



Original article



Green synthesis and evaluation of anti-microbial, antioxidant, anti-inflammatory, and anti-diabetic activities of silver nanoparticles from *Argyrea nervosa* leaf extract: An *invitro* study

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ABSTRACT

The aim of the study is to develop an environmentally friendly development of isolating silver nanoparticles using *Argyrea nervosa* plant leaves and examine their potential pharmacological properties, including antibacterial, anti-inflammatory, antioxidant, and anti-diabetic activities. Various analytical techniques were employed to examine the isolated silver nanoparticles, encompassing UV spectroscopy, XRD, SEM, EDX, FTIR, zeta potential measurement, and particle size determination. Furthermore, *Argyrea nervosa*-mediated silver nanoparticles exhibited strong antioxidant, anti-inflammatory activity, and good anti-diabetic activity. *Argyrea nervosa*-mediated nanoparticles showed a significant antibacterial activity against selected pathogens. The findings indicate that *Argyrea nervosa* demonstrates encouraging pharmacological properties. However, additional *in vivo* research focusing on molecular-level investigations is necessary to substantiate these findings and explore the potential of *Argyrea nervosa*-mediated silver nanoparticles as a viable drug for antioxidant, antibacterial, and anti-diabetic purposes.

1. Introduction

Promising and multi-disciplinary area of biotechnology combined with nanotechnology known as nanobiotechnology. Nanotechnology is an emerging and rapidly expanding field that is revolutionizing science and technology by focusing on the development of novel nanomaterials (Albrecht et al., 2006). In recent years, biosynthetic techniques based on nanoparticle (NP) preparation have used biological microbes like fungi, bacteria, and plant extracts. Metal-based nanoparticles can be produced using numerous chemical, biological, physical, and hybrid processes (Tiwari et al., 2008). Nanotechnology has applications in many fields including medicine, biotechnology, chemistry, physics, and material science. In parallel, metal-based nanoparticles such as gold (Au),

selenium (Se), silver (Ag), zinc sulfide (ZnS) and zinc oxide (ZnO) gained more attention due to their specialty and specificity (Malarkodi et al., 2014; Vanaja et al., 2014). Among these NPs, silver nanoparticles (AgNPs) are easy to synthesize and user-friendly metals. Moreover, AgNPs are effective, chemically stable, and has been shown to have antibacterial, antiviral, antifungal, and easily synthesized (Balavijayalakshmi and Ramalakshmi, 2017; Palanisamy et al., 2023). Silver-impregnated topical dressings are now often used to treat wounds, burn-related infections, and chronic ulcers. Since the chemical methods-based synthesis of nanoparticles have harmful effects as they are environmentally hazardous, in the current study, we used physical method-based nanoparticle preparation as these methods are user-friendly, easy to synthesize, and harmless methods (Raveendran et al., 2003).

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Medicinal herbs and plant extracts are now widely recognized as effective medications worthy of respect and value, and they play an important part in modern medicine. According to the WHO, 80% of the total population uses herbs for primary healthcare. The Indian subcontinent is home to a wide variety of herbal plants used in the Indian system of medicine (Mukherjee et al., 2012; Saminathan et al., 2022). Plant-based treatments have traditionally been recognized as the very first line of defense in sustaining health and treating disease (Ballabh and Chaurasia, 2007). *Argyrea nervosa* (*A. nervosa*) is one of the most medically valuable plants, it has pharmacologic activities like hypoglycemic, anti-inflammatory, antiulcer, antimicrobial, immunomodulatory, anticonvulsant, etc. *A. nervosa* is commonly known as morning glory, Hawaiian Baby Wood Rose, and elephant Creeper. The plant has the potential to attain a height exceeding 10 m. It is a woody shrub, mostly found edges of the lake and semi-deciduous forests of the Western Ghats of India (Thombre et al., 2014). Traditionally used for gonorrhoea and chronic ulcer management. This is also used for an ornamental plant because of its attractive leaves and flower colors (Paulke et al., 2015). Leaf has potential anti-cancer and anti-bacterial activity (Subramanyam et al., 2021). Bloom is funnel-shaped violet or lavender in color. while leaves contain, β -sitosterol, 1-tricentanol, flavonoids, lipids, and quercetin (Singhal et al., 2011). Roots are used for obesity management, diabetes, tuberculosis, ulcer, and wound management (Milimita et al., 2013). The seeds in the dried-out flower pods are black in color. Each flowering pod contains three to five seeds (Meher and Padhan, 2011). Based on these realities, current research focused on AgNPs isolated from *A. nervosa* leaf extracts for their characterization, and analysis of *in-vitro* biological activities.

2. Methodology

2.1. Chemicals

In this study, silver nitrate extra pure AR, 99.9 %, DPPH, ABTS, potassium persulfate, ascorbic acid, α -amylase, α -glucosidase, and p-NPG were purchased from Sigma. Standard drugs ciprofloxacin and acarbose were procured from the pharmacy. The microbial pathogens such as *Escherichia coli* (*E. coli*), and *Pseudomonas aeruginosa* (*P. aeruginosa*) were sourced from Pharmacology Department, Saveetha dental college, Chennai.

2.2. Extract preparation

The fresh *A. nervosa* leaves were collected from Velliangiri Hills, India. The plant sample was authenticated by the National Institute of Siddha Ministry of Ayush, Chennai (certificate no: NISMB5592023, supplementary Fig. S1). The leaves were cleaned with normal water to prepare the plant extract, followed by a rinse with distilled water. After the air-drying process, the leaves samples were in the shade for 5–7 days, they were finely powdered using a mixer grinder. 5 g of the powdered leaves were mixed with sterilized water (100 ml) and heated to 60 °C for 30 min and then allowed to cool. After filtration through Whatman filter paper, the resultant clear extract was collected and kept at 4 °C for the following process.

2.3. AgNPs synthesis from the *A. nervosa* extract

5 ml of prepared *A. nervosa* aqueous extract was added to 20 mM pure aqueous silver nitrate (AgNO_3) solution and kept for 60 min to reduce silver nitrate to silver nanoparticles (AgNPs). The color of the solution changed from yellow to dark brown after the reduction of Ag^+ by Ag^0 nanoparticles were confirmed that the synthesis of AgNPs (Balavijayalakshmi and Ramalakshmi, 2017).

2.4. Nanoparticle characterization

The confirmation of AgNP synthesis was done using UV–Vis absorption spectra. To investigate the structure and composition of the synthesized AgNPs, XRD analysis was conducted using a Bruker instrument, Germany. FTIR spectroscopy was used to study the possible functional groups involved in the synthesis and stabilization of AgNPs. The shape and size of the AgNPs were characterized using SEM, and the presence of elemental Ag was confirmed using EDX. Zeta potential was measured using a Microtra instrument, which is an important parameter for characterizing stability in aqueous nanosuspensions. The particle size investigation method determined the average particle size of the synthesized AgNPs.

2.5. Inoculum preparation and anti-bacterial activity of *A. nervosa* AgNPs

A disc diffusion process was employed to estimate the bacterial efficiency of AgNPs derived from *A. nervosa*. In this study, ciprofloxacin was used as an antibiotic agent. The concentration range of AgNPs tested was 10–30 $\mu\text{g/ml}$. The agar plates were incubated at 37 °C for a duration of 12 h. On a subsequent day, the inhibition zone for each sample was measured and recorded in millimetres (mm) (Shanmugam et al., 2021).

2.6. DPPH activity

A range of concentrations for *A. nervosa* AgNPs (100–500 $\mu\text{g/ml}$) and plant extracts were obtained by performing serial dilution with a 1 mg/ml stock solution. Subsequently, the mixture was incubated for half an hour and read at 517 nm using a spectrophotometer (Hatano et al., 1990). The inhibition (%) for scavenging activity was calculated using the formula:

$$\% \text{ of inhibition} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100 \quad (1)$$

2.7. ABTS antioxidant assay

Test sample concentrations range of 100–500 $\mu\text{g/ml}$ for both AgNPs and plant extract were used for antioxidant assay. Briefly, 50 μl of the sample and 950 μl of the solution ABTS made up of the 1 ml reaction mixture of the standard and extracts. For 10 s, the reaction solution was vortexed and read at 734 nm. In this analysis, ascorbic acid served as the standard, and the percentage (%) was calculated using Eq. (1).

2.8. Hydrogen peroxide (H_2O_2) activity

Concentrations ranging from 100 to 500 $\mu\text{g/ml}$ of leaf extract and AgNPs were employed in the experiment with ascorbic acid as the standard. Briefly, a mixture containing 50 μl of H_2O_2 (5 mM) solution was prepared and incubated at room temperature (26 ± 2 °C) for a duration of 20 min and read at 610 nm. Eq. (1) was utilized to calculate the H_2O_2 radical scavenging activity.

2.9. Albumin denaturation inhibition

The albumin denaturation assay was carried out as described by Mizushima and Kobayashi (1968) Diclofenac sodium was used as a standard for this procedure. The reaction mixture consisted of sample concentrations levels ranging from 100 to 500 $\mu\text{g/ml}$, along with 1 % aqueous solution of albumin fraction. The absorbance of the mixture was measured within the range of 660 nm. The percentage (%) of inhibition was determined using Eq. (1) for calculation purposes.

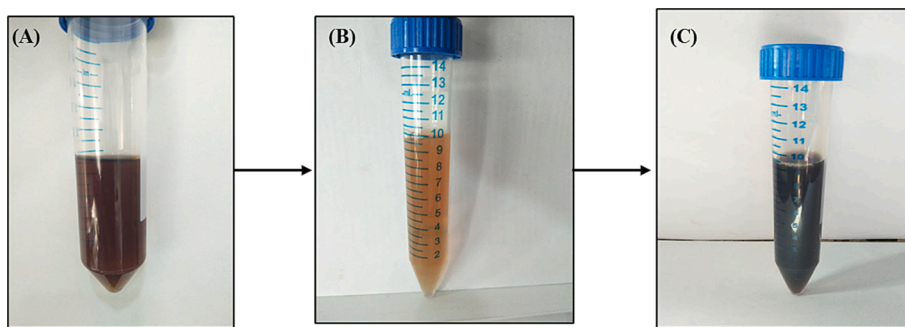


Fig. 1. Represents AgNPs synthesized in *A. nervosa* leaf extract. (a) Leaf extract without the addition of silver nitrate (AgNO_3), (b) After adding Ag solution, (c) *A. nervosa* Leaf extract with Ag solution.

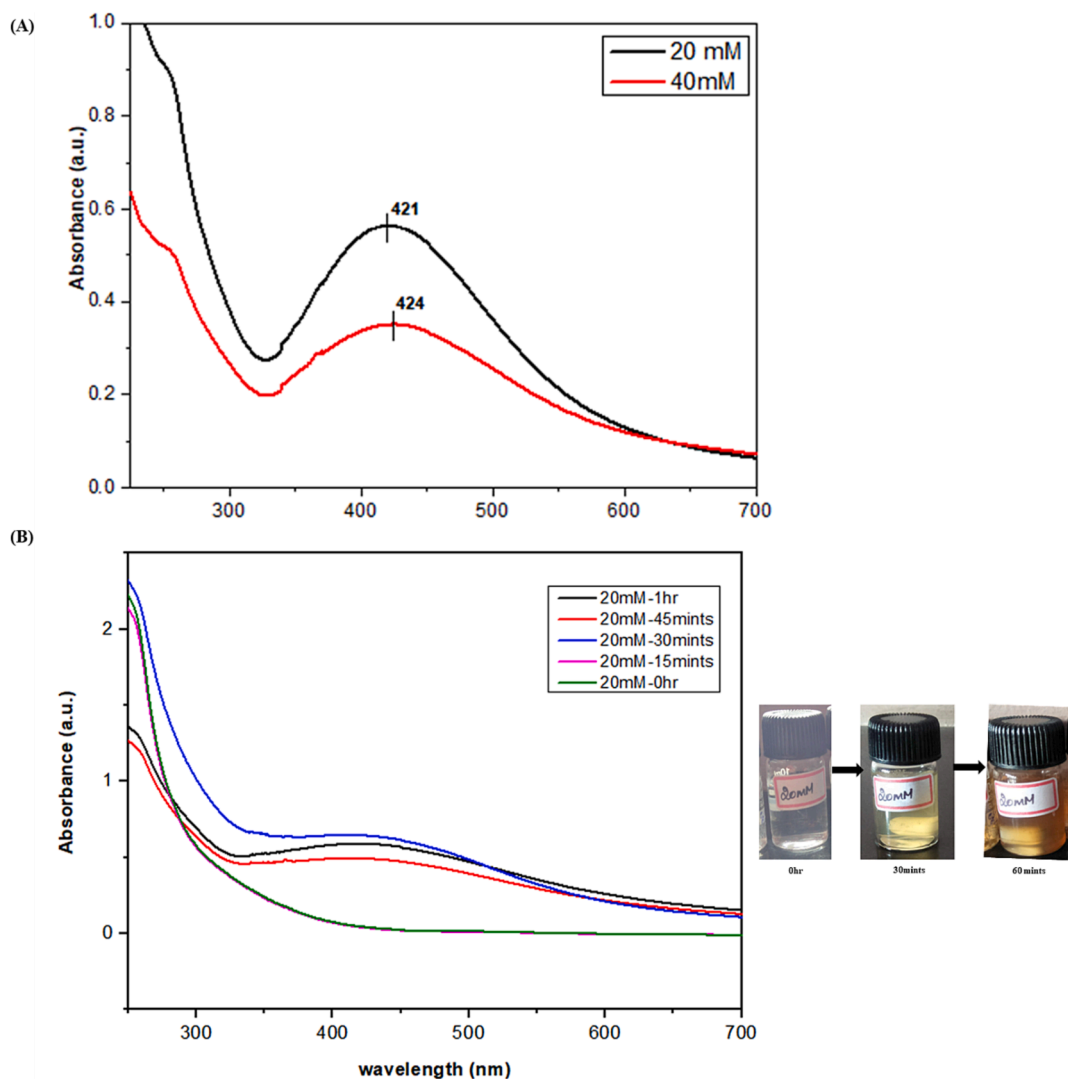


Fig. 2. (a) UV- spectrum record of *A. nervosa* AgNPs in a concentration-dependent manner (20 mM and 40 mM). (b) Time-dependent manner analysis was performed on the duration of 0 hr, 15, 30, 45 min, and 1 hr.

2.10. α -Amylase inhibition

The antidiabetic activity of *A. nervosa*-mediated AgNPs was estimated *in vitro* by a standard protocol with acarbose as a reference substance and the absorbance was subsequently measured at 540 nm (Hansawadi et al., 2000). Results were expressed as % of inhibition of α -amylase inhibitory activity.

2.11. α -Glucosidase activity

The α -glucosidase enzyme inhibitory activity was done by standard (Yarrappagaari, et al., 2020; Yin et al., 2008). The results were expressed in % of inhibition of α -glucosidase activity.

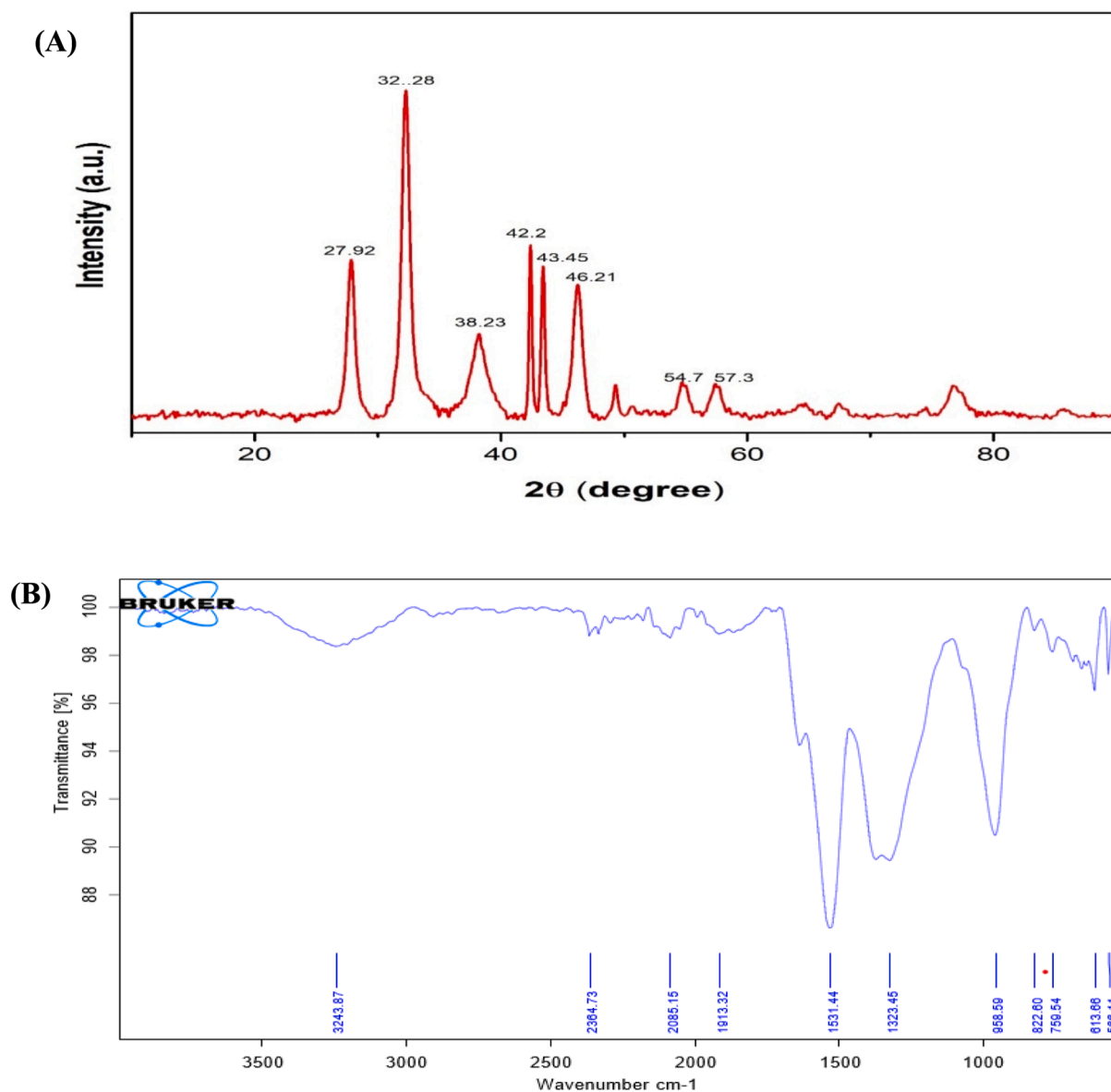


Fig. 3. (a) The XRD pattern of *A. nervosa* mediated AgNPs, where the X-axis indicates the 2θ scale, and the Y-axis denotes the intensity of the AgNPs. (b) The FTIR spectrum of *A. nervosa* mediated AgNPs. Where the X-axis denotes the wavenumber in cm^{-1} , and the Y-axis shows the transmittance of the AgNPs.

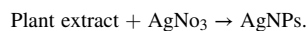
3. Statistical analysis

The data were expressed as the mean \pm SEM of three replicates. One-way analysis of variance (ANOVA) was used, followed by Tukey's test. $p < 0.05$ had been considered statistically significant.

4. Results

4.1. Observation of color changes of AgNPs

The synthesized AgNPs are generally confirmed by their, range, shape, and surface area with dispersity. Apart from these characteristics, simple color development is also used to identify the presence of AgNPs. When silver solution was added to the extract, the plant aqueous extract color was altered from light brown to dark and deep brown color (Fig. 1). These color changes indicate the presence of AgNPs. In this linking, earlier studies showed comparable color changes to form dark brown color (Subramanyam et al., 2021). Due to the reduction of Ag^+ ions converted to Ag^0 atoms.



4.2. UV-Vis spectral analysis of AgNPs

To find the exact concentration for the AgNPs preparation initially, we need to find the metal solution reactivity to our plant extract. At the preliminary stage of the synthesis, the reduction of Ag ions in the aqueous solution of the Ag complex was observed through UV-Vis spectroscopy, indicating the interaction with the components presents in the *A. nervosa* extract. For this UV spectroscopy analysis, 20 mM and 40 mM of Ag solution were used for the preparation of the *A. nervosa* mediated AgNPs preparation (Fig. 2a). The UV spectrum absorbance measurement for a 20 mM Ag solution yielded an absorbance value of 421 nm, while the 40 mM concentration exhibited an absorbance value of 424 nm. Based on these results, time-dependent analyses were conducted for the 20 mM concentration at different time intervals, including 0 h, 15 min, 30 min, 45 min, and 1 h (Fig. 2b).

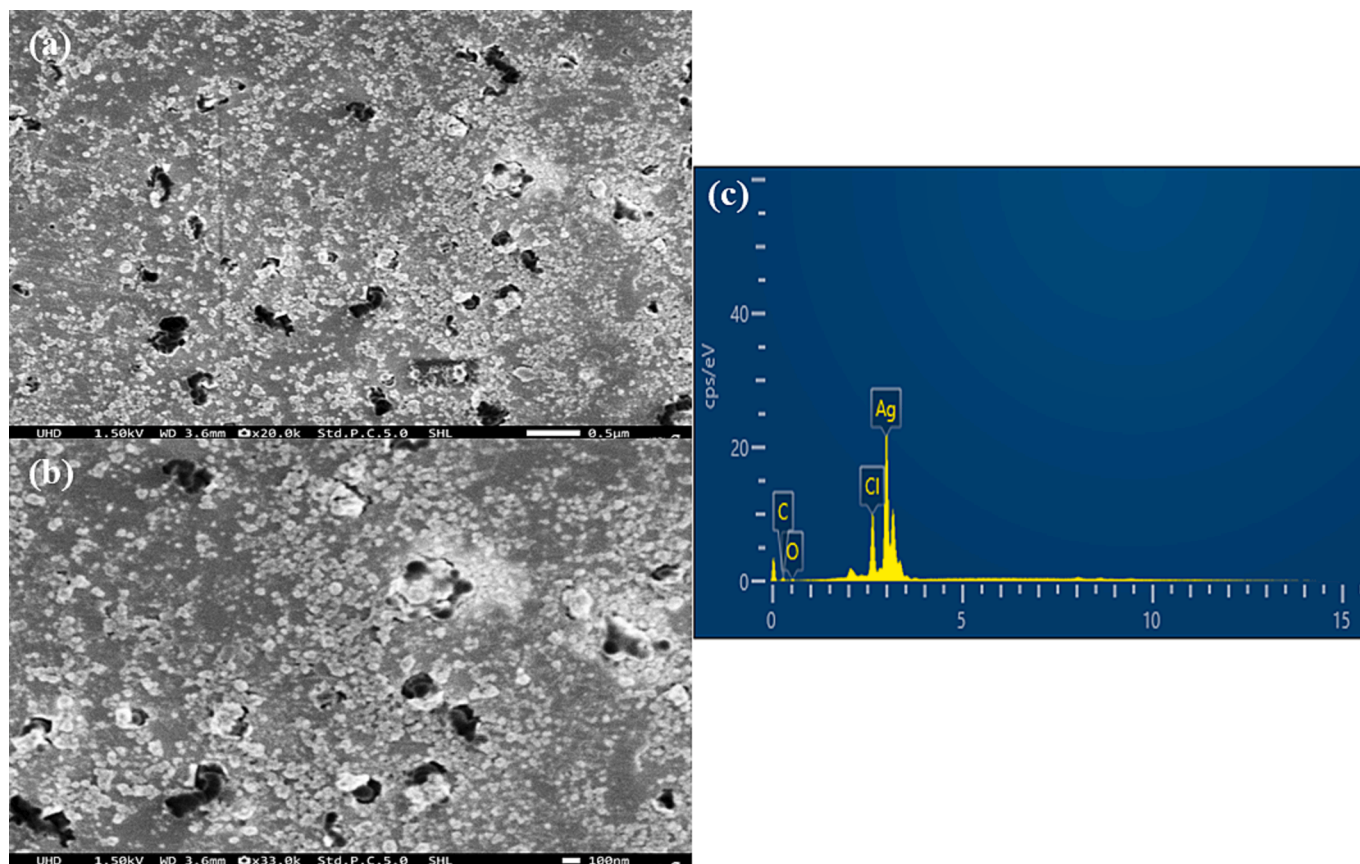


Fig. 4. SEM Analysis of *A. nervosa* mediated AgNPs (a) 50 μm , (b) 100 nm. (c) EDX analysis of AgNPs prepared with *A. nervosa* leaf extract.

4.3. XRD and FTIR investigation

The nature of crystalline AgNPs was verified by the XRD diffraction diagram (Fig. 3a). The calculation of the crystallite sizes of AgNPs synthesized using *A. nervosa* extract was performed using Debye-Scherrer's equation, as described by Gnanajobitha et al. (2013).

$$D = \frac{K\lambda\beta}{\cos\theta}$$

The inorganic biomolecules in the fucoidans responsible for AgNP formation were analyzed using the FT-IR analysis (Fig. 3b).

4.4. SEM and EDX analysis

Fig. 4a and b depicts the morphology of *A. nervosa*-mediated AgNPs. For SEM analysis, dried nanoparticles were utilized, and two magnification ranges were employed to visualize their presence namely 50 μm , and 100 nm. EDX results demonstrate the purity and the complete chemical composition of *A. nervosa* mediated AgNPs. The percentage of elements followed by Ag is 82.3 %, chlorine (Cl) 10 %, carbon (C) 5.2 %, and oxygen (O) 2.6 % respectively. The additional elements assisted as capping organic agents bound to the surface of the AgNPs (Fig. 4c).

4.5. Zeta potential measurement and particle size analysis

The synthesized AgNPs were dispersed into the double distilled water to analyze the zeta potential. The zeta potential of the prepared AgNPs was -16.4 mV and the zeta deviation was 7.89 mV as shown in Fig. 5a. The particle size investigation results clearly demonstrated the distribution of AgNPs in the reaction mixture according to intensity, number, and volume of AgNPs respectively. Before analyzing particle size, NPs were suspended in 10 ml of water and sonicated for 30 s. The

average particle size detected in 69.54 nm (Fig. 5b).

4.6. Antimicrobial activity

The present study investigated the antibacterial action of *A. nervosa* AgNPs against *E. coli* and *P. aeruginosa*. This study was carried out using the following samples: *A. nervosa* AgNPs at 10, 20, and 30 μg concentration, ciprofloxacin (antibiotic control, 30 μl concentration) (Fig. 6). Increasing concentration of *A. nervosa* AgNPs formed improved the ZOI for both tested species. *A. nervosa*-mediated AgNPs showed increased antimicrobial activity in terms of ZOI in both tested organisms (Table 1).

4.7. DPPH antioxidant assay

The *A. nervosa* leaf extract and AgNPs antioxidant activity conformed via performing the DPPH free radical scavenging activity (Fig. 7a and Table 2a). This is a commonly used method for quantifying free radical scavenging action. A decrease in DPPH is indicated by a change in color from violet to yellow, demonstrating the capability of the extract to donate a proton to the DPPH radical (Kawra et al., 2020).

4.8. ABTS assay

The current study examined the ABTS test on both AgNPs and extract of *A. nervosa* (Fig. 7b and Table 2b). ABTS is recognized as a protonated radical capable of accepting electrons from antioxidant compounds, resulting in a color change from blue to pink, as described by Gangwar et al. (2013).

4.9. H_2O_2 scavenging activity

H_2O_2 activity of *A. nervosa* leaves extract and AgNPs capped with

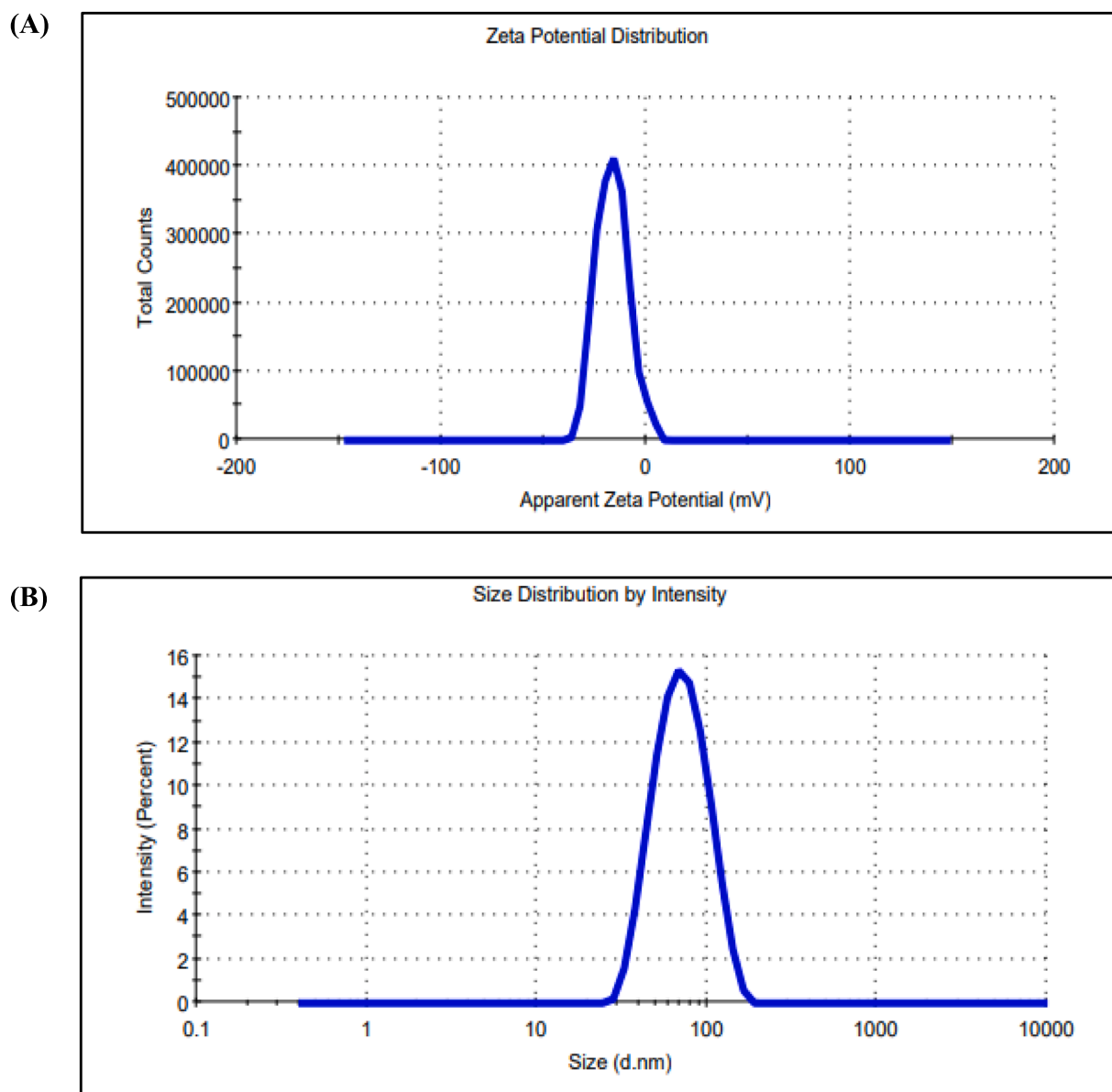


Fig. 5. (a) Zeta potential measurement and (b) Particle size of AgNPs with respect to intensity, number, and volume of AgNPs.

plant extract showed H_2O_2 scavenging activity by transferring electrons to H_2O_2 and neutralizing it into water- H_2O_2 inhibition activity assay plays a dynamic part in the determination of antioxidant activity. The results obtained for the H_2O_2 scavenging activity closely resembled those obtained for the DPPH radical scavenging activity. The *A. nervosa* AgNPs displayed potential H_2O_2 radical action in a dose-dependent manner (Fig. 7c and Table 2c).

4.10. Albumin denaturation inhibition

In the present study, anti-inflammatory action was performed by albumin denaturation assay. The outcomes were compared to the standard drug diclofenac sodium. The assay confirmed the anti-inflammatory action of *A. nervosa* AgNPs whose efficacy was nearest to the standard drug as shown in Fig. 8a and Table 3a.

4.11. The activity of α -amylase inhibition

The study found that AgNPs can reduce the activity of enzymes that break down complex carbohydrates and increase glucose consumption,

as previously described by Sengottaiyan et al. (2016) and Balan et al. (2016). In the current study, it was observed that an increase in the concentration of AgNPs resulted in a corresponding dose-dependent increase in the inhibition percentage of α -amylase (Fig. 8b and Table 3b).

4.12. α -Glucosidase inhibition

The α -glucosidase is an important enzyme in carbohydrate metabolism, according to research, inhibiting α -glucosidase can delay carbohydrate digestion, and absorption, and decrease blood sugar levels (Khan et al., 2014). AgNPs synthesized from *A. nervosa* showed a significant increase in α -glucosidase inhibitory action in a dose-dependent manner (Fig. 8c and Table 3c).

5. Discussion

Currently, there is a global focus among researchers to develop eco-friendly and cost-effective medicine. The current study purposed to investigate the synthesis and characterization of *A. nervosa* AgNPs, as

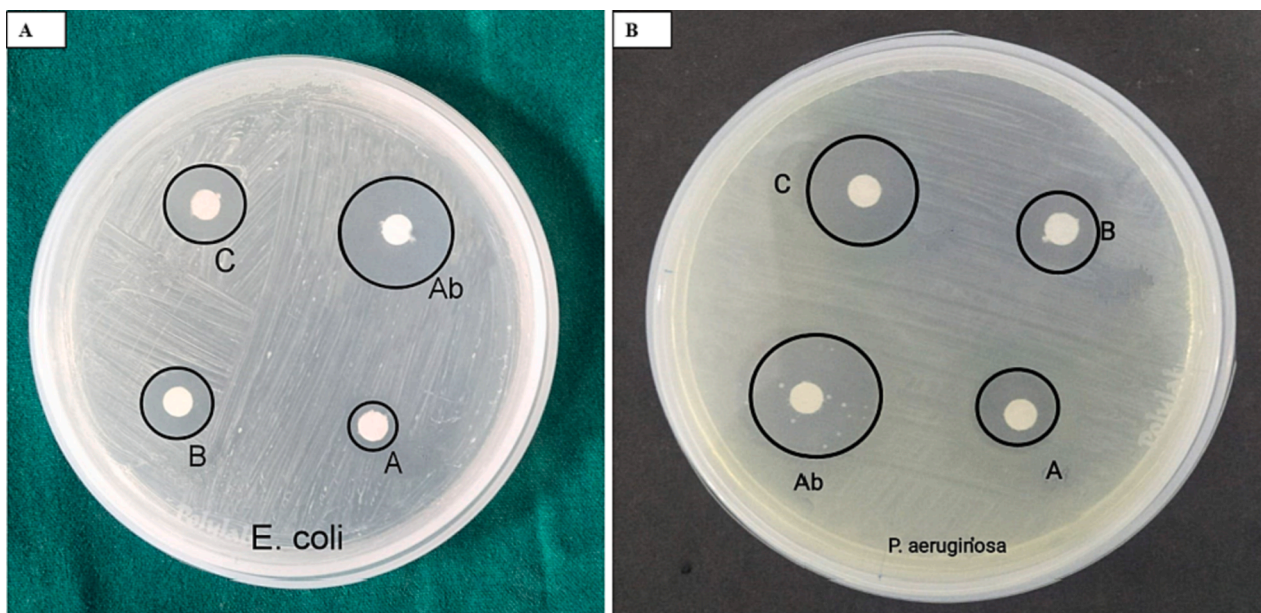


Fig. 6. Photograph of petri dishes with loaded samples of *A. nervosa* mediated AgNPs against (a) *E. coli*, (b) *p. aeruginosa*. Ab-antibiotic drug (30 μ l), a-10 μ l, b-20 μ l, c-30 μ l concentrations.

Table 1

The ZOI of *A. nervosa* AgNPs and standard using different concentrations such as 10, 20, 30 μ l, and standard 30 μ l.

Name of the pathogen	Standard (30 μ l)	AgNPs ZOI (mm)		
		10 μ l	20 μ l	30 μ l
<i>E. coli</i>	12.5	3.5	6	7.8
<i>p. aeruginosa</i>	15.5	5.5	7	10.5

well as their potential therapeutic effects in terms of antibacterial, antioxidant, anti-inflammatory, and anti-diabetic properties. By utilizing a green synthesis approach, the current study aimed to produce nanoparticles that are both effective and environmentally safe. The outcome of these findings could contribute to the development of new and more accessible treatments for patients in need.

The UV-visible spectroscopy also enables the quantitative analysis of AgNPs. By measuring the absorbance at a specific wavelength and comparing it to a calibration curve obtained from known concentrations of AgNPs, the concentration of the nanoparticles in a sample can be determined. This quantitative analysis is valuable for various applications, including nanoparticle synthesis, quality control, and assessing

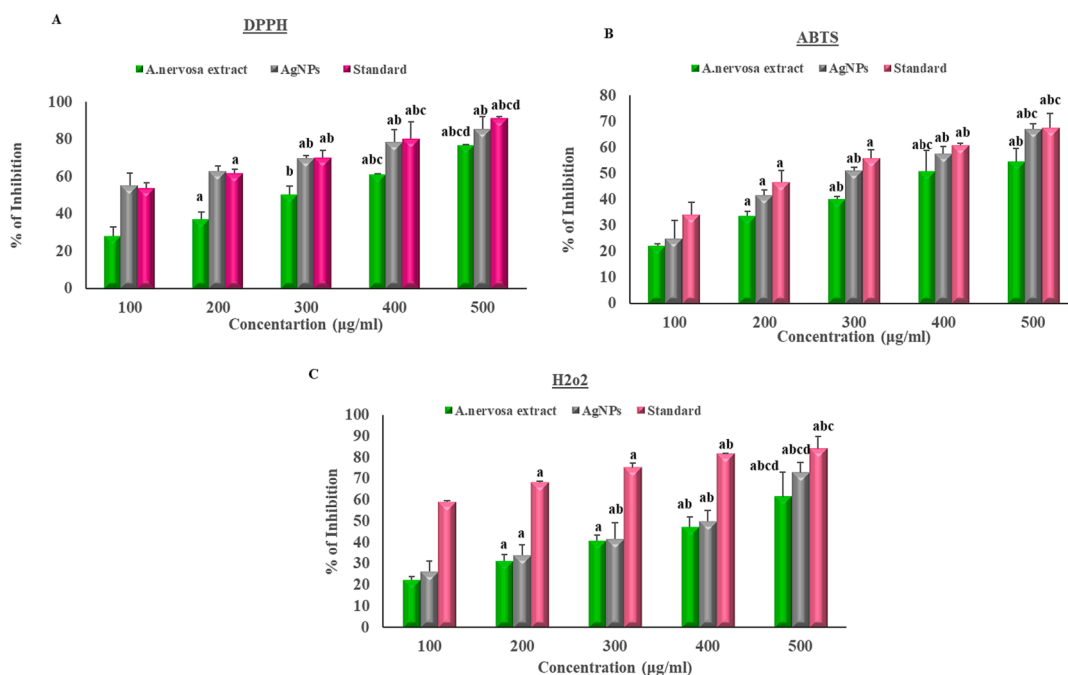


Fig. 7. Effect of *A. nervosa* and AgNPs on (a) DPPH activity, (b) ABTS assay, (c) H_2O_2 . The X-axis shows the different concentrations of AgNPs and extract and the Y-axis denotes the % of inhibition. (a-significance to 100 μ g/ml, b-significance to 200 μ g/ml, c-significance to 300 μ g/ml, and d-significance to 400 μ g/ml).

Table 2

(a) DPPH activity, (b) ABTS assay, (c) H₂O₂ activity of the extract, *A. nervosa* mediated AgNPs and standard drug, (a-significance to 100 µg/ml, b-significance to 200 µg/ml, c-significance to 300 µg/ml, and d-significance to 400 µg/ml). Values are expressed in mean ± SEM of 3 replications.

(A)			
Concentration (µg/ml)	DPPH % of scavenging activity		
	Extract	AgNPs	Standard
100	27.92 ± 5.1	55.33 ± 6.4	53.81 ± 2.7
200	37.15 ± 3.8 ^a	62.81 ± 2.8	61.83 ± 2.1 ^a
300	50.23 ± 4.8 ^b	69.8 ± 1.3 ^{ab}	70.13 ± 3.8 ^{ab}
400	61.23 ± 0.2 ^{abc}	78.33 ± 6.8 ^{ab}	80.1 ± 9.1 ^{abc}
500	76.91 ± 0.1 ^{abcd}	85.43 ± 6.6 ^{ab}	91.3 ± 0.7 ^{abcd}
(B)			
Concentration (µg/ml)	ABTS % of scavenging activity		
	Extract	AgNPs	Standard
100	22.04 ± 1	25 ± 7	34.19 ± 4.8
200	33.63 ± 1.6 ^a	41.66 ± 2 ^a	46.5 ± 4.7 ^a
300	40.04 ± 1 ^{ab}	51.13 ± 1.2 ^{ab}	55.9 ± 3.2 ^a
400	50.95 ± 8 ^{abc}	57.63 ± 2.6 ^{ab}	60.8 ± 0.9 ^{ab}
500	54.56 ± 5.1 ^{ab}	67.09 ± 2 ^{abc}	67.5 ± 5.7 ^{abc}
(C)			
Concentration (µg/ml)	H ₂ O ₂ % of scavenging activity		
	Extract	AgNPs	Standard
100	22.48 ± 1.5	26.26 ± 5	58.98 ± 0.7
200	31.07 ± 3.1 ^a	33.95 ± 5 ^a	68.15 ± 0.7 ^a
300	40.72 ± 2.7 ^a	41.63 ± 7.5 ^{ab}	75.15 ± 2 ^a
400	46.94 ± 4.9 ^{ab}	49.91 ± 5 ^{ab}	81.58 ± 0.2 ^{ab}
500	61.50 ± 11.6 ^{abcd}	72.60 ± 5 ^{abcd}	83.97 ± 6 ^{abc}

the stability of AgNPs in different conditions. In addition to size and concentration, UV-Vis spectral analysis can also provide an insight into the stability and surface chemistry of AgNPs. Changes in the absorption peak position, shape, or intensity over time or under different

environmental conditions can indicate nanoparticle aggregation, surface modifications, or interactions with other molecules. UV-vis spec analysis was performed for two different concentrations, from those ranges, 20 mM time-dependent analyses were performed for various time durations such as 0 hr, 15 mints, 30 mints, 45 mints, and 1 hr absorbance peak was observed at 421 nm.

In XRD diffraction patterns showed four distinct peaks at $2\theta = 27.784^\circ, 32.192^\circ, 38.095^\circ, 42.429^\circ, 43.400^\circ, 46.165^\circ, 49.079^\circ$, corresponding to (27.92), (32.28), (38.23), (42.2), (43.45), (46.21), (54.7), (57.3) planes respectively. The occurrence of functional groups in *A. nervosa* leaf extract leads to the efficient reduction of Ag ions to AgNPs. To get a good signal-to-noise ratio of AgNPs were taken in the range of 500–3400 cm. In the present study, the bio-synthesized *A. nervosa* AgNPs exposed some prominent peaks in FTIR examination such as 3243, 1531.44, and 1323.45 cm⁻¹. The peaks observed at 3243 cm⁻¹ can be attributed to the presence of O–H bonds, which are associated with both alcohols and phenols in the sample. These findings demonstrated that the leaf extract's various naturally occurring substances, such as tannins, polyphenols, and flavonoids, are actively involved in the reduction of Ag to AgNPs, as well as the capping and stability of the nanoparticles. These results correlated with previous research on green AgNPs production (Adoni et al., 2020; Palle et al., 2020).

The size of the particles ranged between 1 nm and 10 nm, indicating that they are nanoscale particles. The shape of AgNPs appeared to be nearly spherical, which is a desirable characteristic for nanoparticles. However, the SEM images indicated that the particles were agglomerated, which resulted in an uneven shape. Despite this, some particles had a circular shape, which suggests that the synthesis process could generate uniform particles. Overall, the SEM images offer important information about the physical properties of *A. nervosa* AgNPs. However additional characterization and analysis are required to understand their full behavior and properties. Analysis through EDX analysis confirmed the presence of elemental silver. The Zeta and particle size analysis results clearly demonstrated the distribution of AgNPs in the reaction

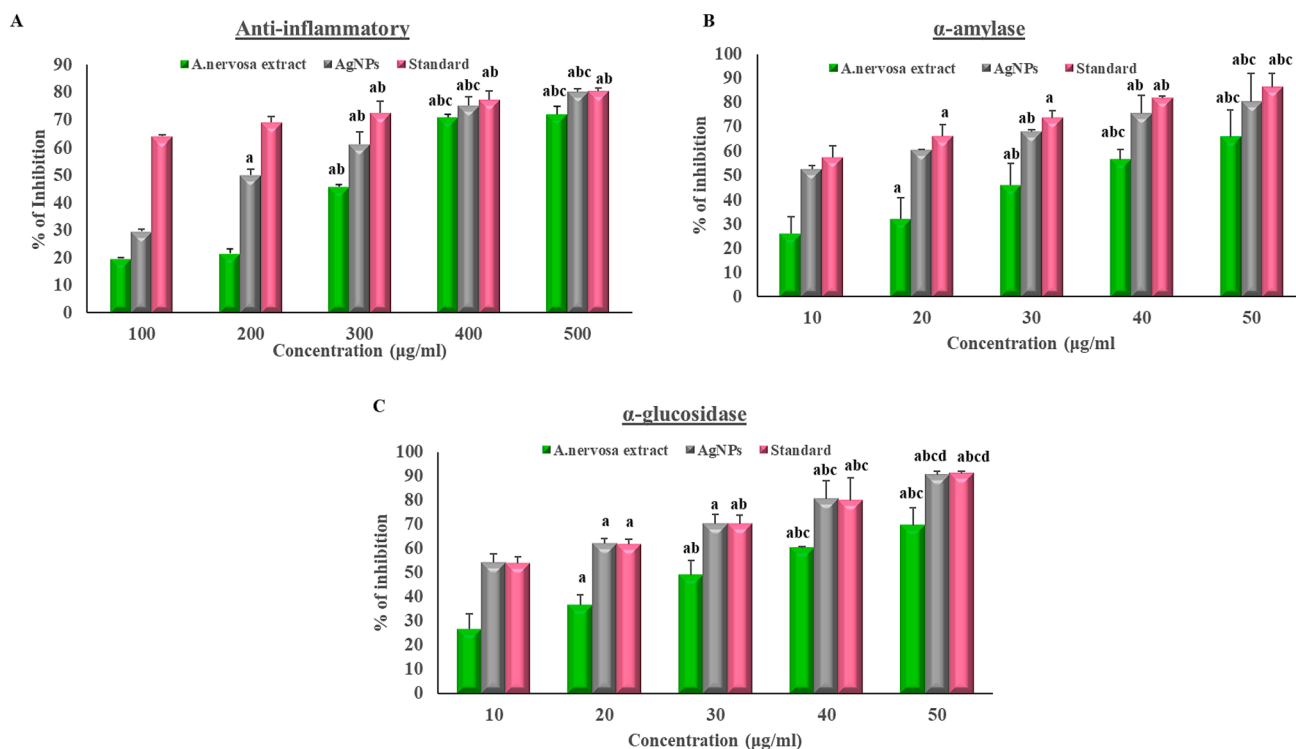


Fig. 8. (a) Anti-inflammatory activity, (b) α -amylase assay, and (c) α -glucosidase assay. (The X-axis shows the different concentrations of AgNPs and extract and the Y-axis denotes the % of inhibition, (a-significance to 100 µg/ml, b-significance to 200 µg/ml, c-significance to 300 µg/ml, and d-significance to 400 µg/ml).

Table 3

(a) Anti-inflammatory activity, (b) α -amylase assay, and (c) α -glucosidase assay of the extract, *A. nervosa* mediated AgNPs and standard drug, (a-significance to 100 μ g/ml, b-significance to 200 μ g/ml, c-significance to 300 μ g/ml, and d-significance to 400 μ g/ml). (Values are expressed in mean \pm SEM of 3 replications).

(A)			
Concentration (μ g/ ml)	Anti-inflammatory % of scavenging activity		
	Extract	AgNPs	Standard
100	19.82 \pm 0.2	29.71 \pm 0.7	63.89 \pm 0.7
200	21.63 \pm 1.6	50.08 \pm 2.1 ^a	68.99 \pm 2.1
300	45.86 \pm 0.9 ^{ab}	61.30 \pm 4.3 ^{ab}	72.45 \pm 4.3 ^{ab}
400	70.97 \pm 1 ^{abc}	75.21 \pm 3.2 ^{abc}	77.31 \pm 3.2 ^{ab}
500	71.93 \pm 2.9 ^{abc}	80.19 \pm 1.2 ^{abc}	80.40 \pm 1.2 ^{ab}
(B)			
Concentration (μ g/ ml)	Alpha-amylase % of scavenging activity		
	Extract	AgNPs	Standard
10	26 \pm 7	52.65 \pm 1.7	57.62 \pm 4.8
20	36.25 \pm 8.8 ^a	60.63 \pm 0.3	66.08 \pm 4.7 ^a
30	46.15 \pm 8.9 ^{ab}	67.95 \pm 0.9 ^{ab}	73.56 \pm 3.2 ^a
40	56.7 \pm 4.3 ^{abc}	75.4 \pm 7.6 ^{ab}	81.75 \pm 0.9 ^{ab}
50	65.65 \pm 11.4 ^{abc}	80.4 \pm \pm 11.6 ^{abc}	86.35 \pm 5.7 ^{abc}
(C)			
Concentration (μ g/ ml)	Alpha-glucosidase % of scavenging activity		
	Extract	AgNPs	Standard
10	26.6 \pm 6.4	54.17 \pm 3.7	53.81 \pm 2.7
20	36.7 \pm 4.3 ^a	62.03 \pm 2.1 ^a	61.83 \pm 2.1 ^a
30	49.1 \pm 5.9 ^{ab}	70.4 \pm 3.8 ^a	70.13 \pm 3.8 ^{ab}
40	60.35 \pm 0.6 ^{abc}	80.5 \pm 7.6 ^{abc}	80.1 \pm 9.1 ^{abc}
50	69.65 \pm 7.3 ^{abc}	90.6 \pm 1.4 ^{abcd}	91.3 \pm 0.7 ^{abcd}

mixture according to intensity, number, and volume of AgNPs respectively.

One of the major healthcare challenges is antibiotic resistance, which is caused by improper or excessive use of antibiotics. Hence, there is a demand on herbal remedies possessing substantial antibacterial potentials that hold enormous significance (Jansen et al., 2006). Nanotechnology offers desirable opportunities to improve the efficacy of herb-based drugs in antibiotic therapy. Nanoparticles can potentially increase the bioavailability, stability, and targeted administration of herbal constituents, enhancing their antibacterial activity.

The strong antimicrobial properties of Ag are well-established and have been extensively reported (Liu et al., 2010; Subramanyam et al., 2021). Earlier studies have provided an evidence of the antibacterial activity of AgNPs through mechanisms such as attachment to the bacterial cell wall or the generation of free radicals. Furthermore, the release of Ag ions from AgNPs is believed to be a significant contributor to their antibacterial effects, particularly through their interaction with thiol groups found in enzymes (Kim et al., 2007). The antibacterial activity of Ag can occur through several mechanisms which mechanism involves the attachment of AgNPs to the surface of bacterial cells, leading to structural damage and disruption of vital functions. This attachment can interfere with essential cell processes and ultimately result in bacterial cell death. Another mechanism involves the generation of reactive oxygen species (ROS) by AgNPs. These free radicals, such as can induce oxidative stress within bacterial cells. Further, oxidative stress can cause damage to cellular components, including, lipids, proteins, and DNA this leads to cell death or inhibition of microbial growth. Moreover, the release of Ag from AgNPs is a crucial aspect of their antimicrobial activity. The silver ions can interact with thiol groups present in enzymes that are essential for bacterial metabolism and cellular processes. This interaction disrupts the enzymatic activity, impeding crucial biochemical pathways and compromising bacterial survival (Yu et al., 2013). Overall, the antibacterial activity of Ag can be attributed to their attachment to bacterial cells, generation of free

radicals, and their interaction with thiol groups of enzymes.

In this study *A. nervosa* leaf extracts exposure showed free radical scavenging action of 27 %, 37 %, 50 %, 61 %, and 76 % with the concentration of 100–500 μ g/ml. Further, biologically synthesized *A. nervosa* AgNPs demonstrated free radical scavenging activity was 55 %, 62 %, 69 %, 78 %, and 85 % respectively on a concentration of 100–500 μ g/ml. As the concentration level increased, there was a continuous increment in the inhibition level of DPPH radical formation. A similar finding was reported for *A. nervosa* leaf extract biosynthesized AgNPs, which demonstrated a significant ability to scavenge DPPH free radicals (Subramanyam et al., 2021).

The *A. nervosa* leaf extract revealed ABTS radical scavenging activity by 22 %, 33 %, 40 %, 50 %, and 54 % on a concentration of 100–500 μ g/ml. Further, biologically synthesized *A. nervosa* AgNPs demonstrated free radical scavenging activity of 25 %, 41 %, 51 %, 57 %, and 67 % on a 100–500 μ g/ml concentration. Standard drug inhibition (%) attained a range of 34 %, 46 %, 55 %, 60 %, and 67 % on a concentration of 100–500 μ g/ml. Similar ABTS action of biologically produced AgNPs was found in earlier studies (Nallappan et al., 2021). *A. nervosa* root aqueous extract has previously proven that it has the potential ABTS radical scavenging activity against standard drugs (Shreedhara et al., 2009).

The *A. nervosa* leaf extract revealed H₂O₂ radical scavenging activity by 22 %, 31 %, 40 %, 46 %, and 61 % on a concentration of 100–500 μ g/ml. Further, biologically synthesized *A. nervosa* AgNPs demonstrated free radical scavenging activity of 26 %, 33 %, 41 %, 49 %, and 72 % on a 100–500 μ g/ml concentration. The accumulation of H₂O₂ in the biological system leads to the generation of hydroxy free radicals, which cause significant impairment of the cell membrane. The existing compounds capable of inhibiting these radicals have significant potential as therapeutic agents for alleviating the symptoms of oxidative stress (Kamalanathan et al., 2015). *A. nervosa* mediated AgNPs exhibited an improved scavenging effect than *A. nervosa* extract alone (Shanmugam et al., 2021).

The anti-inflammatory assay revealed extract maximum inhibition (%) obtained was 19 %, 21 %, 45 %, 70 %, and 71 % on a concentration level of 100–500 μ g/ml. Maximum anti-inflammatory activity of *A. nervosa* AgNPs was detected as 29 %, 50 %, 61 %, 75 %, and 80 % respectively at a concentration of 100–500 μ g/ml concentration levels. This finding is in accordance with the outcomes of other studies using AgNPs synthesized from diverse medicinal plants (Kedi et al., 2018). *A. nervosa* AgNPs were shown to be a good anti-inflammatory agent biosynthesized in an eco-friendly technique, without any kind of negative impacts as in chemically synthesized medications. In future *A. nervosa* AgNPs may be used as an alternative to chemically generated anti-inflammatory drugs used to treat inflammation-related disorders.

The α -amylase activity of *A. nervosa* extract was found to be 26 %, 36 %, 46 %, 56 %, and 65 % at concentrations of 10–50 μ g/ml. The maximum α -amylase activity of *A. nervosa* AgNPs was observed as 52 %, 60 %, 67 %, 75 %, and 80 % at 10–50 μ g/ml concentrations. The inhibition percentage of the standard drug ranged from 57 % to 86 % at concentrations of 10 to 50 μ g/ml. Furthermore, the AgNPs synthesized from *A. nervosa* showed significantly higher levels of α -amylase inhibition activity compared to acarbose in all tested concentrations. This is consistent with the findings of other studies using AgNPs synthesized from different therapeutic plants (Jini and Sharmila, 2020). In the α -glucosidase assay *A. nervosa* leaves extract inhibition (%) obtained was 26 %, 36 %, 49 %, 60 %, and 68 % on a of 10–50 μ g/ml concentrations. Maximum α -glucosidase activity of *A. nervosa* AgNPs was observed in the range of 54 %, 62 %, 70 %, 80 %, and 90 % at 10–50 μ g/ml concentration. Green synthesized Nps from other medicinal plants showed similar outcomes (Jini and Sharmila, 2020). Additional research is essential to find out the possible activity of *A. nervosa* AgNPs role in diabetic management in animal or cell line studies.

6. Conclusions and recommendations

This study was focused on the production of NPs using plant materials, an emerging technique in nanotechnology. To develop a cost-effective and environmentally friendly method for synthesizing AgNPs using aqueous plant leaf extract from *A. nervosa*. We characterized the synthesized *A. nervosa* AgNPs using various techniques. Furthermore, our *in vitro* assay results showed that *A. nervosa*-mediated AgNPs have potential anti-bacterial, antioxidant, anti-inflammatory, and anti-diabetic activities. This green synthesis technique is a promising and more sustainable alternative to conventional methods. Further studies on animal and cell line models are warranted to explore the potential biological activities of *A. nervosa*-mediated AgNPs.

7. Limitation of the study

The study has some boundaries, over all the discoveries may be specific to *Argyrea nervosa* leaf extract-derived AgNPs and may not be applicable to AgNPs synthesized from other plant extracts. The properties of *Argyrea nervosa* leaf extract may vary depending on factors like geographical location, environmental conditions, and the time of harvest. These variations may affect the reproducibility of the green synthesis process. Furthermore, the potential toxicity of the AgNPs to humans and the environment may not be comprehensively addressed. Additional research on their safety profiles, including cytotoxicity and environmental impact, is essential.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary material to this article can be found online at <https://doi.org/10.1016/j.jksus.2023.102955>.

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