

PHYTOFABRICATION OF *BORRERIA HISPIDA*-MEDIATED SILVER NANOPARTICLES: PHYSICOCHEMICAL CHARACTERIZATION AND IN VITRO BIOACTIVITY ASSESSMENT

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ABSTRACT:

In the present study, silver nanoparticles (AgNPs) were synthesized using an aqueous extract of *Borreria hispida* and characterized in terms of their physicochemical properties, as well as preliminary in vitro anti-inflammatory and α -amylase inhibitory activities. The dried plant material was extracted with distilled water and reacted with 0.001 M silver nitrate at room temperature. A yellow-to-brown colour change was observed as an indicator of the formation of nanoparticles which was then investigated through UV-visible and Fourier-transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM). The UV-visible spectrum exhibited a surface plasmon resonance peak at 425 nm and the FTIR spectrum showed the characteristic bands of plant derived functional groups used in the preparation of nanoparticles at 3367.71 cm⁻¹, 2893.22 cm⁻¹, 1639.49 cm⁻¹, 1363.67 cm⁻¹, 1209.37 cm⁻¹, 696.30 cm⁻¹, 605.65 cm⁻¹ and 443.63 cm⁻¹. It was observed by SEM that the particles size ranged from 67.21 nm and 92.47 nm with clustered regions. The protein-denaturation assay showed a decrease in absorbance in all the samples tested from the absorbance of the blank (1.976) to the lowest reported sample volume (0.540) or 72.67% apparent inhibition. The apparent inhibition in the α -amylase assay was 18.38% at 1 mL absorbance was reduced from 1.795 to 1.465. Based on these results, *B. hispida* mediated AgNPs could be investigated further in a controlled manner, especially by carrying out concentration normalized dose-response experiments, replicate experiments and complementary characterization of AgNPs.

KEYWORDS: *Borreria hispida*; silver nanoparticles; green synthesis; anti-inflammatory activity; α -amylase inhibition

1. INTRODUCTION

Nanoparticles have size dependent physicochemical properties different from bulk materials, and these have been the subject of much research in biomedical applications, environmental and technological uses. In particular, silver nanoparticles (AgNPs) have attracted particular interest due to their optical and surface characteristics which can be adjusted by synthesis conditions and because of their biological activity, which has led to research on antimicrobial, antioxidant, anti-inflammatory and enzyme-inhibitory properties. The plant-mediated synthesis is an alternative approach to conventional chemical method for synthesis of nano materials as plant metabolites can be used as reducing agents as well as stabilizing agents and aqueous method can minimize the use of hazardous reducing agents (Dhir et al., 2024; Zulfiqar et al., 2024; Fahim et al., 2024). Other recent reviews also highlight the fact that the size, shape, stability and biological activity of AgNPs can be affected significantly by the pH, temperature, concentration of precursors, and composition and reaction time of the extract (Shahzadi et al., 2025; Eker et al., 2025).

Spermaceoce hispida/borreria hispida is a member of the Rubiaceae family that has been documented as having multiple classes of secondary metabolites and traditionally used for medicinal purposes. Of particular interest is that the synthesis of AgNPs using *S. hispida* has already been reported, specifically Vennila et al. (2018) used an aqueous leaf extract of *S. hispida* to synthesize AgNPs and characterized them using UV-visible, SEM, FTIR, XRD and EDX. It aimed to synthesize AgNPs from *B. hispida* preparation in aqueous media, characterize the product by UV-Visible, FTIR and SEM, and evaluate the preliminary in vitro anti-inflammatory activity by protein-denaturation inhibition and antidiabetic related activity by α -amylase inhibition. The study is not intended to prove efficacy of the treatment, but rather as a preliminary characterization and screening investigation.

The Rubiaceae *Borreria hispida* (also mentioned as *Spermacoce hispida*) belongs to the plants described as medicinal herbs in the dissertation and is characterized by the presence of flavonoids, alkaloids, tannins and saponins. A biological source for synthesizing nanoparticles and a source of biological activity, these phytochemicals offer justification for considering the plant as a source. Characterization is essential for the confirmation of nanoparticle formation and the link to the physicochemical characteristics to the biological performance. The surface plasmon resonance can be demonstrated by using UV–visible spectroscopy, FTIR can be used to obtain information on surface-associated functional groups and electron microscopy can be used to assess the particle morphology and size. In recent plant mediated AgNP studies, the results of UV–visible, FTIR and electron microscopy were also correlated with the biological assays, thus providing structure–activity relationships (Mohamed et al., 2024; Lekkala et al., 2025). Hence, the aim of the present study was to synthesize the AgNPs with aqueous extract of *B. hispida*, characterize the synthesized material by UV-Visible, FTIR and SEM analysis and test the preliminary anti-inflammatory activity of the synthesized material using a protein-denaturation assay and antidiabetic activity by analyzing the α -amylase inhibition activity.

2. MATERIALS AND METHODS

The extraction of plant material and water-based extraction

The plant materials were washed with distilled water followed by surface sterilization in 70% ethanol. The material was shade dried at room temperature (around 25 °C) for 7-10 days, ground to fine powder and stored in an air tight container at 4 °C. The powdered material was dissolved in 100 mL of distilled water and heated for 10 min at 60–80 °C with constant stirring, for aqueous extraction. The extract was filtered through Whatman No. 1 filter paper and kept at 4 °C till further use.

2.2 Green synthesis of AgNPs

0.08 g of AgNO₃ was dissolved in 100 mL distilled water to prepare silver nitrate and it was kept in an amber bottle. 10 mL of *B. hispida* extract was added to 90 mL of 0.001 M AgNO₃ in a 250-mL conical flask for the synthesis of nanoparticles. This mixture was stirred at 150 rpm and incubated in the dark at about 25 °C. The development of the yellow-to-brown colour of the solution was visually seen as a sign of AgNPs formation and the results confirmed by UV-visible absorption spectroscopy. The reaction mixture was centrifuged at 10,000 rpm for 15 min at 4 °C, the pellet was washed with deionized water, ethanol three times and redispersed in deionized water and stored at 4 °C.

2.3 Characterization

The UV–visible spectra were recorded with a Shimadzu, UV-1800 spectrophotometer, in the range of 300–700 nm. The spectra of the freeze dried AgNPs in KBr were recorded on FTIR using Bruker Alpha II instrument in the range 4000–400 cm⁻¹. The material was examined by SEM using JEOL, JSM-IT500. The dissertation describes two measurements of particle size made from SEM images. The zeta-potential analysis is described in the methods, but no numerical result for the zeta-potential was available, so it is not interpreted here.

2.4 In vitro assay on anti-inflammatory activity

The protein denaturation inhibition was measured by combining 1 mL of 0.2% BSA solution in phosphate-buffered saline (PBS, pH 7.4) with AgNP solution. The positive control was described as the aspirin at 100µg/mL and the negative control was described as PBS. Samples were incubated for 15 min at 37 °C followed by 10 min at 60 °C. Absorbance of the cooled samples was measured at 660 nm. The results of the experiment are reported here in terms of volume of AgNP sample, as these units are reported in the dissertation and a conversion to nanoparticle concentration is not provided.

2.5 In vitro antidiabetic assay

The α -amylase inhibition activity was evaluated by the addition of 500 µL of AgNP sample to 500 µL of α -amylase (1 mg/mL in phosphate buffer saline (PBS), pH 6.9) and incubated at 37 °C for 10 min. 1% starch solution (1 ml) was then added and the reaction was incubated another 10 min. DNS reagent (500 µl) was then added and heated at 90 °C for 5 min. Absorbance was then measured at 540 nm. The positive control of acarbose was described as 100 µg/mL. The experimental input of AgNPs for the dissertation is given as 0.2–1.0 mL of AgNP.

The manuscript contains the unprocessed absorbance data that was published in the dissertation. Apparent inhibition was determined from the reported blank value by using the following formula: $[(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$. No replicate measurements, standard deviations, confidence intervals or statistical tests are reported, so only descriptive analysis is done. The reported sample inputs are doses, which are not concentration-normalized, thus the term “apparent inhibition” is used and no implied IC₅₀ or potency comparison is made.

3. RESULTS AND DISCUSSION

AgNP synthesis was performed using 10 mL of the aqueous extract of *B. hispida* and 90 mL of 0.001 M AgNO₃ solution and was stirred at 150 rpm and maintained at approximately 25 °C in dark conditions for 5 days. The extraction, nanoparticle synthesis, purification and instrumental characterization conditions were summarized in Table 1. The aqueous extract was prepared by adding 10 g of plant material to 100 mL of distilled water and then heating at 60-80 °C for 10 min and filtered through a Whatman No. 1 filter paper. After the synthesis, the mixture was centrifuged at 10,000 rpm for 15 min at 4 °C and the obtained material was washed three times prior to characterization (Table 1).

The yellow-to-brown colour change of the reaction mixture was a first sign of the formation of the nanoparticle preparation. The UV–visible spectrum taken at the wavelength range of 300–700 nm revealed a prominent absorption peak at 425 nm (Fig. 1). This absorption is consistent with the surface plasmon resonance characteristic of Ag nanoparticles (AgNPs) and is spectroscopic evidence of the formation of AgNPs. The value of the 425 nm maximum should be used especially in connection with the synthesis conditions summarized in Table 1. The characteristics of crystalline structure, elemental composition, surface charge and a full particle-size distribution, however, cannot be determined by UV–visible spectroscopy alone, but must be complemented.

Table 1. Experimental conditions used for aqueous extraction, AgNP synthesis and characterization.

Parameter	Reported condition
Plant extract	10 g plant material in 100 mL distilled water
Extraction	60–80 °C for 10 min; Whatman No. 1 filtration
AgNO ₃ precursor	0.001 M; 90 mL
Extract:AgNO ₃	10 mL : 90 mL
Reaction	150 rpm; ~25 °C; dark
Purification	10,000 rpm, 15 min, 4 °C; three washes
UV–visible	300–700 nm
FTIR	4000–400 cm ⁻¹
SEM	JEOL JSM-IT500

3.1 Green synthesis and UV–visible characterization

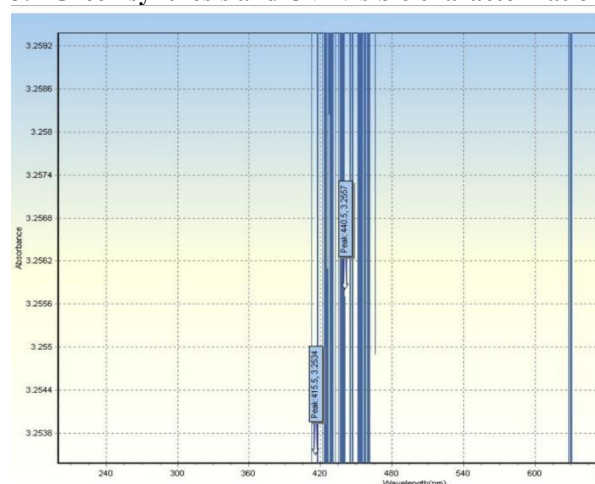


Figure 1. UV–visible spectrum of *B. hispida*-mediated AgNPs showing the reported absorption maximum at 425 nm.

The observed maximum is at 425 nm, which is in general agreement with the synthesis of AgNPs using plants where Ag⁺ ions could be reduced in the presence of plant metabolites that stabilize the surface of the AgNPs. The literature also shows that the optical response and physicochemical properties of the green-synthesized AgNPs can be influenced by the extract composition, concentration of precursors, temperature, pH and reaction time (Dhir et al., 2024; Fahim et al., 2024; Zulfiqar et al., 2024; Shahzadi et al., 2025). The present UV–visible result should thus not be considered as a definitive particle size or biological performance indicator, but rather as evidence for the formation of nanoparticles. Based on the results of the 425-nm SPR observation and the synthesis conditions in Table 1, subsequent FTIR and SEM analysis are conducted.

3.2 FTIR characterization

The FTIR spectrum contained eight reported bands at 3367.71, 2893.22, 1639.49, 1363.67, 1209.37, 696.30, 605.65 and 443.63 cm⁻¹. These bands correspond to several different environments in the range of functional groups involved in the preparation of the nanoparticles; namely, the ones containing oxygen and nitrogen and the fingerprint region (Fig. 2; Table 2). Individual assignments are only tentative since no complementary phytochemical profiling was reported.

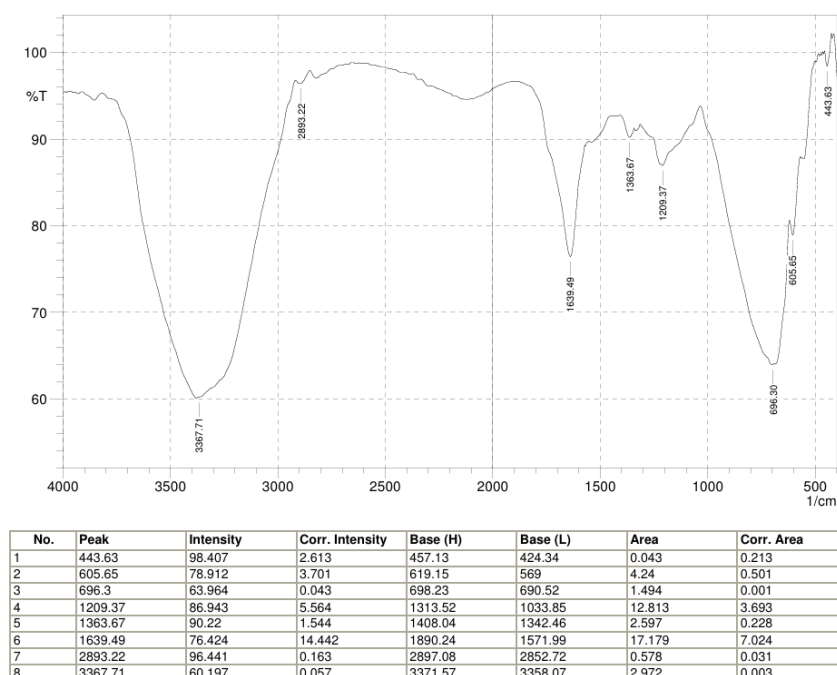


Figure 2. FTIR spectrum and peak table of the synthesized AgNPs

Table 2. FTIR bands recorded for *B. hispida*-mediated AgNPs and cautious functional-group interpretation.

FTIR band (cm ⁻¹)	Cautious interpretation
3367.71	O–H/N–H stretching region
2893.22	Aliphatic C–H stretching region
1639.49	C=O/C=C or amide-associated region
1363.67	C–H bending / fingerprint region
1209.37	C–O/C–N stretching region
696.30	Fingerprint-region vibration
605.65	Fingerprint-region vibration
443.63	Low-wavenumber fingerprint/metal-associated region; tentative

The FTIR profile supports the other indications of organic functional groups involved in the preparation of the nanoparticles shown below (Fig. 2; Table 2). The band at 3367.71 cm⁻¹ is in the O–H/N–H stretching region, and the band at 1639.49 cm⁻¹ is in a region which could be associated with carbonyl or C=C or amide related vibrations. The other signals are primarily in fingerprint areas. These assignments are deliberately tentative as it is not possible to identify individual flavonoids, alkaloids, tannins or other phytochemicals using FTIR only. Recent reviews also highlight that biomolecules derived from plants can also play a role in the reduction and stabilization of AgNPs, however, a complementary chemical profiling is needed to definitively identify (Dhir et al., 2024; Eker et al., 2025). The surface morphology and particle size of SEM were examined. SEM surface morphology and size were analyzed. SEM images revealed that nanoscale particles with two particle-size measurements were reported in the dissertation which is 67.21 and 92.47 nm. These are all smaller than 100 nm, which would be considered nanoscale. These two measurements are not a full particle size distribution, however, clusters can be seen in the data (Fig. 3; Table 1).

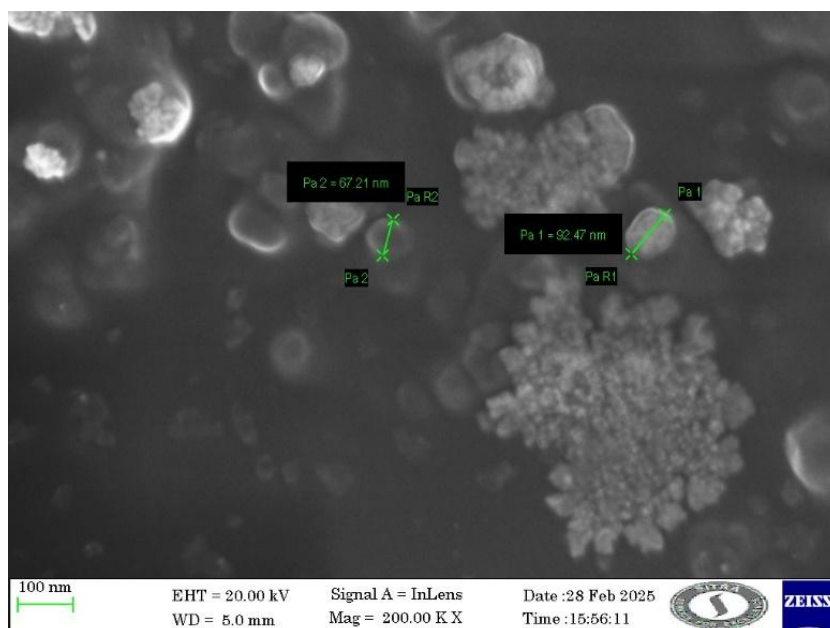


Figure 3. SEM image of *B. hispida*-mediated AgNPs with the reported size annotations.

Two reported particle-size measurements were obtained by SEM (67.21 and 92.47 nm) (Fig. 3; Table 1). The two observations are in the nano range, but in the micrograph clustered areas are visible, so these two measurements cannot be considered as a representative particle-size distribution. Vennila et al. (2018) previously reported the synthesis of AgNPs using *Spermacoce hispida* under optimized conditions, and described the predominantly rod shaped particles that resulted from the synthesis condition, showing that synthesis conditions can generate different nanoparticle characteristics from the same plant source. The anti-inflammatory activity was assessed in vitro. The anti-inflammatory activity was measured in vitro. As per the protein-denaturation assay, with increase in the reported AgNP sample volume, there was a significant decrease in absorbance. The absorbance decreased from 1.976 to 0.540 at 1mL, corresponding to 72.67% apparent inhibition, based on the absorbance of the blank (Table 3; Fig. 4). The data is reported as sample volume not AgNP concentration, so the response is reported as apparent inhibition not concentration-based potency.

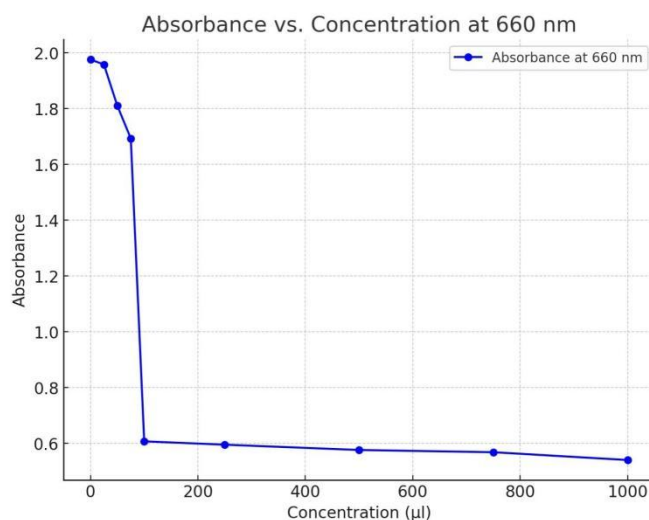


Figure 4. Absorbance at 660 nm as a function of the reported AgNP sample volume.

The protein-denaturation assay demonstrated a significant decrease in absorbance with the reported sample volume of AgNP (Fig. 4), with 72.67% apparent inhibition at 1 mL. Fig. 4 shows that the highest change was observed between the range of 75 and 100 µL, absorbance values ranging from 1.693 to 0.607. Protein-denaturation assays are early screening test in the assessment of anti-inflammatory activity using plant mediated AgNP (Mohamed et al., 2024; Diallo et al., 2024; Kar et al., 2025). However, the present data are not presented as dose of AgNP per concentration of AgNP, and replicates, standard deviations and a numerical response for the positive-control were not available. Hence, the 72.67% is not a potency estimate based on the IC₅₀ or an evidence of anti-inflammatory activity but rather apparent assay inhibition. **3.5 Antidiabetic effect in vitro.** An α -amylase assay was performed to determine the effect of the reported AgNP sample volume and absorbance was observed to decrease as the volume increased. Absorbance of the blank was 1.795, while Absorbance at 1.0ml was 1.465, which is 18.38% apparent inhibition compared to blank (Fig. 5). The result suggests an assay response for the conditions reported, but does not demonstrate therapeutic antidiabetic activity.

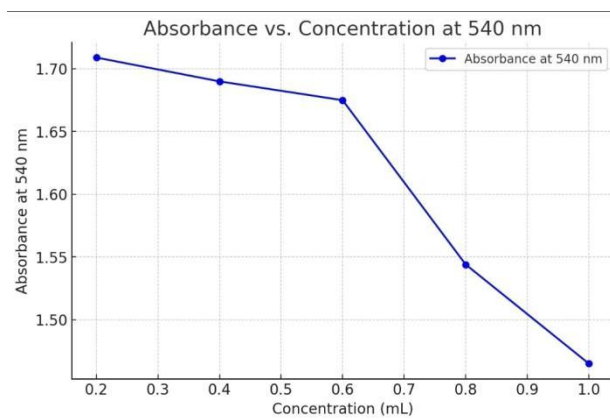


Figure 5. Absorbance at 540 nm as a function of the reported AgNP sample volume.

Table.3 Comparison of the present study with selected previous studies on plant-mediated silver nanoparticles

Study	Plant source / route	Key characterization or assay	Main reported finding	Relevance to present study
Vennila et al. (2018)	Spermacoce hispida aqueous leaf extract	UV-Vis, SEM, FTIR, XRD, EDX; antioxidant, anti-inflammatory and other assays	AgNPs formed under optimized 1 mM AgNO ₃ , pH 8, 40 °C, 10 min; predominantly rod-shaped	Directly relevant species-level precedent; confirms this is not the first <i>S. hispida</i> -mediated AgNP report.
Mohamed et al. (2024)	Four plant extracts	UV-Vis, SEM, FTIR; protein-denaturation anti-inflammatory assay	Plant-mediated AgNPs showed measurable anti-inflammatory activity	Supports protein-denaturation assay as preliminary screening, not proof of mechanism.
Bhardwaj et al. (2024)	Balanites aegyptiaca leaf/seed extracts	UV-Vis, FTIR, SEM, TEM, DLS, zeta potential; α -amylase	AgNPs showed α -amylase inhibition up to 72.71–74.04% in their assay	Shows the value of concentration-based dosing and broader characterization for antidiabetic claims.
Diallo et al. (2024)	Aphania senegalensis leaf extract	Green synthesis and anti-inflammatory testing	Plant-mediated AgNPs demonstrated anti-inflammatory activity	Provides recent support for evaluating plant-mediated AgNPs using anti-inflammatory assays.
Kar et al. (2025)	Clerodendrum thomsoniae leaf extract	UV-Vis, XRD, EDX; protein-denaturation assay	SPR at 427 nm; mean particle size 47 nm; IC ₅₀ 53.58 $\pm$ 17.78 μ g/mL for protein-denaturation inhibition	Illustrates the stronger evidence obtained when particle characterization and concentration-based IC ₅₀ analysis are available.
Present study	Borreria hispida aqueous preparation	UV-Vis, FTIR, SEM; protein-denaturation and α -amylase assays	SPR maximum 425 nm; SEM measurements 67.21 and 92.47 nm; apparent inhibition 72.67% and 18.38% at highest reported volumes	Preliminary dataset using a distinct synthesis protocol; concentration-normalized and replicate-based confirmation is needed.

It is seen that there is a small decrease between 0.2 to 0.6 mL followed by a larger decrease at 0.8–1.0 mL (see figure 5). This is consistent with the α -amylase inhibitory activity of plant mediated AgNPs, but higher studies tend to employ concentration based dosing and if done, the wider physicochemical characterization. For instance, Bhardwaj et al. (2024) performed α -amylase testing and SEM, TEM, DLS and zeta-potential characterization. Therefore, the present response does not qualify for a therapeutic anti-diabetic comment. The characterization and the assay results together

with the present work clearly indicate that this is not the first report of the synthesis of AgNP using *B. hispida*/*S. hispida*. Previous works by Vennila et al. (2018) already reported the synthesis of AgNPs using the aqueous extract of *S. hispida*, and recent works highlight concentration-normalized dosing, repeated measurement and complementary characterization methods for a better understanding of the biological interpretation (Bhardwaj et al., 2024; Kar et al., 2025; Shahzadi et al., 2025).

Thus, the novelty of the present study lies in the synthesis procedure using an aqueous extract, the 425-nm SPR profile, the SEM observations and the preliminary anti-inflammatory and α -amylase assay responses. Comparisons should not be made directly between studies as there is variation in plant sources, extraction methods, concentrations of the precursor and assay methods. Study limitations and recommendations 4.1 Major drawbacks are the lack of biological replicates, standard deviations, inferential statistics, IC50 values, and full numerical results for the positive controls, and reporting the biological assays as sample volumes rather than concentration-normalized AgNP doses, along with only two SEM size measurements that are visible aggregation, and lack of reported DLS/TEM size distributions, XRD, and numerical zeta potential. These restrictions limit the quantitative potency and mechanistic interpretation, but not the preliminary observations. Independent replicates, concentration based dosing, complete controls, statistical analysis, DLS/TEM, XRD, zeta potential, elemental analysis and quantitative phytochemical profile and toxicity evaluation are suggested for future investigations. In future, novelty is not to be claimed as the synthesis of AgNPs using *S. hispida* has already been reported, but based on other factors such as optimized synthesis conditions, physicochemical properties, and stronger biological evidence.

5. CONCLUSION

The aqueous extract of *B. hispida* was able to support the green synthesis of AgNPs under the reported experimental conditions. The results of UV–visible spectroscopy exhibited a peak at 425 nm (Fig. 1; Table 1), FTIR revealed multiple bands for the functional groups associated with the plants (Fig. 2; Table 2), and SEM yielded two reported particle size measurements: 67.21 and 92.47 nm (Fig. 3). The apparent inhibition in the protein-denaturation assay was 72.67% at the highest volume of the sample reported (Table 3; Fig. 4), while in the α -amylase assay, the apparent inhibition was 18.38% at 1.0 mL volume of the sample (Fig. 5). The study offers preliminary evidence that the selected *B. hispida* preparation is able to produce a biologically capped AgNP preparation that can be detected by an in vitro assay. Concentration-normalized dosing, replicate-based statistics and complete positive-control results, as well as several advanced physicochemical measurements, are lacking, however, which limits the ability to interpret the results as proof of therapeutic anti-inflammatory or antidiabetic efficacy. Furthermore, synthesis of AgNP with *S. hispida* has already been reported (Vennila et al., 2018) and thus the novelty of the present work is the specific conditions used for the synthesis as well as the characterization data set and the preliminary profile of the bioactivity of the synthesized nanoparticles. Additional controlled studies are needed to demonstrate reproducibility, mechanism of action, and biological activity and safety.

Declarations

Ethics approval and consent to participate: Not applicable; the present manuscript reports in vitro assays and no human or animal experiments.

Consent for publication: Not applicable.

Data availability: The numerical data reported in this manuscript were derived from the dissertation dataset. Additional raw laboratory records should be made available by the authors upon reasonable request, subject to institutional policies. Competing interests: The authors should confirm the appropriate competing-interest statement before submission.

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