



Research Article

Synthesis of silver nanoparticles from *Dicranopteris linearis* extract: Physicochemical, cytotoxic, and antimicrobial characterization

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ABSTRACT

Extracts from plants are rich in phytochemicals that can be used as biogenic reducing agents for the synthesis of Ag (silver) nanoparticles (AgNPs). In this research, we synthesized AgNPs using *Dicranopteris linearis* leaves extract and assessed their cytotoxicity and antimicrobial activities. The aqueous extract of *D. linearis* (DL) leaves was analyzed using liquid chromatography-mass spectrometry (LC-MS). The extracts served as reducing agents for silver nitrate, producing AgNPs-DL. The nanoparticles were characterized by UV-visible spectrophotometry, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and dynamic light scattering (DLS), and cytotoxicity was assessed against human breast adenocarcinoma cells line (MCF-7) using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Antimicrobial activity was tested against four bacteria, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus*, using the disc diffusion method. AgNPs-DL were successfully synthesized from *D. linearis* leaves extract. LC-MS/QTOF analysis revealed a diverse phytochemical profile, supporting the extract's role in nanoparticle synthesis. SEM analysis showed predominantly spherical formation of AgNPs with size of 279.3 nm in average. Cytotoxicity assay indicated low toxicity against MCF-7 cells. AgNPs-DL also demonstrated inhibitory activity against *E. coli*, *P. aeruginosa*, *B. subtilis*, and *S. aureus*. Phytochemicals in *D. linearis* leaves extract contribute to stabilization and reduction of silver ions, resulting in AgNPs with distinct physicochemical properties and notable bioactivity.

1. Introduction

Antibiotic resistance has become a critical health concern, driven by genetic mutations, environmental pollution, and ecological changes that promote the multidrug-resistant microorganism and viral strains (Verma & Mehata, 2016; Bakon et al., 2023). The increase of this resistance reduced the effectiveness of conventional antimicrobial therapies, underscoring the urgent need for new strategies to combat infectious diseases (Siddiqi et al., 2018).

Nanotechnology has emerged as a promising field in this context. Nanoparticles possess unique physicochemical properties, particularly a high surface-to-volume ratio, which enhances their interactions with microbial cells and enables broad-spectrum antibacterial activity. Silver (Ag) nanoparticles (AgNPs) are the frequently studied due to their potent antimicrobial activity, antioxidant activity, and cytotoxicity (Ipek et al., 2023) (Verma and Mehata, 2016b). Mechanistic studies indicate that AgNPs can inhibit cancer cell proliferation by binding to the cell membranes, disrupting respiration, and altering membrane permeability (Ipek et al., 2023).

Recent research has increasingly focused on green synthesis of AgNPs using bio-based systems, such as plants and microorganisms, as eco-friendly and cost-effective alternatives to conventional chemical processes (Bakht Dalir et al., 2020; Salem & Fouda, 2021). Plant-mediated synthesis is particularly attractive because plant extracts have a wide range of biomolecules that act as reducing and stabilizer agents in nanoparticle formation with minimal impurities. This approach reduces the use of hazardous chemicals and is scalable for industrial applications (Suriyakala et al., 2022).

Dicranopteris linearis (Burmese f.) Underw., domestically known in Malaysia as 'resam,' is a fern tree commonly found in secondary forests and widely used in traditional medicine. Its leaves have been applied to reduce body temperature, manage fever, and treat skin disorders (Ahmad et al., 2023; Baharuddin et al., 2018; Lai et al., 2021). Phytochemical analysis of the *Gleicheniaceae* family has shown that terpenoids and flavonoids are predominant constituents, alongside alkaloids, saponins, tannins, steroids, and cardiac glycosides (Baharuddin et al., 2021; Balagot et al., 2023). These biomolecules are known to contribute to the reduction and stabilization of silver ions during nanoparticle synthesis (Anbumani et al., 2022; Raj et al., 2021). Based on this background, the

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present study aimed to synthesize AgNPs using *D. linearis* leaves extract and evaluate their physicochemical properties, antibacterial activity, and cytotoxic potential.

2. Materials and Methods

2.1 Materials

D. linearis leaves were collected from Kuantan, Pahang, Malaysia, in October 2023. Ethanol (95% purity) was purchased from HmbG Chemicals. An ultrasonic sonicator (Qsonica Ultrasonic Sonicator Converter Model CL-334) was used for extraction, and a Supra 22 K centrifuge (Hanil Science Industrial) was used for nanoparticle separation.

The test microorganisms included the bacteria of *Bacillus subtilis* and *Staphylococcus aureus* (Gram-positive); *Pseudomonas aeruginosa* and *Escherichia coli* (Gram-negative), *Candida albicans* (yeast), and *Aspergillus niger* (filamentous fungus), which were obtained from ATCC, US. The cell line of MCF-7 (human breast cancer) was also obtained from ATCC, US.

Silver nitrate, dimethyl sulfoxide, ethanol, and methanol were obtained from EMSURE®, Merck (analytical grade), sodium hydroxide (R&M Chemicals), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT reagent, Molecular Probes), tamoxifen citrate (Calbiochem, Merck), and amoxicillin disc (Oxoid, Thermo Fisher).

Dulbecco's Modified Eagle's Medium (DMEM, Gibco), TrypLE Express (Gibco), fetal bovine serum (FBS, Gibco), penicillin-streptomycin (Nacalai Tesque, Kyoto, Japan), and phosphate-buffered saline (PBS, Sigma-Aldrich). For microbial growth, tryptic soy agar/broth (TSA, TSB, Merck), nutrient agar/broth (NA, NB, Merck), and Sabouraud dextrose agar/broth (SBA, SDB, Merck) were used.

UV-visible spectrophotometer (Shimadzu UV-1800), liquid chromatography- mass spectrometry/quadrupole time of flight (LC-MS/QTOF) (Agilent 1200 LC system with a 6520 QTOF, Agilent Technologies), Fourier-transform infrared (FTIR) spectrometer (PerkinElmer Dual), scanning electron microscope (SEM) with energy-dispersive X-ray (EDX) (JSM-IT200, JEOL), particle size analyzer (Zetasizer Nano ZS, Malvern), X-ray diffractometer (XRD) (PHI 5000 VersaProbe II, ULVAC-PHI), thermogravimetric analyzer (TGA) (Hitachi STA7000), high-speed centrifuge (Supra 22K, Hanil Science Industrial), microplate reader (Azure Biosystems), rotary evaporator (Rotavapor R-300, BÜCHI) and ultrasonic sonicator (Qsonica) were used. Additional equipment and apparatus included Petri dishes (90 mm × 15 mm), CO₂ incubator (5% CO₂, 37°C, BB15, Thermo Fisher), and 96-well flat-bottom tissue culture plates (Falcon, Becton Dickinson) (Zeheri et al., 2025).

2.2 Preparation of *D. linearis* leaves extract

Fresh leaves of *D. linearis* were sorted and washed three times with running water, oven-dried at 40°C for 2 days, and ground into powder using a mechanical grinder (Patra et al., 2020). Then, 10 g of leaf powder was extracted with 100 mL of deionized water. Five extracts were prepared by boiling for 5, 15, 30 min, 1 h, and 2 h, respectively. Extracts were cooled to room temperature (24°C), filtered using a NICE filter paper (102 Qualitative), and kept at 4°C.

2.3 Liquid chromatograph- mass spectrometry analysis

Aqueous extract of DL was concentrated at 100°C, 150 rpm using a rotary evaporator (IKA HB 10 basic). The concentrate extract was reconstituted in methanol (10 mg/mL), then diluted to 1 mg/mL, followed by filtration through a syringe filter (0.22-µm). The separation was run using an Agilent ZORBAX Eclipse Plus column (C18 Rapid Resolution HT) (dimension 2.1 × 100 mm, particle size 1.8 µm) at 40°C. The mobile phase was made with 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient was programmed at 5-95% B (0-18 mins), 95% B (18-23 mins), followed by re-equilibration for 2 min. Flow rate was set at 0.25 mL/min, and the injection volume was 2 µL with a total run time of 30 min. The MS was operated in the

positive electrospray ionization (ESI) mode using a gas temperature of 325°C, a gas flow of 11 L/min, and a nebulizer pressure of 35 psi. The data were analyzed using the software of Agilent Mass Hunter Qualitative Analysis and the METLIN database.

2.4 Synthesis of AgNPs

For this, 0.0153 g silver nitrate (AgNO₃) was dissolved in 90 mL of deionized water to obtain 1 mM AgNO₃. Each *D. linearis* extract (10 mL) was mixed with 90 mL of the AgNO₃ solution and incubated at 25°C under continuous stirring for 24 h. Nanoparticles were collected using centrifugation at 15,000 rpm for 30 min at 4°C, washed with deionized water, and passed through a Millipore membrane filter with 0.22-µm pore size.

2.5 Characterization of AgNPs

UV-visible spectra of AgNPs were recorded between 300-800 nm at 1 nm resolution (UV-1800, Shimadzu) using deionized water as the blank. Samples were analyzed at the following time intervals (1, 2, 4, 24, 48 h). The extract producing the highest absorbance was used for further characterization and biological assays.

Morphology was examined by SEM (EVO-50X, Zeiss). For zeta potential analysis, filtrates were measured 13 times at 25°C using a Zetasizer (particle size analyzer) (Zetasizer Nano ZS, Malvern). Functional groups were identified by FTIR spectroscopy (Frontier, PerkinElmer).

2.6 Cytotoxicity assay

MCF-7 cell lines were cultured in complete medium (containing DMEM, 10% FBS, and 1% penicillin-streptomycin) at 37°C in a 5% CO₂ incubator. At 80% confluency, cells were trypsinized, seeded into a 96-well plate (15,000 cells/well), and treated with AgNPs, *D. linearis* extract, and tamoxifen (positive control) at different concentrations of 250, 125, 62.5, 31.25, 15.625, 7.8125, and 3.90625 µg/mL. After incubation (24 h), 20 µL MTT reagent (5 mg/mL) was added per well, then the plates were incubated for 30 min. Formazan crystals were dissolved with 100 µL DMSO and incubated for 30 min at 37°C. Absorbance was measured at 570 nm using a microplate reader (Shelembe et al., 2022). Cell viability was calculated as:

$$\text{Cell viability (\%)} = \frac{\text{Absorbance}_{\text{treated}}}{\text{Absorbance}_{\text{untreated}}} \times 100 \quad (1)$$

2.7 Antibacterial assay

Antibacterial activity was tested using the disc diffusion method using *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa*, which were cultured in tryptic soy medium at 37 ± 1°C for 18 h. 10 mL of colloidal AgNPs solution (2 mg/mL) or AgNO₃ (2 mg/mL) was applied to discs, with amoxicillin serving as the positive control. After incubation (37 ± 1°C, 18 h), inhibition zones were measured in mm.

2.8 Statistical analysis

The experiments were conducted in triplicate. Data are presented as mean ± standard deviation (SD). The statistical analysis was performed using one-way analysis of variance (ANOVA) in SPSS, with significance set at $p < 0.05$.

3. Results and Discussion

3.1 LC-MS-QTOF analysis of *D. linearis* leaves extract

Preliminary LC-MS analysis was performed to profile the compounds in the *D. linearis* leaf extract that are potentially responsible for AgNPs synthesis. Chromatographic profiles (Fig. 1) were compared against entries in the METLIN database. Details of the 10 major compounds together with names, molecular masses, m/z values, and their class have been provided in Table 1. Identified compounds include dicarboxylic

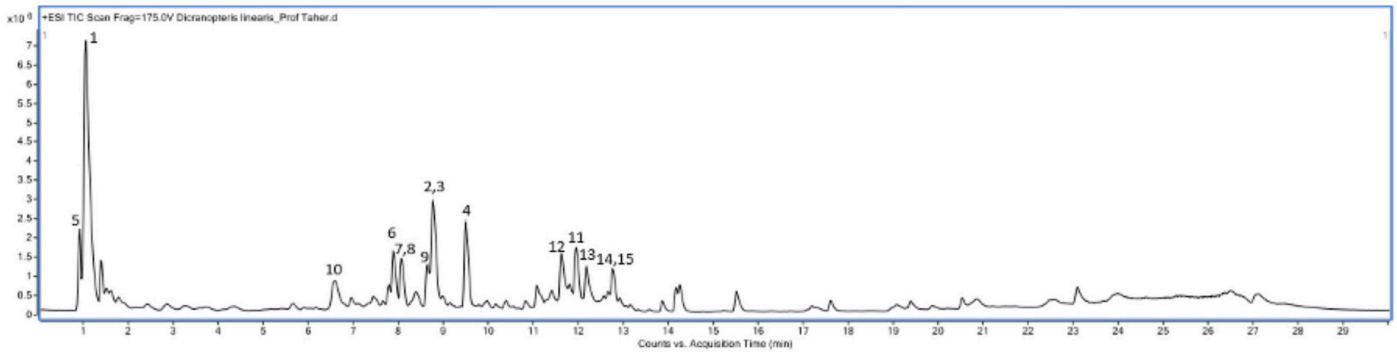


Fig. 1. LC-MS/QTOF of *Dicranopteris linearis* leaf extract.

Table 1.

The 15 major compounds in *D. linearis* leaf extract were analyzed using LC-MS-QTOF analysis.

| Peak No | Compound name | Molecular formula | m/z | Mass (g/mol) | Classification |
|---------|--|---|----------|--------------|--|
| 1 | 3-hydroxy-sebacic acid | C ₁₀ H ₁₈ O ₅ | 236.1483 | 218.1145 | Dicarboxylic acid (Sebacic acid) |
| 2 | Melanoxetin | C ₁₅ H ₁₀ O ₇ | 303.0505 | 302.0432 | - |
| 3 | Isovitexin | C ₂₁ H ₂₀ O ₁₀ | 433.1135 | 432.1061 | Flavonoid (Flavone glycoside) |
| 4 | 5,7,2',3'-Tetrahydroxyflavone | C ₁₅ H ₁₀ O ₆ | 287.0556 | 286.0483 | Flavonoid |
| 7 | 8-Hydroxyluteolin 8-glucoside | C ₂₁ H ₂₀ O ₁₂ | 465.1021 | 464.0948 | Flavonoid (Luteolin derivative) |
| 8 | 3,5,7,2',5'-Pentahydroxyflavone | C ₁₅ H ₁₀ O ₇ | 303.0492 | 302.0419 | Flavonoid (pentahydroxyflavone) |
| 9 | 6-C-Galactosylisoscuteallarein | C ₂₁ H ₂₀ O ₁₁ | 449.1082 | 448.1009 | Flavonoid (Isoscutellarein derivative) |
| 10 | Epicatechin-(4beta->6)-epicatechin-(2beta->7,4beta->8)-epicatechin | C ₄₅ H ₃₆ O ₁₈ | 865.1966 | 864.1891 | Condensed tannin (Oligomeric proanthocyanidin) |
| 12 | Ent-kaur-16-en-19-al | C ₂₀ H ₃₀ O | 287.2371 | 286.2298 | Diterpene |
| 14 | Axerophthene | C ₂₀ H ₃₀ | 271.2423 | 270.2349 | - |
| 15 | Pantoyllactone glucoside | C ₁₂ H ₂₀ O ₈ | 293.2266 | 268.2193 | Pantothenic acid glucoside |

Peaks 5, 6, 11c, and 13 are unknown

acids, condensed tannins, diterpenes, pantothenic acid glucosides, and several flavonoids such as flavone glycosides, luteolin derivatives, hydroxyflavones, and isoscutellarein. Additional classes detected were polysaccharides, vitamins, amino acids, proteins, phenolics, saponins, alkaloids, and terpenes (Khurshheed et al., 2023). Four compounds could not be identified as their mass data did not correspond to entries in the database.

D. linearis is known to be rich in terpenoids and flavonoids (Baharuddin et al., 2021), and previous studies have also reported the presence of saponins, alkaloids, tannins, steroids, and cardiac glycosides (Balagot et al., 2023). Glycosides with aglycone components, such as diterpenes, flavanols, and monoaromatic compounds, have also been detected (Duong et al., 2023). These phytochemicals, including both macro- and micromolecules, play essential roles in the reduction and stabilization of silver ions (Anbumani et al., 2022; Raj et al., 2021). The identified metabolites therefore support the potential of *D. linearis* extract as an effective biogenic agent for AgNPs synthesis.

3.2 Synthesis of silver nanoparticle (AgNPs)

The reduction of silver ions in AgNO₃ solution by *D. linearis* extract was indicated by color changes during incubation (1, 2, 4, 24, and 48 h). The solution gradually turned from yellow to dark brown with increasing incubation time (Fig. 2), consistent with the reduction of Ag⁺ ions to AgNPs (Baranitharan et al., 2021; Kumar et al., 2022). UV-visible spectroscopy further confirmed nanoparticle formation.

3.3 Characterizations of AgNPs

3.3.1 UV-vis spectrophotometry

UV-vis spectra were recorded at 5, 15, 30 min, and 2 h showed increasing absorbance over time, with a peak at 350 nm (Fig. 3a). Previous reports of AgNPs synthesized with *D. linearis* extract observed surface plasmon resonance at 450 nm (Rajaganesh et al., 2016). Spectra

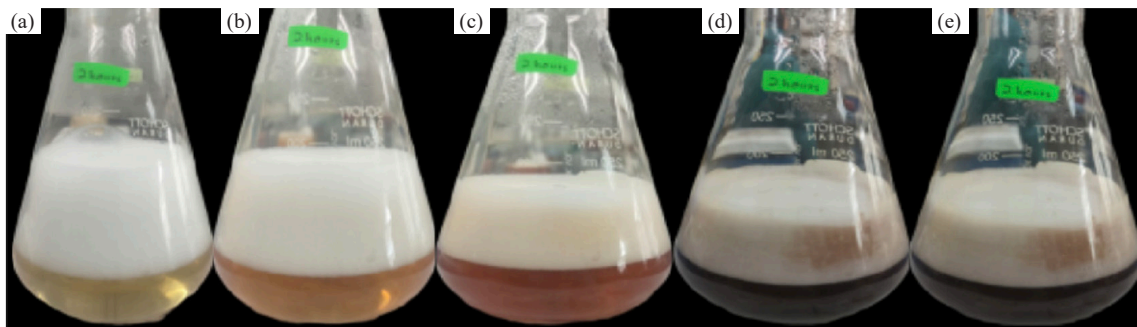


Fig. 2. AgNP production using *Dicranopteris linearis* leaf extract. *D. linearis* extract (10 mL) was mixed with 90 mL of 1 mM silver nitrate solution in an Erlenmeyer flask and incubated at room temperature (24°C) for 24 h. Color changes after (a) 1, (b) 2, (c) 4, (d) 24, and (e) 48 h of incubation were observed.

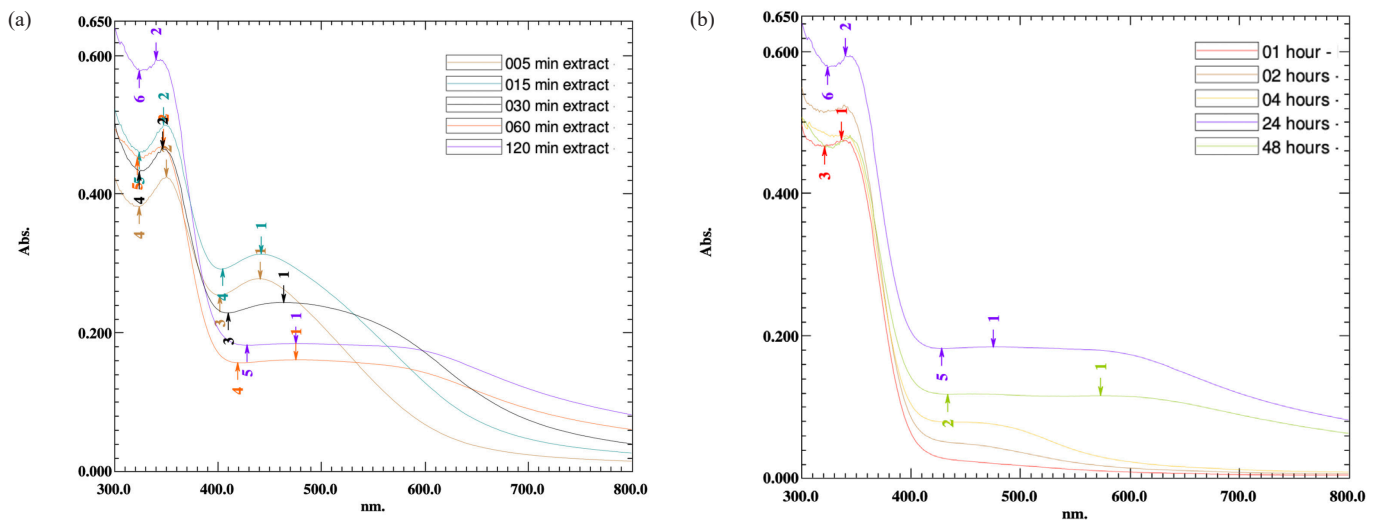


Fig. 3. (a) UV-visible spectrophotometry of silver nanoparticles synthesized using *Dicranopteris linearis* leaf extract (AgNPs-DL). Nanoparticle synthesis using different extracts at 24 h. (b) UV-visible spectrophotometry of AgNPs synthesized using *Dicranopteris linearis* leaf extract (AgNPs-DL). Nanoparticle synthesis at 1, 2, 4, 24, and 48 h using extract (2 h extraction time).

at different incubation times (Fig. 3b) confirmed peak absorbance at 350 nm. Absorbance increased from 1-24 h but decreased at 48 h, likely due to nanoparticle aggregation and colloidal instability resulting from extended stirring (Badiyah et al., 2019).

3.3.2 SEM analysis

SEM images revealed that AgNPs-DL were predominantly spherical, with sizes ranging from 120-145 nm (Fig. 4). Previous studies also reported spherical AgNPs of smaller size (40-60 nm) with some aggregation (Rajaganesh et al., 2016). Nanoparticle morphology is affected by several factors, including reaction temperature, time, and the nature of phytochemicals in the extract. The present results suggest that organic constituents in *D. linearis* extract play a major role in determining nanoparticle morphology (Badiyah et al., 2019; Taher et al., 2023; Rajaganesh et al., 2016).

3.3.3 Zeta potential and particle size, and analysis

Dynamic light scattering (DLS) analysis indicated an average particle size of 279.3 ± 5.55 nm (Table 2). While nanoparticles are generally

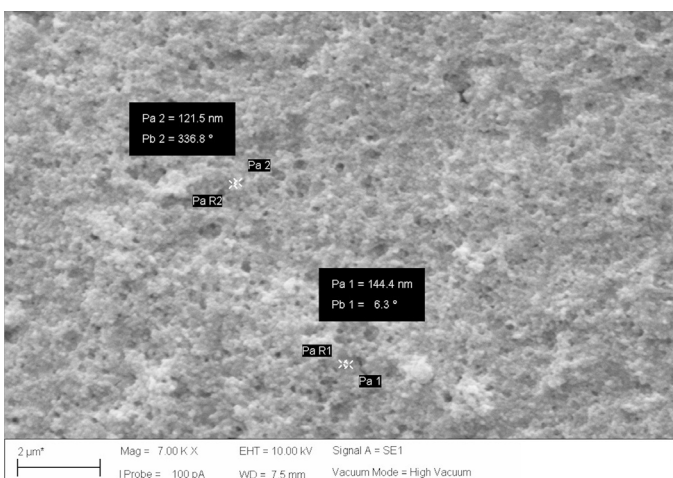


Fig. 4. Morphological characteristics of AgNPs synthesized using *Dicranopteris linearis* leaf extract (AgNPs-DL), observed via SEM analysis ($\times 7000$ magnification).

Table 2.

Average particle size and zeta potential of AgNPs synthesized using *D. linearis* leaf extract.

| Particle size analysis | Measurements |
|------------------------------|------------------|
| Particle size average (d.nm) | 279.3 ± 5.55 |
| PdI | 0.248 |
| Zeta potential analysis | |
| Zeta POTENTIAL (mV) | -57.4 ± 1.27 |
| Zeta DEVIATION (mV) | 30.3 |
| Conductivity (mS/cm) | 0.547 |

defined as being within 1-100 nm in pharmaceutical applications (Susanti et al., 2022), even particles up to 1000 nm are often considered acceptable (Mazayen et al., 2022). The discrepancy between SEM and DLS values may reflect the principles of measurement, as DLS relies on Rayleigh scattering and often detects hydrodynamic diameters (Suriyakala et al., 2022).

The polydispersity index (PdI) of 2.48 indicated a relatively narrow distribution of size. A zeta potential of -57.4 mV suggested strong electrostatic stability, reducing the likelihood of aggregation (Suriyakala et al., 2022). However, the high zeta deviation (30.3 mV) implied some heterogeneity in particle surface charge.

3.3.4 FTIR spectroscopy

FTIR spectra of AgNPs-DL (Fig. 5) revealed several functional groups that play in nanoparticle stabilization. The broad peak at 3193.79 cm^{-1} corresponded to OH stretching of $-\text{COOH}$ or $\text{N}-\text{H}$ groups (Rajaganesh et al., 2016). Peak at 2919.70 and 2162.71 cm^{-1} were attributed to alkyne $\text{C}-\text{H}$ and $\text{C}\equiv\text{C}$ stretching (Dada et al., 2018; Dalir et al., 2020). Absorptions at 1601.63 , 1509.20 , and 1493.15 cm^{-1} represented $\text{C}=\text{C}$ interaction (stretching in aromatic compounds), while 1200.38 and 1025.78 cm^{-1} indicated $\text{C}-\text{N}$ stretching. A band at 694.62 cm^{-1} suggested $\text{C}-\text{Br}$ stretching (Dada et al., 2018) (Hamida et al., 2020). These functional groups confirm the presence of phytochemicals involved in producing of AgNPs, preventing aggregation (Trivedi et al., 2021).

3.4 Cytotoxicity study

Treatment with AgNPs-DL reduced the percentage of viability of MCF-7 cell lines (Fig. 6). Viabilities following exposure to 3.90625 , 7.8125 , 15.625 , 31.25 , 62.5 , 125 , and 250 $\mu\text{g}/\text{mL}$ of

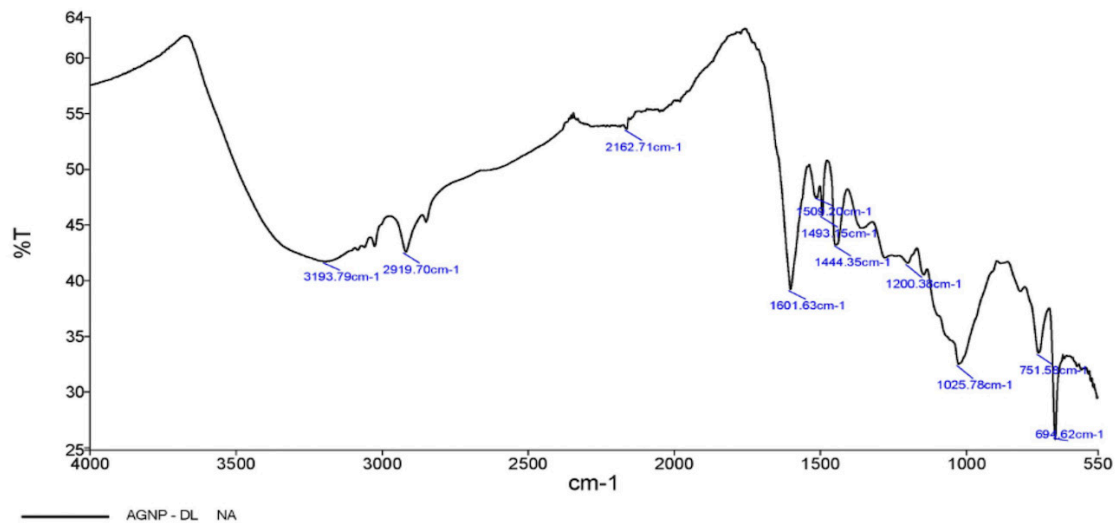


Fig. 5. FTIR spectrum of AgNPs synthesized using AgNPs-DL.

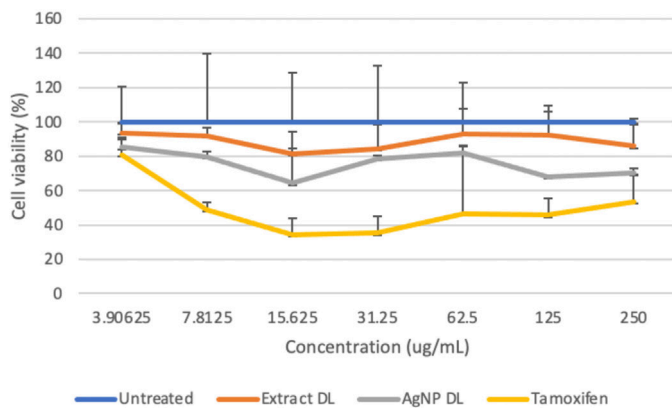


Fig. 6. Percentage viability of MCF-7 cells after treatment with various concentrations of *Dicranopteris linearis* extract, AgNPs, and tamoxifen at concentrations ranging from 3.90625 to 250 $\mu\text{g/mL}$.

AgNPs-DL were 85.17, 79.82, 64.16, 78.27, 82.09, 67.92, and 70.12%, respectively. However, the reduction was not strictly dose-dependent, preventing calculation of an IC_{50} value. The observed inconsistencies may reflect confounding factors such as uneven cell densities, variations in incubation conditions during the MTT assay, wavelength calibration differences, or culture medium variability (Ghasemi et al., 2021).

3.5 Antimicrobial assay

AgNPs-DL demonstrated activity against Gram-positive and Gram-negative bacteria. Using the disc diffusion method, inhibition zones were largest against *B. subtilis* (10.33 ± 0.58 mm), followed by *S. aureus* (8.67 ± 0.58 mm), *E. coli* (8.67 ± 0.58 mm), and *P. aeruginosa* (7.33 ± 0.58 mm) (Fig. 7). By comparison, AgNO_3 produced stronger inhibition against *S. aureus* (11.0 ± 1.73 mm) and *P. aeruginosa* (8.33 ± 0.58 mm). Amoxicillin showed significantly higher inhibition against *B. subtilis*, *S. aureus*, and *E. coli*, although less potent than conventional antibiotics.

4. Conclusions

In this research, AgNPs-DL were successfully synthesized using *D. linearis* leaves extract. Phytochemicals such as dicarboxylic acids, condensed tannins, and flavonoids likely contributed to the silver ions reduction during nanoparticle synthesis. The nanoparticles exhibited a characteristic dark brown color and distinct absorbance in the visible spectrum. SEM analysis confirmed a predominantly spherical morphology with some aggregation, while DLS indicated

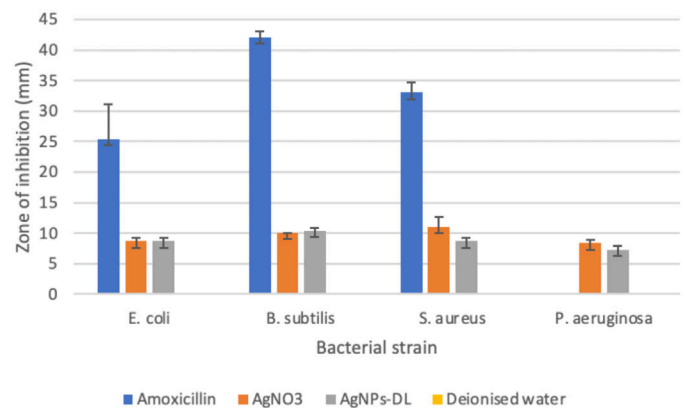


Fig. 7. Zone of inhibition (mm) of Gram-positive and Gram-negative bacteria following treatment with amoxicillin (positive control), silver nitrate (AgNO_3), AgNPs synthesized using *Dicranopteris linearis* extract (AgNPs-DL), and deionised water (negative control), based on the disc diffusion method.

the particle size of 279.3 nm, a narrow size distribution ($\text{PdI} = 0.248$), and a negative zeta potential (-57.4 mV), suggesting good colloidal stability. FTIR spectroscopy further verified the involvement of several functional groups from the extract in silver ion reduction and nanoparticle stabilization. Biological evaluations demonstrated that AgNPs-DL exerted variable cytotoxic activity against MCF-7 cell lines and demonstrated antibacterial effect against both Gram-positive and Gram-negative bacteria, with *B. subtilis* being the most susceptible. These findings demonstrate the potential of *D. linearis* leaves extract as a sustainable source for the biogenic synthesis of AgNPs with distinct physicochemical properties as well as promising antimicrobial activity.

CRediT authorship contribution statement

Muhammad Taher, Deny Susanti, Muhammad Taufiq Mohd Jailani: Designed the project. **Muhammad Taher, Muhammad Taufiq Mohd Jailani:** Supervised this study. **Nur Hannah Zainal Abidin:** Performed the study and collected the data. **Nur Hannah Zainal Abidin:** Drafted the manuscript. **Junaidi Khotib, Muhammad Taher:** Reviewed the manuscript. All the authors have read the manuscript.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work presented in this paper.

Declaration of Generative AI and AI-assisted technologies in the writing process

The authors confirm that there was no use of Artificial Intelligence (AI)-Assisted Technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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