ($n = 3$). '–' indicates no measurable inhibition zone

flavonoids. Structural attributes like 3', 4'-dihydroxy substitution patterns and 5-OH groups support electron donation and radical neutralization (Heim *et al.*, 2002). Antioxidant efficacy was also compared with other plant-mediated nanoparticles, showing superior or competitive performance (Ahmed *et al.*, 2016). All findings were statistically significant ($p < 0.05$).

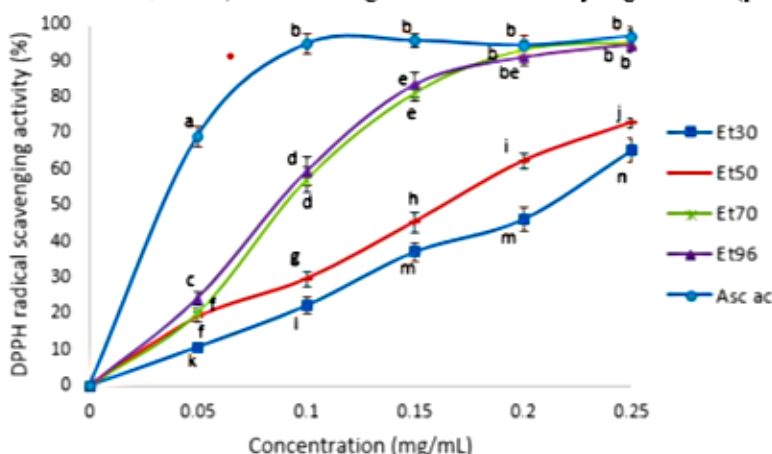


Fig. 4: DPPH radical scavenging activity of ascorbic acid and *T. foenum-graecum* seed extracts. Treatments not sharing the same letter are significantly different ($p < 0.05$). Asc ac = ascorbic acid. treatments: et30 = extract with 30% ethanol; et50 = extract with 50% ethanol; et70 = extract with 70% ethanol; et96 = extract with 96% ethanol

Table 5: DPPH radical scavenging activity of methanol and ethanol extracts, and AgNPs at different concentrations

Extracts	IC ₅₀ (mean ± SE)	Statistical group
Et30	0.203 ± 0.008	a
Et50	0.166 ± 0.012	b
Et70	0.093 ± 0.005	c
Et96	0.088 ± 0.006	c
Ascorbic acid	0.036 ± 0.003	d

Values are mean ± SD ($n = 3$). IC₅₀ values were determined by nonlinear regression analysis. Et30, Et50, Et70 and Et96 indicate fenugreek seed extract with based on 30, 50, 70 and 96% ethanol, respectively.

Antioxidant assay

Oxidative stress, caused by excess ROS, contributes to the various diseases. The antioxidant properties of fenugreek seed extracts were evaluated using DPPH assay. The 70 and 96% ethanolic extracts showed highest radical scavenging activities, with IC₅₀ values of 0.093 and 0.102 mg mL⁻¹, respectively. These values were significantly lower (indicating stronger activity) than ~350 μg mL⁻¹ reported earlier by Kaviarasan *et al.* (2007). This was correlated with rich presence of phenolic acids and

The IC₅₀ values in DPPH radical scavenging activity were also determined. Higher the IC₅₀ value, weaker is the antioxidant activity of a compound. The antiradical activity of 30% fenugreek seed extracts was significantly weaker ($p < 0.05$). The DPPH scavenging activity was dose-dependent. IC₅₀ values reflect the antioxidant potency.

Comparative and practical implications

The enhanced bioactivity of fenugreek-derived AgNPs can be attributed to a synergistic effect of phytochemicals and the nanoscale properties of silver. The underlying mechanisms- membrane

disruption, ROS generation, DNA/enzyme interactions were clarified for antimicrobial activity, while antioxidant effects stem from electron donation and metal chelation. A comparison with other plant-based AgNPs (e.g., neem, green tea) was included, showing that fenugreek-based AgNPs perform competitively due to a distinct flavonoid-rich profile (Sharma *et al.*, 2009; Song and Kim, 2009). The practical applications include green nanomaterials for wound healing, antimicrobial coatings for medical devices, and functional foods (Iravani, 2011). The eco-friendly synthesis and strong bioefficacy highlight their potential for scalable biomedical applications.

Conclusion: This study successfully demonstrated the green synthesis of AgNPs using *Trigonella foenum-graecum* seed extract, presenting an eco-friendly, cost-effective, and sustainable method of nanoparticle fabrication. FTIR analysis confirmed the involvement of key functional groups, like hydroxyl, amide, alkene, and phenolic moieties, in the bioreduction and stabilization of AgNPs. Phytochemical screening validated the presence of bioactive compounds including flavonoids, phenols, tannins, and saponins, which contributed not only to nanoparticle formation but also to their enhanced biological activity. TLC profiling provided insights into the chemical diversity and polarity of phytoconstituents involved in the synthesis, while antimicrobial assays demonstrated significant antibacterial activity, particularly against *Escherichia coli* and *Staphylococcus aureus*. The DPPH assay further confirmed potent antioxidant activity, with ethanol extracts (Et70 and Et96) exhibiting IC₅₀ values comparable to standard ascorbic acid. Taken together, these findings underscore the potential of fenugreek-mediated AgNPs as multifunctional agents with strong antimicrobial and antioxidant properties, driven by the synergistic action of phytochemicals and nanoscale effects. Such nanoparticles hold promise for diverse biomedical, pharmaceutical, and environmental applications. Future studies need to be aimed to elucidate underlying mechanisms through molecular docking and cellular pathway analyses, along with *in vivo* and cytotoxicity evaluations to facilitate clinical or industrial translation.

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