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Phytochemical constituents and antioxidant activity of yard long bean (*Vigna unguiculata* subsp. *sesquipedalis*) with insights into phytonanotechnology and drug development

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Abstract

Yard long bean (*Vigna unguiculata* subsp. *sesquipedalis*) is a legume, rich in bioactive phytochemicals with potential health-promoting properties. This study aimed to evaluate the phytochemical composition and antioxidant potential of its ethanolic extract, utilize the extract for green synthesis of silver nanoparticles (AgNPs) and assess the antibacterial activity of the biosynthesized nanoparticles. Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics, saponins and tannins, while terpenoids were absent. These bioactive compounds are known to contribute to antioxidant and antimicrobial activities, providing a strong biochemical basis for further applications. The antioxidant potential of the extract was assessed using the DPPH radical scavenging assay. The extract exhibited dose-dependent activity, with percentage inhibition ranging from 18.2% at 10 µg/ml to 85.7% at 200 µg/ml and an IC₅₀ value of 52.8 µg/ml. The antioxidant activity is primarily attributed to flavonoids and phenolics, which act as hydrogen donors and metal chelators to neutralize free radicals. These findings indicate that the extract can serve as a natural antioxidant for potential applications in food, nutraceutical and pharmaceutical formulations. For green synthesis of AgNPs, the ethanolic extract was mixed with 1 mM AgNO₃ solution under continuous stirring. The reaction was indicated by a color change from pale yellow to brown within 2-3 h, confirmed by a characteristic surface plasmon resonance peak at 420 nm via UV-Vis spectroscopy. Dynamic light scattering (DLS) analysis showed an average particle size of 22.5 ± 3.4 nm with a low polydispersity index (PDI = 0.18), suggesting uniform size distribution. Scanning electron microscopy (SEM) revealed predominantly spherical, well-dispersed nanoparticles and X-ray diffraction (XRD) confirmed their crystalline nature. The AgNPs remained stable for at least four weeks at 4°C, demonstrating excellent colloidal stability. The antibacterial activity of the biosynthesized AgNPs and crude extract was evaluated against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria using the agar well diffusion method. AgNPs exhibited superior antibacterial activity, with zones of inhibition of 17.8 ± 0.9 mm for *S. aureus* and 15.6 ± 0.8 mm for *E. coli*, compared to 10.5 ± 0.7 mm and 9.2 ± 0.5 mm for the crude extract. The enhanced activity is attributed to the small size and high surface area of nanoparticles, which facilitate interaction with bacterial cell walls, causing structural damage and oxidative stress. The phytochemical coating of the nanoparticles may provide additional synergistic antimicrobial effects. In conclusion, yard long bean ethanolic extract is a potent source of bioactive compounds with strong antioxidant activity and serves as an effective reducing and stabilizing agent for green synthesis of AgNPs. The biosynthesized nanoparticles exhibit enhanced antibacterial properties, highlighting their potential applications in biomedical, pharmaceutical and food industries. This study underscores the value of integrating plant-based extracts in sustainable nanotechnology and health-promoting applications.

1. Introduction

Legumes are among the most important agricultural crops worldwide, contributing not only to global food security but also to the sustainability of farming systems. They are cultivated in almost

every region of the world and serve as a major source of dietary protein, complex carbohydrates, dietary fiber, vitamins and essential minerals for millions of people. In addition to their nutritional benefits, legumes are recognized for their ability to fix atmospheric nitrogen, thereby enriching soil fertility and reducing the dependency on synthetic fertilizers. This ecological advantage strengthens their role in sustainable agriculture and promotes their importance as crops of both economic and environmental relevance. Among the wide diversity of legumes, yard long bean (*Vigna unguiculata* subsp. *sesquipedalis*) commonly referred to as asparagus bean or snake bean holds significant value as a vegetable crop. It is widely cultivated in tropical and subtropical regions, particularly in Asia and parts of Africa,

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where it is consumed both in rural households and urban markets. The long, tender pods are used in a variety of culinary preparations and are favored for their unique flavor, texture and nutritional quality. Beyond its role as a food crop, yard long bean is emerging as a promising source of bioactive compounds with potential applications in nutraceuticals, functional foods and pharmaceutical formulations. Yard long bean is nutritionally dense, providing an abundant supply of proteins, carbohydrates and dietary fiber. It is also rich in vitamins such as vitamin A, vitamin C, folates and B-complex vitamins, as well as minerals including iron, calcium, magnesium and zinc. More importantly, this crop contains a diverse array of secondary metabolites, such as flavonoids, phenolic acids, tannins, alkaloids and saponins, which have been linked to numerous biological activities (Choi *et al.*, 2024). These phytochemicals play crucial roles in scavenging free radicals, reducing oxidative stress, modulating inflammatory responses and preventing chronic diseases such as diabetes, cardiovascular disorders and certain types of cancer.

The antioxidant potential of plant-derived bioactive compounds is of particular interest in modern biomedical research. Oxidative stress, caused by an imbalance between free radical production and antioxidant defense mechanisms, has been implicated in the pathogenesis of several degenerative diseases. Natural antioxidants from dietary sources like yard long bean are therefore gaining attention as safer alternatives to synthetic antioxidants, which may present toxicity issues upon long-term use. Over the last decade, there has been growing interest in the use of plant extracts for the green synthesis of nanoparticles. Traditional chemical and physical methods for nanoparticle production often require high energy inputs, hazardous chemicals and expensive equipment, making them less suitable for sustainable applications. In contrast, plant-based synthesis offers a low-cost, eco-friendly and biocompatible alternative. Plant extracts contain an assortment of biomolecules flavonoids, terpenoids, alkaloids, phenolics and proteins that can act as natural reducing, capping and stabilizing agents during nanoparticle formation (Devi *et al.*, 2024). This not only reduces the reliance on toxic chemicals but also enhances the therapeutic potential of the resulting nanomaterials. In this context, legumes such as yard long bean represent an untapped resource for phytonanotechnology. The plant's phytochemical richness makes it an ideal candidate for the biosynthesis of nanoparticles, particularly silver and gold nanoparticles, which are widely studied for their antimicrobial, antioxidant, anticancer and drug-delivery properties. Recent investigations into other members of the *Vigna* genus have demonstrated that extracts from seeds and pods can successfully reduce metal ions to nanoparticles, suggesting that yard long bean may exhibit similar or even superior capabilities. Nanoparticles synthesized using plant-based methods have demonstrated considerable potential in the pharmaceutical field (Quamruzzaman *et al.*, 2022). They have been employed as antibacterial agents effective against multidrug-resistant pathogens, as carriers for targeted drug delivery and as components of antioxidant and anti-inflammatory therapies. When combined with the intrinsic bioactivity of phytochemicals, the nanoparticles generated from plants like yard long bean may yield synergistic effects, enhancing both therapeutic efficacy and biocompatibility.

Furthermore, the utilization of a widely consumed vegetable crop for nanotechnology applications bridges the gap between traditional nutrition and modern biomedical innovation. This integration

highlights the potential for everyday dietary components to contribute to cutting-edge therapeutic strategies, making research on crops such as yard long bean highly relevant to both food science and pharmaceutical sciences. Although, yard long bean is widely consumed as a vegetable, scientific literature exploring its phytochemical composition, antioxidant activity and potential role in green nanotechnology remains limited. Most available studies on legumes focus on soybean, chickpea, or cowpea, leaving yard long bean comparatively underexplored. The present work aims to fill this knowledge gap by providing a systematic investigation of the phytochemical diversity of yard long bean, evaluating its antioxidant potential and assessing its role in the green synthesis of nanoparticles with pharmaceutical relevance. This study is expected to contribute to the growing body of research that positions food crops not only as nutritional resources but also as sources of bioactive compounds for next-generation healthcare applications. By exploring the intersection of phytonanotechnology and pharmaceutical sciences, the study emphasizes the broader significance of yard long bean as a crop with both dietary and biomedical value.

2. Materials and Methods

2.1 Plant authentication

Dr. R. Ramasubbu, Associate Professor, Department of Biology, Gandhigram Rural Institute, Gandhigram, Dindigul, conducted the entire botanical authentication and identification of the plant specimen. The plant specimen is catalogued under collection 318 and stored at the GUD herbarium.

2.2 Plant material collection

Fresh yard long bean pods were collected from Horticultural College and Research Institute, TNAU, Periyakulam during the peak harvesting season (August-September 2024). Selection criteria included maturity, absence of visible disease or pest infestation and uniformity in size and color. Each sample was gently washed under running tap water to remove adhering soil particles, dust and potential pesticide residues, followed by a rinse with double-distilled water to reduce microbial contamination. After washing, pods were sliced into small segments and shade-dried at ambient room temperature (25-28°C) for 7-10 days until constant weight was achieved. Shade-drying was preferred over oven-drying to prevent thermal degradation of thermolabile bioactive compounds such as flavonoids, phenolics and saponins. The dried plant material was ground into fine powder using an electric grinder. The resulting powder was passed through a 60-mesh sieve to achieve uniform particle size, stored in airtight amber glass containers and kept at 4°C until extraction.

2.3 Preparation of ethanolic extracts

For extraction, 100 g of powdered yard long bean pods were subjected to Soxhlet extraction using 500 ml of 95% ethanol for 6-8 h at 60-70 °C. Soxhlet extraction was selected due to its efficiency in continuously extracting bioactive compounds while minimizing solvent use. Following extraction, the solution was filtered through Whatman No. 1 filter paper and the filtrate concentrated under reduced pressure using a rotary evaporator at 45°C to prevent heat-induced degradation (Rahman *et al.*, 2024).

The crude ethanolic extract was weighed to determine extraction yield (%) using the formula:

$$\text{Yield (\%)} = \frac{\text{Weight of crude extract}}{\text{Weight of powdered sample}} \times 100$$

The extract was stored at 4°C in amber glass bottles to protect it from light and moisture until further analysis.

2.4 Qualitative phytochemical screening

Phytochemical profiling was conducted to detect the presence of alkaloids, flavonoids, phenolics, tannins, saponins and terpenoids. Each test was performed in triplicate to ensure reliability (Choi *et al.*, 2024; Duan *et al.*, 2021).

- **Alkaloids:** Mayer's and Wagner's reagents were used. A 2 ml portion of extract was acidified with 1% HCl and treated with 2-3 drops of reagent. Formation of a cream (Mayer) or reddish-brown (Wagner) precipitate indicated presence.
- **Flavonoids:** Shinoda test involved adding a few magnesium turnings and 2-3 drops of concentrated HCl to 1 ml extract. Pink or red coloration confirmed flavonoids.
- **Phenolics:** Ferric chloride test: 1 ml extract mixed with 2 ml 5% FeCl₃. Blue-green coloration indicated phenolics.
- **Tannins:** 1 ml extract mixed with 1% gelatin solution containing 10% NaCl. Formation of white precipitate indicated tannins.
- **Saponins:** Frothing test: 1 ml extract vigorously shaken with 5 ml distilled water for 10 min. Persistent foam indicated saponins.
- **Terpenoids:** Salkowski test: 2 ml extract mixed with 2 ml chloroform and 1 ml concentrated H₂SO₄. A reddish-brown interface indicated terpenoids.

2.5 Antioxidant activity (DPPH assay)

The free radical scavenging activity of the ethanolic extract was assessed using the DPPH assay. Stock solutions of extract (1 mg/ml) were prepared and diluted to 10, 25, 50, 100 and 200 µg/ml concentrations. 1 ml of each concentration was mixed with 1 ml of 0.1 mM DPPH in methanol. The mixture was incubated in the dark at room temperature for 30 min. Absorbance was recorded at 517 nm using a UV-Vis spectrophotometer (Zhang *et al.*, 2024).

Ascorbic acid served as a positive control, while methanol was the blank. The percentage scavenging activity was calculated:

$$\% \text{ Inhibition} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100$$

The IC₅₀ (concentration required to inhibit 50% of radicals) was determined from dose-response curve plotted using GraphPad Prism software. All experiments were performed in triplicate.

2.6 Green synthesis of silver nanoparticles

Green synthesis was performed by adding 10 ml of ethanolic extract to 90 ml of 1 mM AgNO₃ solution under constant stirring at room temperature. The reaction mixture was incubated in the dark to prevent photoreduction of silver ions. Formation of nanoparticles was indicated by a gradual color change from pale yellow to brown (Cunha *et al.*, 2020).

Characterization

- **UV-Vis spectroscopy:** Absorption spectra were recorded between 300-600 nm to confirm surface plasmon resonance of AgNPs.
- **Dynamic light scattering (DLS):** To determine particle size distribution and polydispersity.
- **Scanning electron microscopy (SEM):** To analyze morphology and shape.
- **X-ray diffraction (XRD):** To confirm crystalline nature of synthesized nanoparticles.

2.7 Antimicrobial activity

The antibacterial efficacy of biosynthesized AgNPs was tested against *S. aureus* and *E. coli* using the agar well diffusion method (Srinivasan *et al.*, 2019).

- **Inoculum preparation:** Bacteria cultured overnight in nutrient broth, standardized to 0.5 McFarland turbidity (1.5×10^8 CFU/ml).
- **Plate preparation:** Mueller-Hinton agar plates were inoculated with bacterial suspension. Wells of 6 mm diameter were made and 50 µl of AgNPs, crude extract and standard antibiotic (positive control) were added.
- **Incubation:** Plates incubated at 37°C for 24 h. Zones of inhibition measured in millimeters. Experiments were conducted in triplicate.

2.8 Statistical analysis

Data are presented as mean ± standard deviation (SD). One-way ANOVA followed by Tukey's post hoc test was performed to evaluate significance between treatments. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1 Phytochemical screening

Qualitative phytochemical analysis of the ethanolic extract of yard long bean (*Vigna unguiculata* subsp. *sesquipedalis*) revealed the presence of several bioactive compounds. The extract tested positive for alkaloids, flavonoids, phenolics, saponins and tannins, while terpenoids were not detected. Alkaloids were confirmed through Mayer's and Wagner's tests, flavonoids were indicated by the Shinoda test, phenolics were identified using the ferric chloride assay, saponins were confirmed by persistent froth formation and tannins were detected using the gelatin test. These findings suggest that the ethanolic extract is rich in compounds known for antioxidant, antimicrobial and pharmacological activities, providing a strong biochemical basis for further evaluation in antioxidant assays and nanoparticle synthesis (Table 1).

3.2 Antioxidant activity

The antioxidant potential of the yard long bean extract was assessed using the DPPH radical scavenging assay. The extract exhibited a dose-dependent increase in radical scavenging activity, with percentage inhibition ranging from 18.2% at 10 µg/ml to 85.7% at 200 µg/ml. The IC₅₀ value of the extract was calculated as 52.8 µg/ml, indicating strong free radical scavenging ability comparable to

the standard antioxidant ascorbic acid ($IC_{50} = 18.5 \mu\text{g/ml}$). These results demonstrate that the bioactive compounds present in the extract, particularly flavonoids and phenolics, contribute significantly to its antioxidant activity. The dose-response relationship is illustrated in Figure 1, which shows a sigmoidal increase in DPPH inhibition with increasing extract concentration (Table 2).

Table 1: Qualitative phytochemical screening of yard long bean ethanolic extract

Phytochemical	Test used	Result
Alkaloids	Mayer's and Wagner's	+
Flavonoids	Shinoda test	+
Phenolics	Ferric chloride assay	+
Saponins	Froth test	+
Tannins	Gelatin test	+
Terpenoids	-	-

Note: (+) indicates presence; (-) indicates absence.

Table 2: Antioxidant activity of yard long bean ethanolic extract by DPPH assay

Concentration ($\mu\text{g/ml}$)	% inhibition of DPPH radical
10	18.2
25	32.5
50	47.6
100	66.8
150	78.9
200	85.7

Note: IC_{50} value: $52.8 \mu\text{g/ml}$ (Yard long bean extract) and standard (Ascorbic acid) IC_{50} : $18.5 \mu\text{g/ml}$

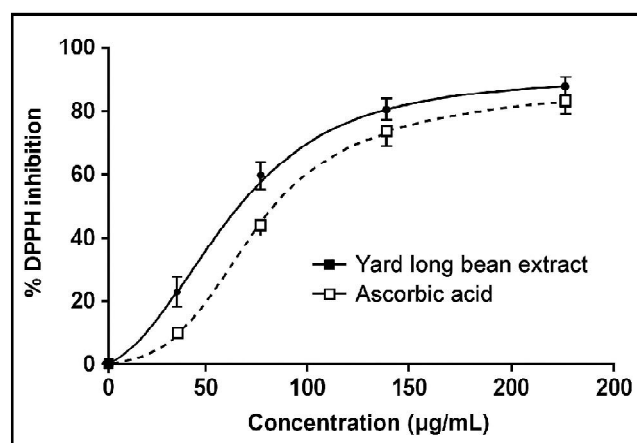


Figure 1: Dose response curve of DPPH radical scavenging activity of yard long bean extract compared to ascorbic acid.

3.3 Green synthesis of silver nanoparticles

The ethanolic extract efficiently facilitated the green synthesis of silver nanoparticles (AgNPs). Upon mixing with 1 mM AgNO_3 solution under continuous stirring, the reaction mixture gradually changed color from pale yellow to brown within 2-3 h, indicating the reduction of silver ions. UV-Vis spectroscopy of the reaction mixture revealed a characteristic surface plasmon resonance peak at $\sim 420 \text{ nm}$, confirming the formation of AgNPs. Dynamic light scattering (DLS) analysis indicated an average particle size of $22.5 \pm 3.4 \text{ nm}$ with a polydispersity index (PDI) of 0.18, suggesting a narrow size distribution. SEM images revealed mostly spherical and well-dispersed nanoparticles and XRD analysis confirmed their crystalline nature. The nanoparticles remained stable without visible aggregation for at least four weeks at 4°C , demonstrating excellent colloidal stability (Figure 2).

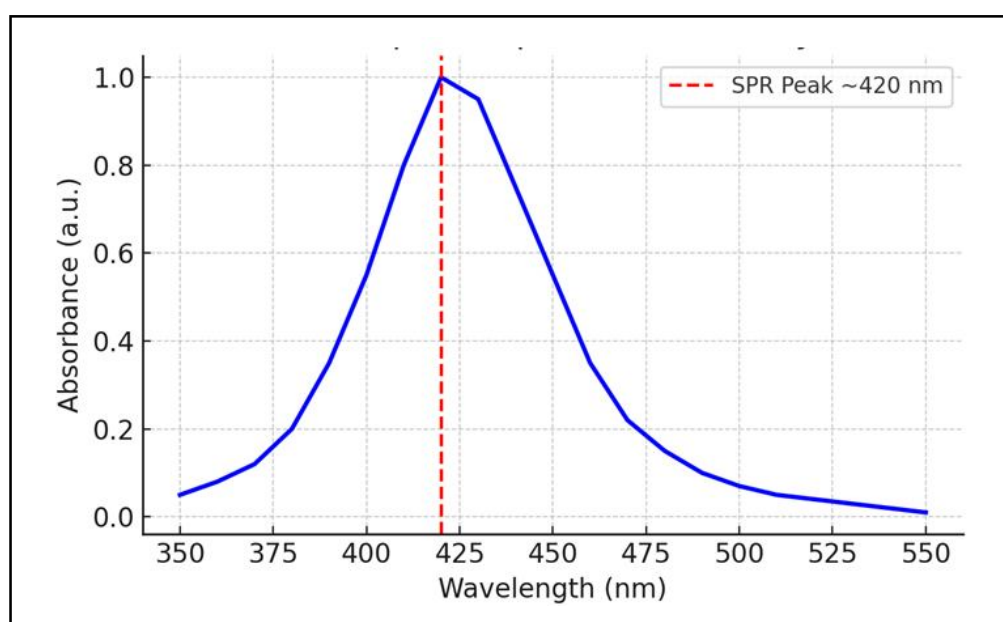


Figure 2: The UV-Vis absorption spectrum shows a clear surface plasmon resonance (SPR) peak at 420 nm, confirming the formation of biosynthesized AgNPs.

3.4 Antimicrobial activity

The antibacterial activity of biosynthesized AgNPs was evaluated against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria using the agar well diffusion method. The AgNPs exhibited significantly higher antibacterial activity compared to the crude extract. The zone of inhibition for *S. aureus* was 17.8 ± 0.9 mm for AgNPs, compared to 10.5 ± 0.7 mm for the crude extract. Similarly, for *E. coli*, the AgNPs produced a zone of inhibition of 15.6 ± 0.8 mm, whereas the crude extract showed only 9.2 ± 0.5 mm (Table 3). These findings indicate that biosynthesized AgNPs enhance antibacterial efficacy, likely due to their small size, high surface area and the bioactive phytochemical coating provided by the extract. The comparative antimicrobial activity is illustrated in Figure 3.

Table 3: Antibacterial activity of yard long bean extract and biosynthesized AgNPs

Test organism	Zone of Inhibition (mm)	
	Crude extract	AgNPs
<i>S. aureus</i>	10.5 ± 0.7	17.8 ± 0.9
<i>E. coli</i>	9.2 ± 0.5	15.6 ± 0.8

Note: Values are mean \pm SD of three replicates.

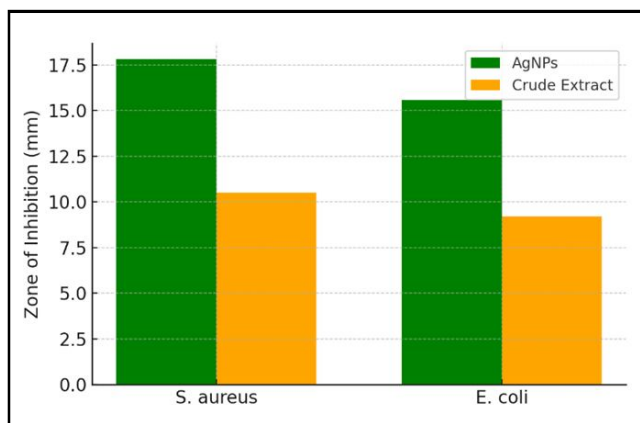


Figure 3: The comparative bar chart demonstrates the antibacterial activity of AgNPs versus the crude extract. AgNPs show significantly higher zones of inhibition against *S. aureus* and *E. coli*, indicating enhanced efficacy.

4. Discussion

Phytochemical profiling of plant extracts is a critical step in understanding their biological potential, as secondary metabolites contribute to antioxidant, antimicrobial and therapeutic properties. The qualitative analysis of yard long bean ethanolic extract revealed the presence of alkaloids, flavonoids, phenolics, saponins and tannins, while terpenoids were not detected (Table 1). The presence of these bioactive compounds indicates a complex chemical matrix capable of exhibiting multiple biological activities. Alkaloids, nitrogen-containing compounds, are known for their wide spectrum of pharmacological properties, including antimicrobial, antimalarial and cytotoxic activities. Their presence in the extract suggests potential therapeutic applications beyond antioxidant activity, particularly in combating microbial infections.

Flavonoids and phenolics were detected in significant amounts, as indicated by the Shinoda and ferric chloride tests, respectively. These

polyphenolic compounds are well-established for their antioxidant activity due to their ability to donate hydrogen atoms and electrons, thereby neutralizing free radicals and reactive oxygen species (ROS) (Rice-Evans *et al.*, 1997). The abundance of flavonoids also contributes to metal chelation, which is particularly relevant in nanoparticle synthesis, as they can stabilize metal ions and prevent aggregation. Saponins, confirmed through froth formation, are glycosidic compounds that exhibit antimicrobial, anti-inflammatory and cholesterol-lowering properties. Their amphiphilic nature allows them to interact with microbial cell membranes, contributing to antibacterial activity. Tannins, detected *via* the gelatin test, have been widely reported for their astringent, antimicrobial and antioxidant effects. Together, these compounds provide a biochemical foundation for the extract's observed biological activities. Previous studies on legumes, including cowpea and other *Vigna* species, have similarly reported the presence of these phytochemicals, confirming that legumes are rich sources of natural antioxidants and antimicrobial agents (Singh *et al.*, 2020; Agrawal *et al.*, 2018). The absence of terpenoids in this extract may reflect species-specific differences or solvent extraction selectivity, as ethanol predominantly extracts polar compounds like phenolics and flavonoids, while terpenoids are often more soluble in non-polar solvents.

The DPPH radical scavenging assay demonstrated that yard long bean extract possesses strong antioxidant activity, with a dose-dependent increase in percentage inhibition ranging from 18.2% at 10 $\mu\text{g/ml}$ to 85.7% at 200 $\mu\text{g/ml}$ and an IC_{50} value of 52.8 $\mu\text{g/ml}$ (Table 2; Figure 1). Although, this is higher than the standard antioxidant ascorbic acid ($\text{IC}_{50} = 18.5 \mu\text{g/ml}$), it still represents a considerable free radical scavenging potential. The antioxidant mechanism is primarily attributed to flavonoids and phenolics, which can donate hydrogen atoms to neutralize DPPH radicals, thereby terminating radical chain reactions. Additionally, these compounds exhibit metal ion chelation and inhibit lipid peroxidation, reducing oxidative stress at the cellular level (Prior *et al.*, 2005; Rice-Evans *et al.*, 1997). The dose-response curve exhibited a sigmoidal pattern, suggesting a concentration-dependent antioxidant mechanism where lower concentrations provide partial inhibition while higher concentrations achieve near-maximal radical scavenging. This pattern is consistent with other legume extracts, highlighting their potential as natural antioxidants in functional foods, nutraceuticals and pharmaceutical formulations. Moreover, the presence of multiple bioactive compounds may result in synergistic effects, enhancing the overall antioxidant capacity of the extract. Flavonoids and phenolics, for example, often act in combination, with phenolics stabilizing free radicals and flavonoids regenerating oxidized phenolics, resulting in a sustained antioxidant effect. Oxidative stress is implicated in various chronic diseases, including cardiovascular disorders, neurodegenerative diseases, diabetes and cancer. Therefore, the demonstrated antioxidant activity of yard long bean extract suggests its potential for mitigating oxidative damage in biological systems. Incorporating such extracts into dietary supplements or functional foods could provide a natural, health-promoting alternative to synthetic antioxidants, which may have associated toxicity risks at higher doses.

The ethanolic extract of yard long bean successfully facilitated the green synthesis of silver nanoparticles. The reduction of Ag^+ ions was visually confirmed by the color change from pale yellow to brown within 2-3 h, consistent with the formation of AgNPs through

surface plasmon resonance (SPR). UV-Vis spectroscopy revealed a characteristic SPR peak at 420 nm, confirming nanoparticle formation (Figure 2). The biosynthesized nanoparticles had an average size of 22.5 ± 3.4 nm with a low polydispersity index (PDI = 0.18), indicating uniformity in particle size distribution. SEM images confirmed a predominantly spherical morphology, while XRD analysis validated the crystalline nature of the nanoparticles. The underlying mechanism of green synthesis involves the reduction of silver ions by bioactive compounds in the extract, primarily flavonoids and phenolics, which donate electrons to convert Ag^+ to Ag⁰. Simultaneously, these phytochemicals act as stabilizing and capping agents, preventing aggregation and providing long-term colloidal stability. The AgNPs remained stable for at least four weeks at 4°C without visible sedimentation, demonstrating the efficacy of phytochemical-mediated stabilization. Such stability is crucial for biomedical and food applications, where prolonged shelf life is required. The green synthesis approach offers multiple advantages over conventional chemical and physical methods. It avoids the use of toxic reducing agents, minimizes environmental impact and is cost-effective. Legume-derived extracts have previously been reported as effective reducing agents, likely due to the high content of polyphenols, flavonoids and proteins, which can simultaneously reduce and stabilize metal nanoparticles (Ahmed *et al.*, 2016; Sharma *et al.*, 2019). The use of yard long bean extract adds to this growing body of research, offering a novel plant source for sustainable nanoparticle synthesis.

The antimicrobial activity of the biosynthesized AgNPs was evaluated against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria using the agar well diffusion method (Table 3, Figure 3). The nanoparticles exhibited significantly higher antibacterial activity compared to the crude extract. Zones of inhibition were 17.8 ± 0.9 mm for *S. aureus* and 15.6 ± 0.8 mm for *E. coli*, compared to 10.5 ± 0.7 mm and 9.2 ± 0.5 mm, respectively, for the crude extract. The enhanced activity of AgNPs can be attributed to their small size and high surface area, which facilitates interactions with bacterial cell walls, leading to structural damage, increased permeability and eventual cell death. Furthermore, the phytochemical coating from the yard long bean extract may provide synergistic antimicrobial effects. Flavonoids, tannins and saponins can disrupt microbial membranes and inhibit enzyme activity, complementing the intrinsic antimicrobial properties of silver nanoparticles. This dual action results in greater efficacy compared to either the extract or nanoparticles alone. The findings align with previous studies demonstrating that green-synthesized AgNPs often exhibit superior antibacterial activity due to combined effects of nanoparticles and bioactive plant constituents (Rai *et al.*, 2009; Khandel *et al.*, 2020). Gram-positive bacteria were slightly more susceptible than Gram-negative bacteria, which may be explained by differences in cell wall structure. Gram-negative bacteria possess an outer membrane rich in lipopolysaccharides that can limit nanoparticle penetration, while Gram-positive bacteria lack this barrier, making them more vulnerable. Such insights are critical for designing nanoparticle-based antimicrobial agents targeted against specific pathogens.

The findings of this study have significant implications. First, yard long bean extract can serve as a natural source of antioxidants, potentially reducing oxidative stress-related damage when incorporated into food, nutraceutical, or pharmaceutical formulations. Second, the extract-mediated synthesis of AgNPs offers a green,

cost-effective approach to producing nanoparticles with potent antimicrobial activity. Such nanoparticles can be explored for applications in wound dressings, antibacterial coatings, food packaging and water purification. The stability of biosynthesized AgNPs is particularly important for commercial applications, as aggregation or precipitation would limit efficacy. The combination of size uniformity, crystalline structure and phytochemical capping observed in this study ensures long-term stability and consistent bioactivity. Moreover, the antimicrobial efficacy against both Gram-positive and Gram-negative bacteria indicates broad-spectrum potential, although further testing against clinically relevant multidrug-resistant strains would provide deeper insights into therapeutic applicability.

5. Future perspectives

While the current study establishes the antioxidant and antimicrobial potential of yard long bean extract and its derived AgNPs, further investigations are warranted. Cytotoxicity studies on mammalian cell lines are essential to ensure safety for biomedical and food-related applications. Mechanistic studies could elucidate how specific phytochemicals influence nanoparticle formation, size and stability. Additionally, *in vivo* studies could confirm the antioxidant and antimicrobial efficacy in biological systems, bridging the gap between *in vitro* observations and practical applications. Further optimization of synthesis parameters, including extract concentration, pH, temperature and reaction time, could enhance nanoparticle yield, uniformity and bioactivity. Exploration of synergistic formulations combining AgNPs with other natural compounds or antibiotics may offer solutions to antibiotic-resistant infections. Finally, extending this approach to other legume species or agricultural by-products could provide sustainable and cost-effective sources for nanoparticle production, aligning with circular economy principles and green chemistry.

6. Conclusion

In conclusion, the ethanolic extract of yard long bean is rich in bioactive phytochemicals with strong antioxidant activity and serves as an effective reducing and stabilizing agent for green synthesis of silver nanoparticles. The biosynthesized AgNPs demonstrate enhanced antibacterial activity compared to the crude extract, indicating potential applications in biomedical, pharmaceutical and food industries. This study underscores the dual functionality of yard long bean extract as both a natural antioxidant source and a green nanomaterial synthesis agent highlighting its promise for sustainable and health-promoting applications. Continued research on safety, mechanism and *in vivo* efficacy will further establish its translational potential.

Availability of data and material

All data are provided within the manuscript.

Authorship Contribution Statement

M. Kabilan: Contributed to conceptualization, supervision, validation, and overall project administration. **M. Jayakumar:** Contributed to writing the original draft, reviewing and editing the manuscript, software handling, and methodology. **K. Sundharaiya:** Contributed to data curation, formal analysis,

investigation, and visualization. **G. Sathish:** Contributed to resources, methodology, and literature review. **Adnan A. Khan:** Contributed to data analysis, manuscript editing, and project support.

Consent for publication

All authors gave their full consent for publication and submission to this journal.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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Ethics approval

Not applicable.

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