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Sivakumar, S, Subban, M, Chinnasamy, R, Chinnaperumal, K, Nakouti, I, El – Sheikh, MA and Purusottapatnam Shaik, J (2023) Green synthesized silver nanoparticles using Andrographis macrobotrys Nees leaf extract and its potential to antibacterial, antioxidant, anti-inflammatory and lung cancer

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1 **Green synthesized silver nanoparticles using *Andrographis macrobotrys* Nees leaf extract**
2 **and its potential to antibacterial, antioxidant, anti-inflammatory and cytotoxicity effects**
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34
35 **Abstract**
36

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

53

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

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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,

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

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

101

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

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

117 Several plant species in the Acanthaceae family have the potentially to be used as
118 medicines. About 28 species of the Andrographis genus are found all over India.
119 *Andrographis macrobotrys* Nees is one of the ethno-medicinal plants that the tribal people in
120 Karnataka, Kerala, and Tamil Nadu use to treat snake bites, fever, muscle pain, and skin
121 diseases. It grows in the locations around Karnataka, Kerala, and Tamil Nadu. The existence
122 of various phytochemicals, such as phenols, flavonoids, tannins, and steroids, is demonstrated
123 by phytochemical analysis on these plants [38]. This plant is important for curing diseases,
124 according to the traditional medical systems of Unani, Siddha, and Ayurveda [39].
125 Andrographolide, deoxy andrographolide, neo andrographolide, 14-deoxy-11, 12-
126 didehydroandrographolide, and iso andrographolide have anti-atherosclerosis, anti-cancer,
127 anti-diabetic, anti-inflammatory, anti-oxidant, immune-stimulant, hepato-protective, and
128 insecticidal properties [40]. As an outcome, the present investigation has been carried out
129 employing a first-report environmentally benign approach of producing silver nanoparticles
130 from *A. macrobotrys* leaf extract. The objectives of present study aimed to synthesised
131 nanoparticles were characterization such as UV-visible spectroscopy, X-ray diffraction
132 (XRD), FTIR spectroscopy, SEM-EDX and TEM. Furthermore, examined the biological
133 applications such as anti-bacterial, anti-oxidant (DPPH and ABTS), cytotoxicity assay using
134 lung cancer (A549) cell lines and anti-inflammatory assay (albumin denaturation and HRBC
135 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

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

163 **2.4. Fourier transforms infrared analysis**

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

170 **2.5. XRD and SEM-EDX analysis**

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

179 **2.6 Antibacterial activity**

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

183 **2.6.1. Disk diffusion method**

184 The disk diffusing method is employed in this work to measure the antibacterial activity [45].
185 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.

211 Scavenging activity/ Inhibition Percentage = ($A_{control}$ –A_{sample}/ $A_{control}$)×100

212

213 **2.7.2. ABTS radical scavenging activity**

214 It uses a modified version of the ABTS radical scavenging ability. The ABTS solution was 215 improved by adding 0.0548g of ABTS to 50 mL of deionized water and 0.0189g of 216 potassium per sulphate (70mM) to 1 mL of deionized water (2 mM). After 2 h of incubation, 217 200 µL of potassium per sulphate and 50 ml of ABTS were added and used. Different sample 218 concentrations (10-50µg/mL) were added to 0.3 mL of the ABTS mixture, along with 1.7 mL 219 of phosphate buffer, and the pH was elevated to 7.4. Then, the tubes were kept at 25 °C for 20 220 minutes of incubation. Utilizing UV, the absorbance was measured at 734 nm. The control 221 was done without a sample using the same process [49].

222 Scavenging activity/ inhibition percentage = ($A_{control}$ –A_{sample}/ $A_{control}$)×100

223 **2.8. Cytotoxicity effects on A549 cell line**

224 The National Centre for Cell Science (NCCS), Pune provided the lung cancer cell line 225 (A549), which was maintained in Eagles Minimum Essential Medium with 10% foetal 226 bovine serum (FBS). The cells were grown at a 37 °C temperature, 5% CO_2, 95% air, and 227 100% relative humidity. The maintained culture medium was replaced weekly. Trypsin-228 EDTA was used to separate monolayer cells so that single cell suspensions could be 229 generated. Viable cells were counted using a haemocytometer and diluted with 5% FBS to 230 give $1x10^5$ cells/mL. 96-well plates were supplied with 100 µL of cell suspension per well at 231 a plating density of 10,000 cells/well and cultured to promote cell adhesion at 37°C, 5% CO_2, 232 95% air, and 100% relative humidity. The test samples were applied to the cells in various 233 concentrations after 24 h. An aliquot of the test solution was diluted to double the final 234 maximum test dosage using serum-free medium. In order to provide a total of 5 different 235 doses, an extra 4 serial dilutions were made. 100 µL of each sample dilution was poured to

236 wells containing 100 µL of medium to have the final sample contents. The plates were
237 incubated for an additional 48 h after the adding of the sample at 37˚C, 5% CO_2, 95% air, and
238 100% relative humidity. For all concentrations, triplicate was achieved and the medium
239 containing no samples was used as the control. Yellow water soluble 2,5-diphenyltetrazolium
240 bromide (MTT) is a tetrazolium salt. Succinate-dehydrogenase, a mitochondrial enzyme
241 found in living cells, breaks the tetrazolium ring, turning the MTT into an insoluble purple
242 formazan. As a result, the amounts of potential cells directly correlate with the amount of
243 formazan produced. Each well received 15 µL of MTT (5 mg/mL) in phosphate buffered
244 saline (PBS), which was added after 48 h, and was then incubated at 37˚C for 4 h. Following
245 the removal of the MTT-containing medium, the formed formazan crystals were dissolved in
246 100 µL of DMSO, and the absorbance at 570 nm was then calculated using an ELISA reader
247 [50,51,52].

248 **2.9. Anti-inflammatory activity**

249 **2.9.1. Inhibition of albumin denaturation**

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

259 $$\text{Percentage Inhibition (\%)} = (Abs_{control} - Abs_{sample}) / Abs_{control} \times 100$$

260

261 **2.9.2. HRBC membrane stabilization assay**

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

279 $$\text{Percentage Inhibition (\%)} = (Abs_{Control} - Abs_{sample}) / Abs_{Control} \; X100$$

280

281 **2.10. Statistical analysis**

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

285

286 **3. Results**

287 **3.1. UV- visible spectroscopy**

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

295 **3.2. FTIR analysis of AgNPs**

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

311 number of peaks that appeared in the FTIR spectrum highlighted the extract's richness (**Table**
312 **1**) [57]

313 **3.3. X-ray diffraction analysis**

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

321 **3.4. SEM and EDX analysis**

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

334

335

336 **3.5. TEM analysis of AgNPs**

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

343 **3.6. Antibacterial activity of *A. macrobotrys* AgNPs**

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

351 **3.7. Minimal inhibitory Concentration assay**

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

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

367 **3.8. Radical scavenging activity**

368 **3.8.1. DPPH assay**

369 AgNPs and conventional ascorbic acid were used to measure the DPPH scavenging activity.
370 The results were represented in **Fig. 8A**. The regression equations produced for the doses of
371 the extracts from percentage inhibition of free radical generation were used to estimate IC_{50}
372 values (concentration of sample necessary to produce 50% of free radicals). Higher
373 antioxidant activity is indicated by a lower IC_{50} value. The inhibition percentage was
374 calculated for different concentrations like 20-100µg/mL was absorbed as 9.23±0.5,
375 19.33±0.3, 33.50±0.7, 44.30±0.3, 58.23±0.4%. The inhibition % of ascorbic acid seems to be
376 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
377 IC_{50} value of 32µg/mL, respectively. This study confirms AgNPs increased antioxidant
378 activity than the aqueous extract.

379 **3.8.2. ABTS scavenging assay**

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

384 40µg/mL (37.09%), and 20µg/mL (29.8%). Based on the results, it can be said that AgNPs
385 have antioxidant capacity that is dose-dependent (**Fig.8B**).

386 **3.9. Cytotoxicity of AgNPs against lung cancer cells**

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

397 **3.10. Anti-inflammatory activity of AgNPs**

398 The *in vitro* anti-inflammatory activity of *A. macrobroyts* fabricated AgNPs by HRBC
399 membrane stabilization procedure showed that the absorbance of the AgNPs and the
400 reference standard to decrease with the increasing dosages of the samples. The absorbance of
401 the test materials was found to be more than reference (standard). The green manufactured
402 AgNPs exhibited more anti-inflammatory activity than the aqueous extract. The leaf aqueous
403 extract derived *A. macrobotrys* AgNPs showed highest of 76% albumin denaturation at
404 250µg/mL to 19% at 50µg/mL, whereas the percentage of albumin denaturation exhibited by
405 aspirin found to be 62% to 15% at a concentration of 50µg/mL to 250µg/mL and is
406 represented in Fig. The IC $_{50}$ value 202.77. The percentage of protection is more in standard
407 than the AgNPs from *A. macrobotrys* (**Fig. 12A**). The maximum % of protection and

408 membrane stabilization indicates by aspirin was 15 % to 62% at a concentration of 50 µg/mL
409 to 250 µg/mL, followed by AgNPs with 20% to 83% protection at the same dosages
410 (**Fig.12B**). The IC_{50} value is 188.37µg/mL. All the experiment was conducted in triplicates.
411

412 **4. Discussions**

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

424 Different analytical methods are used to structurally examine the manufactured NPs. In the
425 present study, nanoparticles were produced from *A. macrobotrys*. The UV-visible spectrum
426 of NPs demonstrated a strong absorption peak at the 450λmax. Similarly, Salayova et al. [65]
427 reported the production of green NPs that were visible at 426 nm in the UV spectrum.
428 Recently, Balachandar et al. [66] studied that the *Glochidion candolleanum* derived silver
429 NPs displayed maximum UV absorption peak at 430nm.A x-ray diffractometer was used to
430 analyse the crystallinity of the manufactured materials (XRD). In the present work, the
431 manufactured *A. macrobotrys* nanoparticles (NPs) showed x-ray diffraction peaks at 2θ
432 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

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

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

483 properties [86]. Recently, Dridi et al. [87] reported that plant-mediated AgNPs showed highly
484 significant antioxidant potential in ABTS, DPPH, and FRAP assays. Additionally, Sahin
485 Yaglioglu et al. [88] investigated the biosynthesis of AgNPs showed prominent antioxidant
486 activity of DPPH and FRAP. In the current study, synthesized AgNPs showed strong
487 antioxidant activity, suggesting promising utility in food and medicine.
488

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

508 albumin denaturation was exhibited dose-dependent manner. Highest inhibitions are shown at
509 61.50% at 250µg/mL. Aspirin (reference) displayed the maximum inhibition 72.50% at 250
510 µg/mL. Moreover, the effects of AgNPs were the most potent and were comparable to the
511 effect of aspirin. These outcomes are in harmony with Azeem et al. [94].

512 **5. Conclusions**

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

526

527 **Acknowledgements** We are thankful to Periyar University in Tamil Nadu, India for
528 supporting us with the necessary infrastructure to complete the project successfully. The
529 authors acknowledge the funding support from Researchers Supporting Project Number
530 (RSP-2022/182), King Saud University, Riyadh, Saudi Arabia.

531

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

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

538 **Funding** This study was funded from University Grant Funds (URF), Periyar University,
539 Salem and Research Supporting Project Number (RSP-2022/182), King Saud University,
540 Riyadh, Saudi Arabia.

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

543 **Declarations**

544 **Ethics approval** Not applicable.

545 **Consent to participate** Not applicable.

546 **Consent for publication** All authors have read the final version of the article and agreed to
547 publish it in Environmental Science and Pollution Research.

548 **Competing Interest** The authors declare no competing interests.

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