Green synthesized silver nanoparticles using *Andrographis macrobotrys* Nees leaf extract and its potential to antibacterial, antioxidant, anti-inflammatory and cytotoxicity effects

Saipraba Sivakumar[a], Murugesan Subban[a*], Ragavendran Chinnasamy[b], Kamaraj Chinnaperumal [c], Ismini Nakouti[d], Mohamed A. El –Sheikh[e], Jilani Purusottapatnam Shaik[f].

[a] Division of Herbal medicine & Nanotechnology Laboratory, Department of Botany, School of Life Sciences, Periyar University, Salem 636 011, Tamil Nadu, India

[b] Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals, Chennai-600 077, Tamil Nadu, India.

[c] Interdisciplinary Institute of Indian System of Medicine (IIISM), Drug Testing Laboratory, Directorate of Research, SRM Institute Science and Technology, Kattankulathur - 603 203, Tamil Nadu, India

[d] Centre for Natural Products Discovery (CNPD), School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool L3 3AF, United Kingdom.

[e] Department of Botany and Microbiology, College of Science, King Saud University, P.O.Box: 2455, Riyadh-11451, Saudi Arabia

[f] Department of Biochemistry, College of Science, King Saud University, P.O.Box: 2455,Riyadh-11451, Saudi Arabia

Abstract

Green silver nanoparticles have received much interest over the years because they are cheap, good for the environment, and easy to use. Present study, first report to synthesized silver nanoparticles from the leaf extract of *Andrographis macrobotrys*, which reduces $AgNO_3$ into Ag through the presence of phytochemicals. The nanoparticles were examined using (UV, spec, FTIR, XRD, TEM and EDAX. The dark brown colour of the *A. macrobotrys* colloidal showed maximum absorbance at 450nm. The TEM images displayed synthesised nanoparticles size were revealed between 20-50nm. The antibacterial activity of Ag-NPs tested show a maximum zone of inhibition of 19 mm for *Escherichia coli* and *Staphylococcus aureus* 17 mm for at 125 µg/mL. Green synthesized AgNPs were assessed for antioxidant activity inhibition rate (DPPH 58.23 % and ABTS 68.87 %). Further, the anticancer activity of AgNPs exhibited 68.15% at 100 µg/mL concentration against A549 lung cancer cells. Additionally, *in vitro* models using the human red blood cells (HRBC) membrane stabilisation method (MSM) were used to assess the anti-inflammatory effects of AgNPs of *A. macrobotrys* and its shown to have a MSM of 76.6% at a dosage of 250 µg/mL. *A. macrobotrys* derived AgNPs possess multi potential activity was used in future pharmaceutical applications.

Keywords AgNPs, *Andrographis macrobotrys*, Antioxidants, Antibacterial, Lung cancer cells (A549).

Corresponding author:

Dr. Subban Murugesan (drsmbtpu@gmail.com)

Assistant Professor, Department of Botany, School of Life Sciences, Periyar University, Salem 636 011, Tamil Nadu, India.

1.Introduction

The greatest gifts that nature has given to humans are medicinal herbs [1]. Because medicinal herbs have a wide range of phytoconstituents that work on many biochemical pathways, they have been used to treat a broad range of diseases since the beginning of history [2,3]. The plant-based medicines are safer and more efficient than synthetic ones in this century of expanding population. In previous decades, the growth rate of synthetic drugs has climbed from 0.5 to 5 million dollars [2]. These medicines not only cost a lot to make but also have a lot of negative health effects [4]. Herbal compounds can be found easily, are less harmful, and can be used as a replacement of synthetic therapies that are sold in the market [5].

Green nanotechnology refers to nature's ability to reduce potential environmental and human health risks and costs associated with nanomaterial creation. Plants, among other biological sources, have sparked considerable interest in the creation of nanomaterials [6]. Silver nanoparticles are utilised often in bioremediation, biomedicine (including drug administration and bio-imaging), optical, and electronic uses because of their distinctive physiochemical characteristics [7]. Recently, the use of silver nanoparticles in everything from home cleaning to clothing, cosmetics, and food manufacturing has expanded [8,910]. Such nanoparticles are also employed in various water treatment cells for their microbial property because silver possesses the antibacterial ability [11]. The fabrication processes and the combination of precursor materials determine the specific properties of the metallic nanoparticles (MNPs) [12,13]. Recent research suggests that bioengineered metal-based nanomaterial films effective of changing the surface of items to give enhanced features, such as advancing the use of antimicrobial textile goods for medical uses, are available [14]. The physical approaches have been studied, but they are expensive, use more energy, and call for sophisticated equipment. Although being generated, nanoparticles need regular external stabilisation to retain stability [15,16]. AgNPs have been made using a variety of techniques,

including chemical-based reduction, nano emulsions, microwave, hybrid-based approaches, photo-chemical reduction and sono-electrical, thermal systems, and a new green fabrication method [17,18,19].

Plant-based nanoparticle synthesis utilising biopolymers is preferable to physical and chemical methods of synthesis because they have less harmful impacts on people and the environment [20], chitosan [21], cellulose [22], gum Arabic [23], phyto extracts [24,25] and essential oils [26] has been encouraged because of its eco- friendly nature. Plant substances including lignin, tannins, and flavonoids, serve as antioxidants and signalling substances for the protective systems. Because of the availability of phenolic compounds, it is known that they have help rule including anti-aging, anti-inflammatory, anti-proliferative, and antioxidants [27]. In "green synthesis," silver nanoparticles mediated by plant extract are manufactured through reduction and stabilisation [28]. Since they are employed in therapeutic systems like the treatment of communicable infectious diseases and involved in tropical remedies, they do not have poisonous substances on their surface and are safer for human cells and the environment [29,30,31].

Human cells are considered to respond defensively by causing inflammation in response to stimuli that harm tissues, such as physical, chemical, immunological, microbial, and biologic disorders, and toxins [32, 33]. The complex process known as the inflammatory reaction comprises the activation of white blood cells as well as the development of immune system chemicals including pro-inflammatory cytokines like IL-1, TNF, INFc, IL-6, IL-12, IL-18, and granulocyte-macrophage colony-stimulating factor (GMS-CF). A signalling pathway known as nuclear factor-kappa b activates multiple genes that produce pro-inflammatory cytokines, adhesion molecules, chemokines, growth factors, and inducible enzymes like COX-2 and iNOS that aid in the formation of pro-inflammatory chemicals (NF-kb) [34].

According to the National Cancer Center (NCC), lung cancer is the fourth most common cancer and has the lowest survival rate at 17.8 percent (NCC, 2011). The two main therapies used on patients to increase survival are chemotherapy and radiotherapy, but these techniques also kill healthy cells neighbouring in addition to the tumour cell [35]. The environmentally friendly synthetic nanoparticles target a specific area for medicine delivery while also lowering the toxicity bring on by the synthetic drugs [36,37]

Several plant species in the Acanthaceae family have the potentially to be used as medicines. About 28 species of the Andrographis genus are found all over India. *Andrographis macrobotrys* Nees is one of the ethno-medicinal plants that the tribal people in Karnataka, Kerala, and Tamil Nadu use to treat snake bites, fever, muscle pain, and skin diseases. It grows in the locations around Karnataka, Kerala, and Tamil Nadu. The existence of various phytochemicals, such as phenols, flavonoids, tannins, and steroids, is demonstrated by phytochemical analysis on these plants [38]. This plant is important for curing diseases, according to the traditional medical systems of Unani, Siddha, and Ayurveda [39]. Andrographolide, deoxy andrographolide, neo andrographolide, 14-deoxy-11, 12-didehydroandrographolide, and iso andrographolide have anti-atherosclerosis, anti-cancer, anti-diabetic, anti-inflammatory, anti-oxidant, immune-stimulant, hepato-protective, and insecticidal properties [40]. As an outcome, the present investigation has been carried out employing a first-report environmentally benign approach of producing silver nanoparticles from *A. macrobotrys* leaf extract. The objectives of present study aimed to synthesised nanoparticles were characterization such as UV-visible spectroscopy, X-ray diffraction (XRD), FTIR spectroscopy, SEM-EDX and TEM. Furthermore, examined the biological applications such as anti-bacterial, anti-oxidant (DPPH and ABTS), cytotoxicity assay using lung cancer (A549) cell lines and anti-inflammatory assay (albumin denaturation and HRBC membrane) stabilization assay were investigated.

2.Materials and methods

2.1. Collection of plant material

The *A. macrobotrys* plant were collected from the Yercaud Hills (Latitude 11.7748° N, 78.2097° E Longitude), Eastern Ghats, Salem (District), Tamil Nadu, India. The Botanical Survey of India (BSI), Coimbatore confirmed the plant and provided it the authentication number BSI/SRC/5/23/2022/Tech/47. The sample herbarium was stored in Department of Botany, Periyar University, Salem-636 011. The leaves of the plant were carefully picked and washed three times in regular tap water to wash of dirt and other debris. The leaves were then dried in the shade under the room temperature and the humidity is about 40- 60 % and powdered into a fine powder for further research. Aqueous was utilised as the solvent for the phytochemical extraction and will be used in subsequent analyses.

2.2. Synthesis of AgNPs

AgNPs nanoparticles were manufactured using a modified procedure [41]. An amber flask was used to carry a 0.1 mM silver nitrate solution. 100 mL of silver nitrate and 10 mL of aqueous extract were combined, then the mixture was stored at room temperature and in the dark for 24 h before the colour change was noticed. Regularly monitoring the solution's colour change, the vial was kept for 48 h at room temperature. The colourless solution turned dark brown, confirms the presence of fabricated AgNPs. After the solution was prepared, the nanoparticles were collected by centrifuging it at 10,000 rpm while filtering the solution via filter paper to remove impurities [42].

2.3. UV-visible spectroscopy

A colour changes from colourless to dark brown denoted the formation of AgNPs, which was then visually validated. The extract is evaluated using a UV-Vis spectrophotometer (Systronics, India Model: 2202) with a slit diameter of 2nm. UV-Vis was used to measure the

sample maximal absorption from 300 to 600 nm. $AgNO_3$ served as the control, and deionized water performed as a blank.

2.4. Fourier transforms infrared analysis

Fourier transforms were used to examine the infrared spectra of produced nanoparticles (Bruker, Germany). In order to pinpoint the location of biological agents involved in particle formation, AgNP samples were manufactured using the KBr crystal as a beam splitter. The material was centrifuged at 10,000 rpm for ten minutes, and the pellets that were produced were then dried at 80 ◦C and pulverized to remove unwanted plant matter and silver with KBr crystal [43].

2.5. XRD and SEM-EDX analysis

Scanning electron microscopy was employed to characterise the biogenic nanoparticles (SEM, JSM-7900F, JEOL Ltd, Japan). Applying carbon or copper tape to place the AgNPs particle on the grid, gold was then sputtered using a sputter coater (Quorum Q150R ES, Quorum Technologies Ltd. Ashford, Kent, England). Diffraction limit was set to 10,000X and voltage at 15 kV. Dispersive energy X-ray (EDAX) evaluations of the attached sample's fundamental characteristics were made (Amtech GmbH, Wiesbaden, Germany). Additionally, the size and shape of the nanoparticles were measured using transmission electron microscopy (TEM, JEOL JEM-1011, Japan) [44].

2.6 Antibacterial activity

The human clinical pathogens named as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* were collected from the Department of Microbiology, Periyar University, Salem-636 011, Tamil Nadu, India.

2.6.1. Disk diffusion method

The disk diffusing method is employed in this work to measure the antibacterial activity [45]. For analysis, the cultures that were inoculated for 24 h in nutrient broth are examined. The

newly prepared nutrition medium is added to the petri plates and allowed 20 min to settle. Next, L-rod was used to distribute the test cultures across the medium. Various concentrations (50, 75, 100 and 125µg/mL) were used in this experiment. Chloramphenicol was used as positive controls (10 µg/mL disc). The growth plates were kept at 37 ◦C in an incubator for 12 h. The diameters of the inhibition zones were measured in millimetres and the test is done in triplicates [46,47].

2.6.2. Minimal inhibitory concentration

Using a modified broth macro-dilution method, the MICs of AgNPs against targeted bacterial strains were determined [48]. Test bsolutions of AgNPs (25, 50, 75, 100, 125 and 150µg/mL) were produced for MIC determination. In two sets, sterile nutrient broth was placed into a sugar test tube (12 X 75 mm) carrying 2.0 mL of a bacterial inoculum (culture density of 5 X 10^2 CFU/mL). Following that, each test tube was mixed with 2.0-mL individual doses of AgNPs, limiting the final tube volume to 4.0 mL, resulting in a 1:2 dilution, followed by 24 h at 37 °C incubation. At 600 nm, an optical density (O.D.) of microbial growth was determined. The MIC endpoint was defined as the lowest dose of AgNPs that showed no growth following incubation.

2.7. Antioxidant assays

2.7.1. DPPH scavenging activity

Methanol is use as a solvent along with 1-diphenyl-2-picrylhydrazyl (DPPH) to access the radical scavenging activity of the aqueous extract and the synthesised materials. A solution of 10 mg per mL was used to prepare the stock solution. Various concentrations of extracts, such as 20- 100µg/mL, were added to 0.1 mM of DPPH solution [46,47]. The solution was thoroughly mixed and left in a chilled, dark room for 30 min. As a control, the identical method was produced and used using ascorbic acid (0.1 mM). The equation was used to study the absorption.

Scavenging activity/ Inhibition Percentage = $(A_{control} - A_{sample}/ A_{control}) \times 100$

2.7.2. ABTS radical scavenging activity

It uses a modified version of the ABTS radical scavenging ability. The ABTS solution was improved by adding 0.0548g of ABTS to 50 mL of deionized water and 0.0189g of potassium per sulphate (70mM) to 1 mL of deionized water (2 mM). After 2 h of incubation, 200 µL of potassium per sulphate and 50 ml of ABTS were added and used. Different sample concentrations (10-50µg/mL) were added to 0.3 mL of the ABTS mixture, along with 1.7 mL of phosphate buffer, and the pH was elevated to 7.4. Then, the tubes were kept at 25 ˚C for 20 minutes of incubation. Utilizing UV, the absorbance was measured at 734 nm. The control was done without a sample using the same process [49].

Scavenging activity/ inhibition percentage = $(A_{control} - A_{sample}/ A_{control}) \times 100$

2.8. Cytotoxicity effects on A549 cell line

The National Centre for Cell Science (NCCS), Pune provided the lung cancer cell line (A549), which was maintained in Eagles Minimum Essential Medium with 10% foetal bovine serum (FBS). The cells were grown at a 37 °C temperature, 5% CO_2, 95% air, and 100% relative humidity. The maintained culture medium was replaced weekly. Trypsin-EDTA was used to separate monolayer cells so that single cell suspensions could be generated. Viable cells were counted using a haemocytometer and diluted with 5% FBS to give $1x10^5$ cells/mL. 96-well plates were supplied with 100 µL of cell suspension per well at a plating density of 10,000 cells/well and cultured to promote cell adhesion at 37°C, 5% CO_2, 95% air, and 100% relative humidity. The test samples were applied to the cells in various concentrations after 24 h. An aliquot of the test solution was diluted to double the final maximum test dosage using serum-free medium. In order to provide a total of 5 different doses, an extra 4 serial dilutions were made. 100 µL of each sample dilution was poured to

wells containing 100 µL of medium to have the final sample contents. The plates were incubated for an additional 48 h after the adding of the sample at 37˚C, 5% CO_2, 95% air, and 100% relative humidity. For all concentrations, triplicate was achieved and the medium containing no samples was used as the control. Yellow water soluble 2,5-diphenyltetrazolium bromide (MTT) is a tetrazolium salt. Succinate-dehydrogenase, a mitochondrial enzyme found in living cells, breaks the tetrazolium ring, turning the MTT into an insoluble purple formazan. As a result, the amounts of potential cells directly correlate with the amount of formazan produced. Each well received 15 µL of MTT (5 mg/mL) in phosphate buffered saline (PBS), which was added after 48 h, and was then incubated at 37˚C for 4 h. Following the removal of the MTT-containing medium, the formed formazan crystals were dissolved in 100 µL of DMSO, and the absorbance at 570 nm was then calculated using an ELISA reader [50,51,52].

2.9. Anti-inflammatory activity

2.9.1. Inhibition of albumin denaturation

Using the prevention of albumin denaturation approach developed with a few minor modifications, the anti-inflammatory effect of nanoparticles was investigated [53,54,55]. The mixture of reactions (0.5 mL; pH 6.3) contained 0.05 mL of distilled water and 0.45 mL of bovine serum albumin (5 percent aqueous solution), pH was adjusted at 6.3 using a small amount of 1 N HCl. Various plants extract volumes were added to the reaction mixture and incubated for 20 min at 37 °C before being boiled for 5 min at 57 °C. After the samples had cooled, 2.5 mL of phosphate buffer saline was then added. At 600 nm, turbidity was measured spectrophotometrically. To calculate the % reduction of protein denaturation, use the equation below:

$$\text{Percentage Inhibition (\%)} = (Abs_{control} - Abs_{sample}) / Abs_{control} \times 100$$

2.9.2. HRBC membrane stabilization assay

The lysosomal enzyme produced during inflammation induces many diseases. These enzymes are thought to have extracellular activity that is connected to either acute or chronic inflammation. The nonsteroidal medications either inhibit these lysosomal enzymes or stabilise the lysosomal membrane in order to exert their effects [56]. The various nanoparticles at the concentration of 50-250 μg/mL respectively, were incubated separately with HRBC solution. Healthy volunteer blood samples (2 mL) were combined with an equivalent volume of sterilised Alsever's solution (2% dextrose, 8% sodium citrate, 5% citric acid, and 0.42% sodium chloride in distilled water) and centrifuged at 3000 rpm. Before usage, a 10 percent v/v suspension of normal saline and was made with an isosaline solution wash for the packed cells. This suspension was then maintained at 4 ˚C unchanged. Synthesized AgNPs at different concentrations (50- 250 µg/0.5 mL) in normal saline, aspirin as a reference (50- 250 µg/0.5 mL), and distilled water as a control (to produce 100% haemolysis instead of hyposaline) were individually added with 1 mL of phosphate buffer, 2 ml of hyposaline, and 0.5 ml of 10% HR. The haemoglobin contents of each test AgNPs was calculated spectrophotometrically at 560 nm after centrifugation at 3000 rpm for 20 min and incubation at 37 °C for 30 min. The formula under was used to estimate the proportion of stability or protection of the HRBC membrane:

$$\text{Percentage Inhibition (\%)} = (Abs_{Control} - Abs_{sample}) / Abs_{Control} \times 100$$

2.10. Statistical analysis

Statistical analysis was done by GrapPad prism software and significance level was obtained through One-way ANNOVA. Each test was performed in triplicate, and the graph was generated using Graph Pad Prism ver. 5.00 (Graph Pad Software, La Jolla, CA).

3. Results

3.1. UV- visible spectroscopy

The colour change from brown to dark brown following the conclusion of the reduction reaction with AM extract and addition in $AgNO_3$ served as indication that AgNPs had been manufactured (1 mM). The constant band at 450 nm of the reaction mixture served as evidence that the AgNPs in **Fig. 1 A-D** were developed. The synthesised Nanoparticles are then purified by centrifugation at 10000 rpm for 15 min, and further washed with distilled H_2O to remove unwanted debris. The yield of the synthesised nanoparticles is about 500 µg/500 ml of the sample mixture.

3.2. FTIR analysis of AgNPs

The various functional groups that are found in the molecules that help in the reduction of silver ions into silver nanoparticles, as well as for capping and stabilising the nanoparticles, are detected using FTIR. It is possible to identify the absorption peak at around 3795.25 cm-1 and 3724.59 cm-1 to O-H stretching vibrations. C-H alkaline and C=O stretch carboxylic acids makes up the band at 3181.44 cm-1. Strong vibrations of carboxylic acids, or the C=O stretch, could be responsible for the intense band at 1650.06 cm-1. Strong alkene C-H group was detected as1337.66, 1198.09, 1158.28, 1069.01, and 853.35 cm-1. 820.94 shows the presence of intense C-O-O phenolic groups. *A. macrobotrys* extract contains carboxyl (-C=O), hydroxyl (-OH), and amine (N-H) groups that are primarily responsible for the reduction of Ag^+ ions to Ag nanoparticles, according to FT-IR study. The presence of proteins in the *A. macrobotrys* extracts provided as a reducing and stabilising agent for the AgNPs and minimized agglomeration. A process to support AgNPs and serving as a stabilising factor to minimize agglomeration in the aqueous medium may form due to the strong complex formation of the amino acid residues' carbonyl group for metal. **Fig. 2** shows the FTIR spectrum of synthesised AgNPs. Plant extract of *A. macrobotrys* acts as a capping agent. The

number of peaks that appeared in the FTIR spectrum highlighted the extract's richness (**Table 1**) [57]

3.3. X-ray diffraction analysis

To determine whether the nanoparticles are crystalline, XRD analysis is done. **Fig. 3** shows the XRD pattern of the synthesised AgNPs. The spectra showed diffraction peaks that matched to standard planes of silver at 31.9◦, 37.05◦, 42.85◦,46.2◦, 62.9◦, and 76.8◦, respectively, with interplanar spacing values of (100), (111), (200), (102), (220), and (311) planes. Additional small peaks are due to the existence of phytochemical over silver nanoparticles. The synthesised nanoparticles have a 63 % pure silver content and 39 percent silver oxide content. The synthesised silver nanoparticles are about 58 nm in mean.

3.4. SEM and EDX analysis

The structure and morphology of the synthesised silver nanoparticles are evaluated using SEM. The ensuing SEM images showed the production of spherical nanoparticles, which are aggregated into clusters roughly 0.5 µm wide (**Fig. 4A**). EDX analysis determines the presence of silver after solvent evaporation agglomerates particles in sample processing. A qualitative and quantitative profile of the elements that could be engaged in the production of AgNPs is revealed by EDX analysis. Due to surface plasmon resonance, the SEM-EDX data demonstrate a mass assessment of the nanoparticles. The strongest and sharpest peak of silver was obtained at 2.6 KeV, which supports the creation of AgNPs. Weaker signals from, C and O atoms were also recorded. These low signals, which might be produced by macromolecules such as proteins or enzymes. However, it is evident from EDX spectra that *A. macrobotrys* reduced AgNPs, giving them a weight percentage of 77.12%, as shown in **Fig. 4B**. The EDX examination showed a strong signal of Ag metal in the evaluated sample.

3.5. TEM analysis of AgNPs

TEM is a very useful instrument for characterisation of nanoparticles, which showed evidence on size and morphology of nanoparticles. The outcomes of the TEM study provided a very clear indication of the size and shape of the nanoparticles. The AgNPs ranged in size from 10.44 to 24.16 nm and were mostly monodisperse (**Fig. 5**). Silver nanoparticles were carefully examined at various magnifications of TEM images, and it was found that the particles are uniform in size (around 24.11 nm).

3.6. Antibacterial activity of *A. macrobotrys* AgNPs

The antibacterial activity of the water extract of *A. macrobotrys* and the manufactured AgNPs was evaluated at various concentrations (50-125 µg/mL) using the well diffusion technique and human pathogens via *E. coli*, *S. aureus*, *E. faecalis*, and *P. aeruginosa*. The results indicate highest zone of antibacterial activity was observed in *E. coli* (19 mm) at 125µg/mL and the lowest antibacterial activity was observed *P. aeruginosa* (7 mm) at 50µg/mL (**Fig. 6; Table 2**). On the basis of the data collected, we suggest that silver nanoparticles might be a promising and secure antibacterial agent.

3.7. Minimal inhibitory Concentration assay

The four human drug resistant clinical pathogens were tested against the standard and various concentrations of AgNPs. The gram negative bacterium *E. coli* and *K. pneumoniae* (23 µg/mL and 20 µg/mL) in the **Fig. 7** exhibits less inhibition than the Gram positive bacteria *B. subtilis* and *S. aureus*(13µg/mL and 14 µg/mL) respectively. The inhibition of bacterial growth may be due to the entry of Ag NPs into bacterial cells. The chloramphenicol is used as a positive control (1 µg/mL). The outcomes of results revealed that a green synthesised AgNPs might inhibit bacterial growth at low doses, implying that AgNPs could be an efficient broad spectrum bactericidal agent. These findings have a good link with previous research of a similar nature, which demonstrated that greenly generated AgNPs might

diminish Gram-negative bacteria in a way that depends on concentration [58]. Because of these properties, the efficiency of synthesised nanoparticles is diminished at low concentrations. AgNPs destroy bacteria based on their size, with small particles becoming far more efficient than larger ones [59]. After passing through the cell membrane of each bacterium, the nanoparticles formed link to multiple biomolecules such as lipid, protein, and DNA, causing oxidative stress and possibly cell death [60].

3.8. Radical scavenging activity

3.8.1. DPPH assay

AgNPs and conventional ascorbic acid were used to measure the DPPH scavenging activity. The results were represented in **Fig. 8A**. The regression equations produced for the doses of the extracts from percentage inhibition of free radical generation were used to estimate IC_{50} values (concentration of sample necessary to produce 50% of free radicals). Higher antioxidant activity is indicated by a lower IC_{50} value. The inhibition percentage was calculated for different concentrations like 20-100µg/mL was absorbed as 9.23±0.5, 19.33±0.3, 33.50±0.7, 44.30±0.3, 58.23±0.4%. The inhibition % of ascorbic acid seems to be 13.23±0.3, 21.23±0.5, 33.45±0.1, 45.60±0.5, 62.33±0.5% and synthesised AgNPs showed the IC_{50} value of 32µg/mL, respectively. This study confirms AgNPs increased antioxidant activity than the aqueous extract.

3.8.2. ABTS scavenging assay

The $ABTS^{+}$ scavenging activity results has inhibition shown in *A. macrobotrys* AgNPs aqueous plant extract (20-100µg/mL) and compared with standard ascorbic acid. The result shows the AgNPs aqueous extract contains maximum free radicals in the higher dose was found in 100µg/mL (68.87%), followed by 80µg/mL (53.64%), 60µg/mL (42.38%),

40µg/mL (37.09%), and 20µg/mL (29.8%). Based on the results, it can be said that AgNPs have antioxidant capacity that is dose-dependent (**Fig.8B**).

3.9. Cytotoxicity of AgNPs against lung cancer cells

The MTT assay was employed to assess the cytotoxicity effects of 48 hrs of exposure of lung cancer (A549) cells to five different doses (6.5-100 µg/mL) of manufactured AgNPs (**Fig. 9**). The finding results with colorimetric assay assessments a significant ($P \leq 0.05$) dosages-dependent enhanced in cytotoxicity against A549 cells. The maximum cytotoxicity (68.15%) was shown at dosages of 100 µg/mL of AgNPs, while at 6.5 µg/mL concentration 17.25% cells as compared to those of control. The IC_{50} value is 33.46µg/mL. In our assessment, fluorescence microscopy has been used to study the morphological abnormalities of (A549) lung cancer cells (**Fig. 10**). It detected a number of changes, such as cell shrinkage, membrane blebbing, and the appearance of apoptotic surfaces and the possible mechanism is given as a schematic illustration (**Fig. 11**).

3.10. Anti-inflammatory activity of AgNPs

The *in vitro* anti-inflammatory activity of *A. macrobroyts* fabricated AgNPs by HRBC membrane stabilization procedure showed that the absorbance of the AgNPs and the reference standard to decrease with the increasing dosages of the samples. The absorbance of the test materials was found to be more than reference (standard). The green manufactured AgNPs exhibited more anti-inflammatory activity than the aqueous extract. The leaf aqueous extract derived *A. macrobotrys* AgNPs showed highest of 76% albumin denaturation at 250µg/mL to 19% at 50µg/mL, whereas the percentage of albumin denaturation exhibited by aspirin found to be 62% to 15% at a concentration of 50µg/mL to 250µg/mL and is represented in Fig. The IC_{50} value 202.77. The percentage of protection is more in standard than the AgNPs from *A. macrobotrys* (**Fig. 12A**). The maximum % of protection and

membrane stabilization indicates by aspirin was 15 % to 62% at a concentration of 50 µg/mL to 250 µg/mL, followed by AgNPs with 20% to 83% protection at the same dosages (**Fig.12B**). The IC_{50} value is 188.37µg/mL. All the experiment was conducted in triplicates.

4. Discussions

Various efforts to introduce silver NPs from bio-based sources, including plants, bacteria, fungi, algae, and proteins, have sprung up as green technologies receive more and more attention [61]. These green synthesised metal-based NPs could be employed as drug carriers in pharmaceutical applications to increase drug delivery. They have flexible architectures that allow for physical property control and increased surface qualities that allow for targeted drug delivery [62]. The synthesis of AgNPs was characterized through UV-Visible spectrum after the confirmation of visible change of colour to dark brown. This happens due to the surface plasmon resonance, an in here nature of metal nanoparticles. The peak value has improved gradually in AgNPs as compared to the crude plant extracts [63]. The phyto-metabolites, such as phenolic component, flavonoids, and glycosides, play an important function as a reducing and stabilising agent [64].

Different analytical methods are used to structurally examine the manufactured NPs. In the present study, nanoparticles were produced from *A. macrobotrys*. The UV-visible spectrum of NPs demonstrated a strong absorption peak at the 450λmax. Similarly, Salayova et al. [65] reported the production of green NPs that were visible at 426 nm in the UV spectrum. Recently, Balachandar et al. [66] studied that the *Glochidion candolleanum* derived silver NPs displayed maximum UV absorption peak at 430nm.A x-ray diffractometer was used to analyse the crystallinity of the manufactured materials (XRD). In the present work, the manufactured *A. macrobotrys* nanoparticles (NPs) showed x-ray diffraction peaks at 2θ values of 31.94˚, 37.05˚, 42.85˚, 62.98˚, and 76.80˚. Recently, Rakesh et al. [67]

433 demonstrated that the XRD peaks of the *Mucuna pruriens*-mediated AgNPs were around 37.6
434 and 43.8 (in 2θ), which suits the crystalline patterns of AgNPs with a fcc structure. The
435 occurrence of element of silver was evidenced by the EDX signals at 2.5keV. Metallic silver
436 nanomaterials exhibit high spectral response mostly between 2.5 and 3.5 keV. Similar results
437 have been shown in a number of investigations [68,69].
438
439 Fourier transform infrared (FTIR) spectroscopy helps us find functional groups like
440 phenolic, amines, carboxyl, and alkyl groups, which are responsibility for the reduction of
441 AgNPs in the green synthesis of AgNPs [70]. The current results of the AgNPs FTIR
442 spectrum produced by *A. macrobotrys* demonstrate peaks for carboxyl, hydroxyl, primary and
443 secondary amine groups, confirming that the liquid served as a capping and stabilising agent
444 in the generation of AgNPs in plant leaf extract. Naveen et al. [71] reported that the *Potentilla*
445 *chinensis* mediated AgNPs exhibited the similar carboxyl and hydroxyl groups. The green
446 formation AgNPs were depicted in SEM images with produced nanoparticle sizes between 20
447 and 60 nm. In other reported work size of AgNPs also exists in this range [72,73].
448 The World Health Organisation (WHO) has identified antibacterial resistance as one of the
449 three primary root causes of human health hazards [74]. The biosynthesized AgNPs had the
450 strongest antibacterial efficacy at the lowest dose against the tested human pathogens. AgNPs
451 cause structural reforms in the bacterial cell wall and nuclear membrane that result in cell
452 death as a result of their strong interaction and ease by which they attach to tissue proteins
453 [75,76]. However, Taha et al. [77] reported that the interaction of the silver ion with the
454 cytoplasm within the cell is what essentially causes its bactericidal effects. Positive charged
455 nanomaterials interacting with negative polarity cells are believed to be the most effective
456 antibacterial agents. Numerous data point to the role of the liberated silver ions (Ag^{+}) from
457 AgNPs in the antibacterial action. The silver ion must be in its ionised state in order to

operate as a possible antibacterial candidate since silver positive charge is thought to be essential for its antimicrobial activities. Recently, Essghaier et al. [78], also investigated the development of *Scabiosa atropurpurea* AgNPs and showed they have a good antibacterial effect against *E. coli*. Additionally, Lubis et al. [79] have been studied the fabrication of AgNPs using *Persicaria odorata* leaf extract showed strong antibacterial effects against *S. epidermidis* and *S. aureus*. The interaction of AgNPs with sulphur-containing proteins found in cell membranes is considered to be the basis for the antibacterial activity of silver nanoparticles produced by biological means [80]. It disrupts the electrical function of the cell, destroys the structure of the membrane, and leaks the contents of the cell. It has been suggested that the free oxygen radicals that are produced when silver nanoparticles interact with bacteria cause cell membrane damage [81]. AgNPs may find possible locations in biological proteins, including enzymes, amino acid residues, and DNA. The potential harm brought on by AgNPs interacting with DNA may have an impact on cell division and DNA replication, ultimately resulting in cell death [82].

Recent research has demonstrated that AgNPs produced with plant extracts, such as aqueous or fruit extracts, have a strong antioxidant capacity [83]. In fact, it is thought that the binding of silver with phytochemicals from plant extracts is responsible for the antioxidant activity of AgNPs [84,85]. DPPH, which offers a simple and quick method to evaluate antioxidant activity, was employed in many investigations. The development of monochromatic solutions was generated by the antioxidant molecule inhibiting the DPPH radical. Secondary metabolic compounds including flavonoids, phenolic acids, and tannins that can donate hydrogen and have antiradical activity are present in plants. The present study, the experimental results shows that the AgNPs synthesized from *A. macrobotrys* possess maximum antioxidant activity nearly reference (ascorbic acid). Nanomaterials produced using green methodologies are well known to have applications and strong antioxidant

properties [86]. Recently, Dridi et al. [87] reported that plant-mediated AgNPs showed highly significant antioxidant potential in ABTS, DPPH, and FRAP assays. Additionally, Sahin Yaglioglu et al. [88] investigated the biosynthesis of AgNPs showed prominent antioxidant activity of DPPH and FRAP. In the current study, synthesized AgNPs showed strong antioxidant activity, suggesting promising utility in food and medicine.

Worldwide, cancer is a major issue challenge which represents 8 percent of the annual cancer mortality. Surgery, radiation, chemo, and targeted therapy are usually used to treat cancer, but there are several drawbacks to this approach, including its large cost and significant side effects [89]. AgNPs for the treatment of tumours is one of the spectacular applications of the developing discipline of nanotechnology. In our investigations, the cytotoxicity activity against lung cancer (A549) cells was directly concentration-dependent manner ($p \leq 0.05$). The literature review indicates that the extract mechanism of suppression towards cancer cell lines is still not fully understood. Interestingly, utilizing the A549 lung cancer line, this is the first study on the anti-cancer effects of AgNPs. Therefore, more investigations should be done to evaluate the potential mechanism responsible for the anticancer effects. AgNPs were assessed against the A549 lung cancer cell line, which concluded in similar reports [90,91,92]. Ag NPs have more cytotoxicity in cancer cells than in normal cells. The ability to quickly penetrate the cells is facilitated by their tiny size and high surface to volume ratio (**Fig.11**). Reactive oxygen species production, Caspase-3 activation, alteration of mitochondrial membrane potential, and DNA damage are the main mechanisms by which metallic nanoparticles minimise cancer cells [93]. Results demonstrated that the element suggested in the present work has strong inhibitory action against the A549 cancer cell line based on the findings. In current study, fabricated AgNPs produced significant anti-inflammatory actions using membrane stabilization and inhibiting

albumin denaturation was exhibited dose-dependent manner. Highest inhibitions are shown at 61.50% at 250µg/mL. Aspirin (reference) displayed the maximum inhibition 72.50% at 250 µg/mL. Moreover, the effects of AgNPs were the most potent and were comparable to the effect of aspirin. These outcomes are in harmony with Azeem et al. [94].

5. Conclusions

In conclusion, AgNPs were manufactured using *A. macrobotrys* aqueous extract as a reducing agent. In tests against clinical pathogens, AgNPs had shown their strongest antibacterial effects. This has low costs and uses eco-friendly methods. The findings obtained using different analytical characterization methods such as UV- visible spectrophotometer, SEM, TEM, EDX, XRD and FT-IR proven the presence of AgNPs. Additionally, the fabricated Ag-NPs exhibited strong 450 nm absorption peak. AgNPs with sphere and oval shapes and sizes between 20-50nm were observed in the SEM and TEM images. The AgNPs demonstrated promising efficacy against the bacterial cultures that were the focus of the study. The maximum AgNPs (125 µg/mL) concentrations exhibited the zone of growth inhibition was around 7-19 mm. These findings imply that the green fabricated AgNPs may be applied as efficient substitute antibacterial agents against infection brought on by MDR resistant bacteria and efficiently inhibit their growth. Finally, we recommended AgNPs as alternative wide-spectrum antimicrobial agents.

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Author contribution Saipraba Sivakumar, Ragavendran Chinnasamy: Investigation, Conceptualization, Methodology, Writing-original draft. Murugesan Subban:

Conceptualization, Date curation, Writing-original draft. Kamaraj Chinnaperumal: Formal analysis, Data curation. Ismini Nakouti: Formal analysis, Data curation. Mohamed A. El-Sheikh and Jilani Purusottapatnam Shaik: Funds provided the characterization of nanoparticles. All the authors read and approved the final manuscript.

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Data availability The datasets used and/or analysed during the current study are available from the authors on reasonable request.

Declarations

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