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Callus-derived silver nanoparticles from myrmecodia tuberosa: green synthesis and antioxidant properties

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ABSTRACT

Green synthesis of Silver nanoparticles (AgNPs) using plant-based materials is an environmentally friendly path for the synthesis of silver nanoparticles instead of traditional chemical methods. This study presents the first biosynthesis of AgNPs using an ethanolic extract obtained from in vitro callus cultures of Myrmecodia tuberosa Jack, an endangered medicinal epiphyte and conservation-friendly biotechnological platform. Cotyledon explants were successfully induced high-frequency friable, green callus and the ethanolic extract acted as reducing and stabilizing agent. UV-Vis spectroscopy revealed surface plasmon resonance peaks at 420–450 nm with a visible color change from light brown to dark brown, confirming the AgNP formation. The most intense and well-defined SPR peak indicating optimal nanoparticle synthesis was observed after 6–8 days static incubation at 35°C using 2 mM $AgNO_3$. In the DPPH radical scavenging assay, it could moderate antioxidant activities, with IC_{50} = 51.80±0.035 µg mL^{-1}) which was more than that of parent callus extract (IC_{50} = 58.91±0.097µgmL^{-1}). This improvement is probably a result of synergistic effects between silver core and phytochemical capping layer. These results indicate that callus cultures of M. tuberosa is an environmentally friendly, sustainable and conservation-friendly source of reducing metabolites for the preparation of green nanomaterials applicable in biomedical practices.

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Introduction

Nanotechnology, a candidate for the next industrial revolution, is poised to have a significant impact on society, the economy, and everyday life. Nanoparticles are particles sized 1–100 nm, differing in character from larger particles of the same material (Balashanmugam and Pudupalayam Thangavelu 2015; Beyene et al. 2017). Nanoparticles are widely applied across science and technology. Silver nanoparticles are favored for medicine, the health sector, and antimicrobial properties in technological and consumer products (Ahmed et al. 2016).

The green synthesis of silver nanoparticles (AgNPs) using plant extracts has gained prominence as an eco-friendly, cost-effective alternative to chemical and physical methods, leveraging phytochemicals as reducing and stabilizing agents. It has become popular for its eco-friendliness and low cost, as well as its lower toxicity relative to chemical risks (Alharbi et al. 2022; Handoko et al. 2017; Handoko et al. 2019). Plant extracts can be obtained from naturally grown plants as well as by tissue culture. Many benefits of plant cell culture technologies include consistent reproduction, controlled metabolite production, renewable resources, and eco-friendliness, all of which are advantageous for the growing trend toward products that

produce secondary metabolites quickly and support rapid growth (Abdulhafiz et al. 2022). Additionally, plant cell culture technology may help conserve endangered or threatened plants in their native habitats (Anis and Faisal 2005).

These approaches come with limitations including restrictions in gene availability, seasonal or discontinuous growth rates, and the harvesting of endangered species (Bennur et al., 2025; Nordine, 2025). Callus cultures provide a promising sustainable alternative as they allow for standardized biomass production with stable metabolite profiles in different seasons independent of environmental fluctuation (Kirwale et al., 2026; Schmitt et al., 2025; Sudheer et al., 2025). Additionally, extracts derived from callus alleviate pressure on threatened wild populations of medicinal plants such as Myrmecodia tuberosa. Currently, the use of callus extracts from this epiphytic species for biosynthesis of silver nanoparticles remains uninvestigated, which is a crucial knowledge gap that this study aims to fill.

Due to the rapid plant metabolism and controlled growth conditions, callus culture represents an efficient approach for producing the applied biomass and bioactive compounds. The ability of these cultures to reproduce, maintain a constant metabolism in the subcultured form and not require whole plants means that they offer consistent production of silver nanoparticles whilst conserving endangered species (Manjkhola et al. 2005; Rani et al. 2017). The secondary metabolites production using callus culture and plant cell culture technologies have many advantages, including rapid biomass accumulation; growing and maintaining the plants under controlled conditions that are independent of seasonal or environmental variations; sustainability for endangered species and/or epiphytic species. Callus cultures should provide a greater yield of metabolites in the appropriate biosynthetic forms and support conservation by lessening dependence on wild collection (Murthy et al. 2017).

The M. tuberosa extract offers numerous pharmacological actions, including antioxidant, antibacterial, cytotoxic, and anticancer activity (Dirgantara et al. 2022). The ant plant callus produces a wide range of secondary metabolites, including flavonoids, phenols, alkaloids, and steroids. Further, extracts from M. tuberosa are rich in flavonoids, phenols, and other polyphenols, contributing to potent antioxidant activity, which is relevant for enhancing the functionality of biosynthesized AgNPs (Sari et al. 2017; Sari et al. 2018), reduce the number of Streptococcus mutans colony in vitro on TYC media containing dental plaque sample (Achmad et al. 2019).

Studies have been conducted into synthesizing AgNPs and their antioxidant activity from various plants such as Tradescantia pallida (Shahzadi et al. 2022), Cestrum nocturnum (Keshari et al. 2020), and callus extracts such as Hyptis suaveolens (Botcha and Prattipati 2023), single garlic (Sari et al. 2023), and sweet lime fruit (Kalia et al. 2020). Furthermore, Kumar et al. (2019) discuss how analytic methods such as UV–Vis spectrometry, transmission electron microscopy (TEM), scanning electron microscopy (SEM), Fourier transmission infrared spectroscopy (FTIR), and X-ray diffraction (XRD) can be used to assess the characteristics of AgNPs produced using plant extract. Recent studies have demonstrated the efficacy of callus extracts in green AgNP synthesis with enhanced bioactivities, yet no reports exist on M. tuberosa callus, a sustainable source of antioxidant-rich metabolites from an epiphytic medicinal plant facing risks of overexploitation. This study aimed to optimise AgNP synthesis parameters using phytochemicals derived from callus which consists of (1) induce and characterize callus from M. tuberosa cotyledon explants using MS medium supplemented with 2,4 D and kinetin; (2) biosynthesize AgNPs using ethanolic callus extract and determine the optimal AgNO3 concentration (0.5-2.0 mM) for maximum nanoparticle formation; (3) confirm nanoparticle formation via UV-Vis spectroscopy; and (4) evaluate and compare the antioxidant activity of AgNPs and callus extract using DPPH assay.

This study was designed as an experimental comparative study to investigate the potential of M. tuberosa callus extract for green AgNP biosynthesis. The reasons for choosing this experimental design are threefold. First, callus culture offers a sustainable, conservation-friendly alternative to wild harvesting of this endangered epiphytic species, thereby resolving the overexploitation issue. Second, callus extracts provide consistent metabolite profiles regardless of season or environment, ensuring reproducible nanoparticle formation. Third, his approach optimizes synthesis parameters ($AgNO_3$ concentration, incubation duration, pH) and enables for direct comparison of AgNPs and crude extract antioxidant properties.

Method

Explant sources and sterilization

All chemicals (analytical grade) used in this study were obtained from Sigma Aldrich, Inc. in the United States. Ant plant seeds were sourced from the Tanjung Palas region, Bulungan, North Kalimantan,

Indonesia. Sterilization followed a stepwise protocol: seeds were immersed in 70% ethanol for 1 min, then in 30%, 20%, and 10% sodium hypochlorite (v/v) (initial concentration 5.25%) for 10 min each, followed by three 5-min rinses in sterile distilled water. The Murashige and Skoog (MS) medium and agar (7.5 g L^{-1}) with 30 g L^{-1} sucrose were used to grow the seeds (Murashige and Skoog, 1962) (Sari et al. 2018).

Callus Induction and Subculture

The seeds of sterile ant plants were grown in glass bottles using MS0 (base media without plant growth regulators) and incubated at 20-25°C with a light intensity of 1000–2000 lux. The seeds used were 100, divided into 10 bottles, with 10 replicates per bottle. One month later, seedlings were selected, and cotyledons were used as explants. One month later, seedlings were selected, and their cotyledon leaves were used as explants. A total of 200 cotyledon explants were used (based on preliminary experiments). For ten weeks, the explants were grown in MS medium supplemented with a mixture of the growth regulators 2,4-D (2 mg L^{-1}) and kinetin (2 mg L^{-1}). This hormone combination was chosen based on its proven efficacy in inducing friable, metabolite-rich callus in Myrmecodia species (Sari et al. 2018). Callus formed in the second week and grew larger each week. The culture was maintained at 20–25°C under a light intensity of 1000–2000 lux (16 hours light/8 hours dark). After three weeks, the large calli were divided into smaller pieces and subcultured in fresh medium. By the following three weeks, the callus had grown large, ready for harvest and extraction. Calluses were studied qualitatively, including their color and texture.

Preparation of Ethanolic Callus Extract

Ethanol served as the solvent for callus extraction. 101.6424 grams of fresh callus were macerated in 1000 mL of ethanol. Extraction was performed using Whatman paper (Whatman 2; Sigma-Aldrich, Germany) until the filtrate was colorless (approximately 48 h), followed by solvent removal by rotary evaporation at 50–55°C under reduced pressure. The residual solvent was removed using a rotary evaporator (Buchi, Switzerland) at 50–55°C. A total of 3.6708 grams of the extracted ethanol was collected. 1 g of the extract was weighed, dissolved in 10 mL of deionized water, and used for the nanoparticle biosynthesis. The extract yield is calculated using the formula: (Weight of extract/Weight of fresh callus) × 100%.

Biosynthesis and Antioxidant Activity of AgNPs

The biosynthesis of AgNPs, was accomplished by treating ethanol callus extract prepared with silver nitrate ($AgNO_3$). In short, 1 mL of aqueous callus extract solution (100 mg mL^{-1}) was mixed with 9 mL $AgNO_3$ solution (final concentrations: 0, 0.5, 1.0, 1.5 and2.0 mM), in sterile glass vials to obtain an extract-to-precursor ratio of 1:9 (v/v). The pH of the reaction mixture before and after was determined using a pH meter. The mixtures were then statically incubated (without shaking or stirring) at 35°C for 8 d. The vials were kept covered loosely as to prevent contamination while permitting some gas exchange and held in ambient laboratory light/dark cycles. The colour was observed visually on a daily basis. Synthesis experiments were performed in triplicate for all of the data presented.

Characterization of AgNPs-As and Antioxidant activity

The nanoparticle colloid was centrifuged at 10,000 rpm for 15 minutes before being freeze-dried (Alpha 1-2 LO, Germany). The AgNPs were characterized using a UV-Vis spectrophotometer (SP-UN52N, Beijing, China). UV-Vis absorption spectra were recorded in the range of 300–700 nm at medium scan speed with a slit width of 2 nm, using quartz cuvettes and deionized water as a blank reference. All measurements were performed in triplicate to ensure reproducibility. Characterization was limited to UV-Vis spectroscopy (SP-UN52N, Beijing, China) to confirm surface plasmon resonance (SPR) peaks at 400–500 nm, as advanced techniques (TEM, SEM) were unavailable in this study. The DPPH radical scavenging activity was measured according to a previously described method. Briefly, 100 µL of sample (AgNPs or callus extract) at various concentrations (3.125–100 µg mL^{-1}) was mixed with 100 µL of 0.1 mM DPPH in ethanol. Specifically, following 30min of incubation in darkness at 25°C absorbance was measured at a wavelength of 514nm using spectrophotometer. Positive control was used by Ascorbic acid. All assays were conducted in triplicate and values are shown as mean ± standard deviation (SD).

The percentage inhibition was determined as follows: % Inhibition = $[(A_0 - A_1)/A_0] \times 100$, where A_0 refers to the absorbance of the control and A_1 refers to the absorbance of each sample. IC_{50} values (concentration needed to inhibit 50% of DPPH radicals) were obtained by Microsoft Excel using linear regression analysis (percentage inhibition vs concentration). The regression equations and coefficient of determination (R^2) are reported for each sample. Statistical differences between samples were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, with $p < 0.05$ considered significant (Arung et al. 2006).

Results and Discussions

Callus Induction

The cotyledon explants grown on MS media supplemented with plant growth regulators, 2,4-D at 2 mg/L and Kinetin at 2 mg/L, produced callus with a fragile texture and a green color. Callus induction occurred from cotyledon explants on MS medium supplemented with 2,4-D (2 mg/L) and kinetin (2 mg/L), resulting in friable, green calli (Figure 1). The explant swelled, and a callus developed at the incision site after a week. The callus kept growing until it completely covered the cotyledon's surface in a month. Formation was evident at explant edges and wounds, with uniform growth across the surface after one month.

(a) (b) (c)

Figure 1 <a. Ant plant callus in MS medium with the addition of 2 ppm 2,4 D + 2 ppm Kinetin, b. Subculture callus, c. Callus collection. Bar = 1 cm>

Friable calli are characterized by a high water content because the wall cells have not yet completed lignification, and the individual cells are easily distinguished from one another. The callus texture of the explant is classified as friable or non-friable. Non-friable callus cells are compact and tight, making separation difficult. In contrast, a friable callus from an explant has loose cell interactions and is easily separated. A total of 200 explants were planted, all of which produced callus (100%); the fresh weight of the callus at the end of the observation period (3 weeks after subculture) was 101.6424 g

Yield Extract of Ant Plant Callus

The yield is the proportion of the product's dry weight to the weight of the raw material. Table 1 shows the results of measuring the yield of ant plant callus extract using 95% ethanol as the solvent.

Table 1<Measuring the Yield of Ant Plant Callus Extract>

Callus Weight (g)	Extract Weight (g)	Yield (%)
101,6424	3,6708	3,6114

The extraction yield of *Myrmecodia tuberosa* callus obtained in this study (3.6114%) using 95% ethanol is relatively moderate. The lower yield observed in callus tissue in this study indicates that the production and accumulation of these chemicals may not yet be fully optimized. The accumulation of bioactive substances in differentiated tissues is linked to the greater yields.

Biosynthesis of Silver Nanoparticles (Color Change and pH stability)

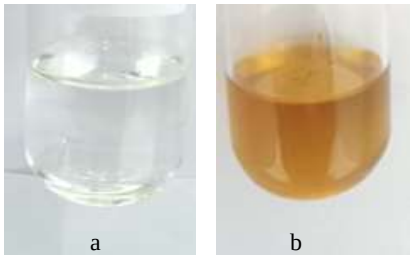


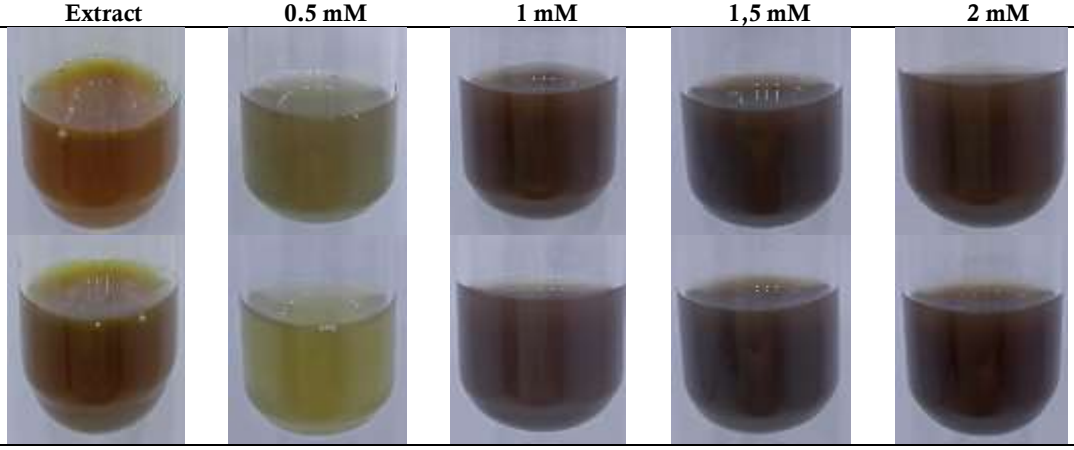
Figure 2 <(a) $AgNO_3$ solution 1 mM (b) Ant Plant Callus Extract>

The $AgNO_3$ solution is colorless, whereas the ant plant callus extract is light brown (Figure 2). Biosynthesis of AgNPs was indicated by a color change from light brown to dark brown at $AgNO_3$

concentrations of 1–2 mM, observed from days 6 to 8 (Figure 3). This serves as preliminary evidence of nanoparticle formation, with color intensity increasing with $AgNO_3$ concentration.

Table 2 <Changes in Color of Biosynthesized Silver Nanoparticles of Ant Plant Callus Extract for 8 Days of Observation>

Days	Ant Callus Extract	Concentrations of $AgNO_3$ 0.5 mM	1 mM	1,5 mM	2 mM
0					
1					
2					
3					
4					
5					
6					

Days	Ant Callus Extract	Concentrations of $AgNO_3$ 0.5 mM	1 mM	1,5 mM	2 mM
7					
8					

The color change in silver nanoparticles biosynthesized with ant callus extract bioreductor is shown over 8 days (Figure 3). The solution turned from light brown to dark brown at a concentration of 1–2 mM $AgNO_3$, starting from day 6–8. The dark brown color became more intense as the $AgNO_3$ concentration increased.

The results of pH measurements for AgNPs and nest callus extract over 8 days show that the pH tended to increase. pH stability of AgNPs over 8 days showed that results remained stable across all treatments, although some fluctuations were still observed (Figure 4).

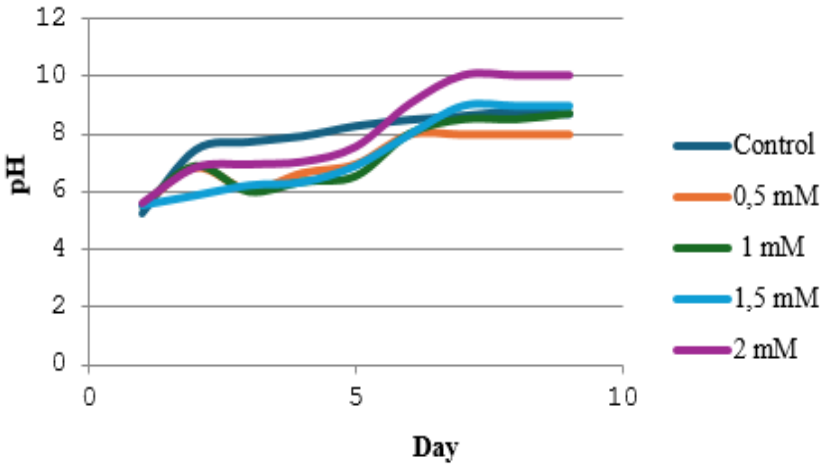


Figure 3 <pH of AgNPs from ant plant callus extract with various concentrations of $AgNO_3$ for 8 days (a) Control, b) $AgNO_3$ 0,5 mM, (c) $AgNO_3$ 1 mM, (d) $AgNO_3$ 1,5 mM, (e) $AgNO_3$ 2 mM>

pH of AgNPs biosynthesized using callus extract of the ant plant (*Myrmecodia tuberosa*) was evaluated over 8 days across five experimental groups: (a) control (no $AgNO_3$), (b) 0.5 mM, (c) 1 mM, (d) 1.5 mM, and (e) 2 mM $AgNO_3$. The control group (without callus extract) showed a pH increase from 5.21 on day 0 to 8.80 on day 7, indicating rapid alkalization. All groups treated with AgNPs showed significantly better pH stability during the first 5 days than the control group. Through day 4, 1.5 mM and 2 mM $AgNO_3$ treatments had lower pH values, showing that the buffering ability was better with these treatments. In contrast, all treatments demonstrated an increasing trend in pH from day 5 onward, with the value of treatment 2 mM reaching the maximum pH (10) on day 6. Between days 0 and 8, the pH values for the 0.5 mM and 1 mM treatments were relatively stable with final pH values of approximately 8.0–8.7 on day 8. This means that with callus extract of selected three species producing even higher concentrations, better pH stability was observed at higher $AgNO_3$ concentration (1.5–2 mM) added for synthesis of AgNPs. In the 2 mM formulation, a mildly alkaline plateau (pH 10) was reached by day 6 indicating complete bioreduction as well as efficient capping by phytochemical constituents.

Characterization of Silver Nanoparticles

The UV-Vis spectral analysis showed characteristic SPR peaks at 420–450 nm, confirming AgNP formation (Figure 4).

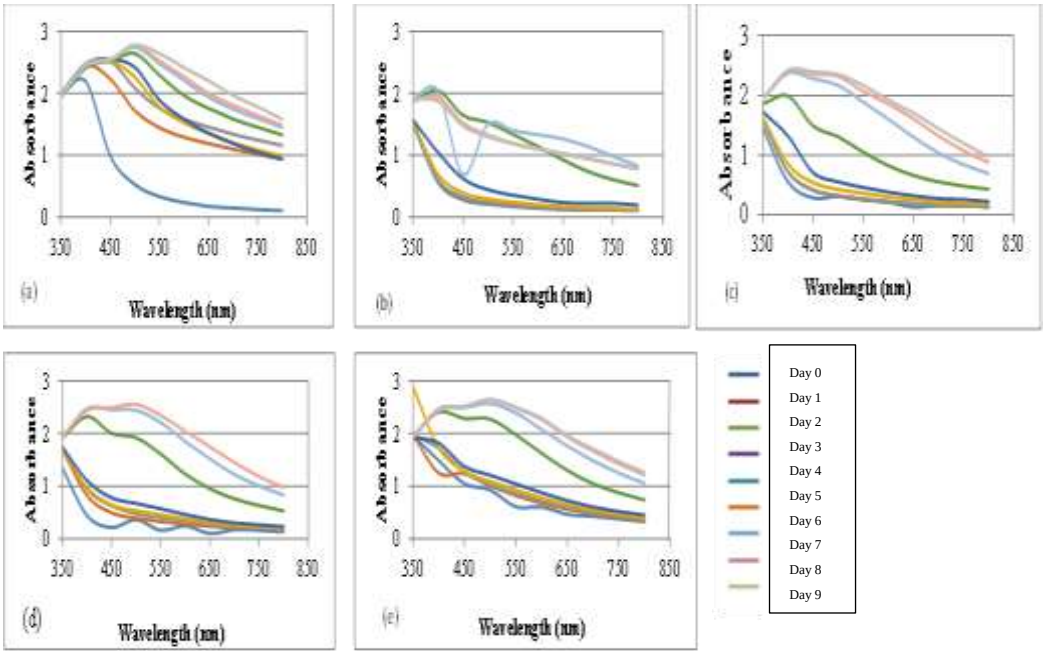


Figure 4 <UV-VIS Spectra of the Biosynthesis of AgNPs using the Ethanolic Extract of Ant Nest Callus with Various Concentrations of $AgNO_3$ for 8 Days (a) Control, b) $AgNO_3$ 0,5 mM, (c) $AgNO_3$ 1 mM, (d) $AgNO_3$ 1,5 mM, (e) $AgNO_3$ 2 mM>

For further insight, maximum absorbance values at the wavelength of the SPR peak (430 nm) were extracted from spectra for each $AgNO_3$ concentration (0.5, 1.0, 1.5 and 2.0 mM) days 0, 2, 4, 6 and day 8 in development. When plotted, the absorbance values increased in a clear dose- and time-dependent manner. After 8 days, the highest absorbance (0.82 ± 0.03) was achieved on treatment with 2 mM $AgNO_3$. The time-dependent development of SPR strength sticks to the repetitive decline of Ag^+ into Ag^0 and steady accumulation of AgNPs in reaction medium. The intensity and definition of the SPR peak increased with $AgNO_3$ concentration up to 2 mM. The reaction mixture with 2 mM $AgNO_3$ exhibited the most intense and sharpest absorption peak, coupled with the most pronounced dark brown coloration, compared to lower concentrations. Therefore, the 2 mM condition was designated as optimal for synthesis under the tested parameters, based on these spectroscopic and visual indicators of nanoparticle yield and stability (Javier and Camacho, 2022).

Antioxidant Activity of Silver Nanoparticles

Table 2 <Antioxidant Activity of Plant Callus Extract and AgNPs>

Sample	Concentration (µg mL-1)	Absorbance (nm)	% Inhibition	IC50 (µg mL^{-1})
Ant Nest Callus Extract	3.125	0.381±0.012	23.340	58.909±0,097
	6.25	0.298±0.021	40.040	
	12.5	0.210±0.030	57.810	
	25	0.189±0.035	61.900	
	50	0.091±0.016	81.620	
	100	0.068±0.006	86.320	
AgNPs	3.125	0.228±0.065	54.060	51.800±0,035
	6.25	0.205±0.035	58.690	
	12.5	0.168±0.006	66.130	
	25	0.157±0.015	68.340	
	50	0.136±0.004	72.640	
	100	0.110±0.002	77.930	
Ascorbic Acid	3.125	0.325±0.022	34.610	14.625±0,055
	6.25	0.279±0.018	43.860	
	12.5	0.210±0.025	57.680	
	25	0.094±0.025	81.15	
	50	0.063±0.008	87.39	
	100	0.004±0.001	99.26	

The IC_{50} values were 58.909 µg mL^{-1} for callus extract, 51.800 µg mL^{-1} for AgNPs, and 14.625 µg mL The antioxidant activity of AgNPs is higher than ant plant callus extract.

DPPH free radical scavenging activity was used to assess the antioxidant activity of both biosynthesized AgNPs and the ant plant callus extract. The AgNPs concentration used is the optimal 2 mM. At 514 nm, the DPPH transitions from violet to colorless in ethanol. Table 2 shows the DPPH activity of AgNPs and the ant plant callus extract.

Callus Induction

The main factor influencing callus formation in the media is the concentration of plant growth regulators. Optimal callus growth is made possible by the proper concentration and combination of plant growth regulators. Juvenile tissues, such as cotyledons explant in this research, have increased cellular plasticity and endogenous auxin levels, which synergize with exogenous 2,4-D and kinetin to enhance dedifferentiation. Callus growth is achieved through a balance between endogenous and exogenous growth regulators (PGRs). In this study, the addition of 2,4-D at 2 mg/L and kinetin at 2 mg/L collaborated with endogenous hormones to produce optimal callus growth. 2,4-D is a group of auxins that plays a role in cell division, and kinetin in cell development. Myrmecodia plant cotyledon explants develop a callus in about a week, which is a quick initial response. Sometimes it takes longer to generate callus in other plants.

Formation was evident at explant edges and wounds, with uniform growth across the surface after one month, indicating effective nutrient uptake (Sari et al. 2018). A single differentiated cell and additional totipotent cells can form a callus capable of regenerating the entire plant body (Fehér 2019). The friability indicates active cell division, making the tissue suitable for suspension culture and metabolite extraction. Compared to compact callus, friable types allow better penetration of elicitors and higher extraction efficiency. In the observed callus formation, the friability is consistent with reports by Syamsudin et al. (2021) who induced callus cultures of Myrmecodia were induced using MS media supplemented with 2,4-D (1–2 mg/L) and BAP (0.5–1 mg/L). Sari et al. (2018) demonstrated that 2 mg/L 2,4-D combined with 2 mg/L kinetin induced friable callus. . The friable callus was observed on leaf explants of Salvia moorcroftiana L. were induced on MS medium supplemented with 1 mg/L 2,4-D and 1 mg/L BAP (Bano et al., 2022), cotyledon explants were cultured on MS (Murashige and Skoog medium 1962) with 1.0 mg L−1 NAA and 1.5 mg L−1 BA (Shwe & Leung, 2020), leaf and hypocotyl explants of Gomphrena globosa on MS medium with 0.5 mg/L 2,4 D + 1.0 mg/L BAP (Pari et al., 2023), leaf explant of Coffea arabica on MS medium supplemented with 1 mg/L 2,4-D and 1 mg/L BAP (Arimarsetiowati et al., 2023) and leaf explant of Gynochthodes umbellata on MS medium with 1 mg/l 2, 4-D (Anjusha. & Appukuttannair, 2016).

The ant plant callus was green due to its chlorophyll concentration (Figure 1). The inclusion of the plant growth regulators 2,4-D and kinetin resulted in the formation of chlorophyll in the callus. Tarrahi and Rezanejad (2013); Xiao et al. (2025) found that chlorophyll production caused the color change in callus to green. Meanwhile, George et al. (2008) revealed that, kinetin plays a role in the formation of chlorophyll, causing the green color to appear. Friable and green callus was also found on Catunaregam spinosa using leaves as explant (Lawrence et al., 2022) Randia echinocarpa using cotyledon explant (Valenzuela-Atondo et al., 2020), Rhodiola dumulosa using internodes explants (Lu et al., 2023).

The successful induction of callus from Myrmecodia tuberosa Jack highlights the plant's amenability to in vitro propagation and its potential as a source of bioactive compounds (Wahyuni et al. 2022). The use of callus tissue rather than whole plants is also consistent with sustainable plant biotechnology approaches, lowering the environmental impact of collecting endangered species from their natural habitats.

Yield Extract of Ant Plant Callus

The extraction yield of *Myrmecodia tuberosa* obtained in this study (3.6114%) using 95% ethanol can be categorized as relatively moderate (Table 1). The lower yield observed in callus tissue in this study indicates that the production and accumulation of these chemicals may not yet be fully optimized. The accumulation of bioactive substances in differentiated tissues is linked to the greater yields. Callus cultures produce fewer secondary metabolites than whole plants because they are undifferentiated (Isah, 2019). Efferth (2019) observed that yields from plant cell and callus cultures frequently fall below 5% unless metabolite production is increased via elicitation or optimal culture conditions.

The yield reflects extraction efficiency but does not directly indicate bioactive compound levels. Further phytochemical analysis would be required to quantify specific bioactives. The basic characteristics that offer enticing opportunities for the production of various secondary metabolite plant products are cell homogeneity, scalability, a reasonably short cell doubling time, minimal space requirements, and ease of

handling. Furthermore, large-scale production of bioactive chemicals is best done by in vitro culture to avoid microbial contamination and seasonal fluctuations (Bapat et al. 2023).

Biosynthesis of Silver Nanoparticles (Color Change and pH stability)

The solution changed color from light brown to dark brown, indicating the formation of silver nanoparticles (Botcha and Prattipati 2019; Govarthanan et al. 2016; Sari et al. 2023; Shkryl et al. 2017). The color shift is due to reduction of Ag^+ to Ag^0 by a reducing agent in the extract. Furthermore, the extract's metabolic compound may contribute to the reduction of Ag^+ to Ag^0, whilst the presence of protein improves the stability of AgNPs (Botcha and Prattipati 2019). During the AgNP production process, the callus extract's secondary metabolites serve as reducing, stabilizing, and capping agents. Silver ions (Ag^+) were changed into elemental silver (Ag^0), which caused the reaction mixture to change color (Vallinayagam et al. 2021). Flavonoids, alkaloids, glycosides, terpenoids, phenols, carbohydrates, proteins, enzymes, and coenzymes are among the phytochemicals that contribute to the green production of AgNPs as bioreductors and stabilizers (Patil et al. 2012).

The AgNPs cationic pH stability is a vital factor controlling their long-term colloidal stability, surface charge and biological activities (Ahmed et al. 2016). The stability of AgNPs synthesized with M. tuberosa callus extract was also confirmed by measuring the pH shape in all concentrations of $AgNO_3$ tested (ranging from 0.05 to 5 mM) after eight days, with no significant fluctuations detected; this indicates that the biomolecules released from callus were efficient at capping AgNPs, inhibiting uncontrolled aggregation. In the case of control (pH 5.8–6.0), it is slightly acidic, which indicates that plant callus extracts contain organic acids, phenolic compounds and amino acids released from metabolically active cells (Hassan et al. 2018). These molecules, not only work as reducing agents but also helps to establish the first pH medium. The pH was initially lower upon addition of $AgNO_3$, because of free Ag^+ ions present in the medium interacting with residual protons and nitrate counterions (Das et al. 2017).

Increase of pH over time is a widely reported phenomenon while carrying out the green synthesis of AgNPs. This happens due to reduction of Ag^+ ions into Ag^0 nanoparticles which will consume protons in the process and lead a shift of equilibrium to less acidic (Khan et al. 2019). Of the concentrations examined, 2.0 mM $AgNO_3$ yielded the most consistent pH trend with minimal oscillation amplitude. This suggests that the reducing capacity of the callus extract was not saturated at low (0.5 and 1 mM) but rather at high (1.5 and 2.0 mM) concentrations of Ag^+ ions were added for a short period resulting in pH being decreased before stabilization. This has been already reported by Roy et al. (2020), who also previously found that supra-optimal precursor concentrations delayed the reduction process, and induced transient pH drops. Thus, the use of 2.0 mM $AgNO_3$ seems to offer a compromise between precursor availability and reducing ability of M. tuberosa callus metabolites.

Characterization of Silver Nanoparticles

Characterizing AgNPs can be done with the help of UV-Vis spectrophotometry (Sharma et al. 2019; Shkryl et al. 2017). The analysis takes a measurement of the amount of light that passes through the sample and compares it to the incident light source's reference measurement. Nanoparticles are typically detected using wavelengths between 400 and 800 nm (Prathna et al. 2011). The UV-Vis spectral analysis showed characteristic SPR peaks at 420–450 nm, confirmed AgNP formation; no inferences were drawn regarding particle size or shape (Patil et al. 2012). It should be noted that UV-Vis spectroscopy alone does not provide information on particle size, shape, or size distribution; such characterization would require additional techniques such as TEM or DLS and is beyond the scope of this study. Silver nanoparticles (AgNPs) were synthesized over eight days using an ethanolic extract from the callus of the ant nest plant, according to the UV-Vis spectral analysis (Figure 5).

The AgNPs were produced earlier, and the production intensified with increasing $AgNO_3$ concentration up to 2 mM. Absorbance intensity increased with $AgNO_3$ concentration up to 2 mM, suggesting a dose-dependent biosynthesis. These phytochemicals, like flavonoids, phenolics, and terpenoids, act as reducing and capping agents The green synthesis of $AgNO_3$ is significantly influenced by the concentration of AgNO3. The absorption increases with increasing $AgNO_3$ concentration (Vanaja and Annadurai 2012). Maarebia et al. (2019) reported that the results of the synthesis of silver nanoparticles from the water extract of the ant plant (Myrmecodia pendans) produced absorbance peaks at wavelengths of 400–500 nm.

Antioxidant Activity of Silver Nanoparticles

The slightly lower IC_{50} (i.e., higher activity) of AgNPs compared to the crude extract indicates an enhancement in radical scavenging capacity upon nanoparticle formation. This enhancement can be attributed to several factors. First, the nanoscale size of AgNPs provides a large surface area-to-volume ratio,

enabling more efficient interaction with DPPH radicals at the molecular level. Second, the phytochemicals (e.g., flavonoids, phenolics) that coat the nanoparticle surface not only stabilize AgNPs but also directly contribute to radical scavenging, creating a synergistic effect between the silver core and the organic layer. Third, the immobilization of bioactive compounds on the nanoparticle surface may protect them from degradation, thereby preserving their antioxidant potential. The observed activity is consistent with previous reports. AgNPs synthesized from callus extracts of *Hyptis suaveolens* exhibited an IC_{50} of 74.66 µg mL^{-1}, with the authors similarly attributing the activity to synergistic capping effects (Botcha and Prattipati 2023). AgNPs from *Boerhavia diffusa* callus were reported by James et al. (2025) to possess potent antioxidant properties linked to surface-bound phenolics and Sudheer et al. (2025) reported spherical *Boerhavia diffusa* AgNPs with an average size of 9 nm that exhibit strong antimicrobial properties. Begum et al. (2020) demonstrated that callus cultures of *Fagonia indica* elicited with biologically synthesized AgNPs showed enhanced biomass accumulation and secondary metabolite production El-Rafie et al. (2022) found that AgNPs from *Rosmarinus officinalis* extract had significantly lower IC_{50} values than the crude extract, supporting the concept of enhanced activity through nanoscale formulation. Collectively, these comparisons validate the moderate enhancement observed in the present study and highlight the potential of callus-derived AgNPs as functional antioxidants.

AgNPs' greater efficacy can also be attributed to their nanoscale size, which enables them to interact with free radicals at the molecular level and penetrate more efficiently. During nanoparticle formation, silver and phytochemicals form a capping layer that stabilizes the nanoparticles while preserving their antioxidant activity (Kumar et al. 2023). This capping enhances radical neutralization by facilitating redox interactions in addition to preventing aggregation. Antioxidants are compounds that can prevent or reduce the oxidative damage caused by free radicals. The mechanism involves the transfer of electrons or hydrogen atoms from antioxidants to free radicals (Khan et al. 2018). Silver nanoparticles generated from medicinal plant extracts are extremely toxic to cancer cells. Antioxidant materials derived from ethnomedicinal plants, such as silver nanoparticles, reduce tumor volume by eliminating free radicals and inhibiting the formation of malignant cells (Radini et al. 2018; Sangami and Manu 2017).

This study demonstrates the successful green biosynthesis of silver nanoparticles (AgNPs) using ethanolic extract from in vitro-derived callus of Myrmecodia tuberosa Jack, an epiphytic medicinal plant. This is the first report on green synthesis of AgNPs using callus extract of Myrmecodia tuberosa, offering a conservation-friendly alternative to wild harvesting. Nanoparticle formation was preliminarily confirmed by surface plasmon resonance peaks at 420–450 nm in UV-Vis spectroscopy and optimal synthesis at 2 mM $AgNO_3$. The biosynthesized AgNPs exhibited moderate DPPH radical scavenging activity (IC_{50} = 51.800 µg/mL), slightly superior to that of the callus extract (IC_{50} = 58.909 µg/mL) and considerably lower than ascorbic acid (IC_{50} = 14.625 µg/mL). This mild enhancement suggests a potential contribution of the nanoparticle form, likely through increased surface area and interaction with phytochemical capping agents. The use of callus culture offers a sustainable alternative to wild harvesting of M. tuberosa, supporting conservation efforts while providing a consistent source of reducing and stabilizing metabolites for green nanotechnology.

Conclusions

This study clearly illustrates the potential of green biosynthesis of AgNPs from Myrmecodia tuberosa callus extracts and presents evidence for increased antioxidant activity. The antioxidant activity of AgNPs slightly higher than callus extract. This mild enhancement suggests a potential contribution of the nanoparticle form, likely through increased surface area and interaction with phytochemical capping agents. However, there are some limitations that need to be addressed. Initially, all characterization of the nanoparticles was limited to UV-Vis spectroscopy as this is a type of instrumentation that can confirm formation and provides details about the SPR peak; however, it does not provide information regarding particle size or morphology, crystallinity, or surface chemistry. Consequently, any conclusions about size, shape or size distribution would be speculative and are therefore avoided in this report; those properties require complementary techniques [transmission electron microscopy (TEM), scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR) or X-ray diffraction (XRD). Second, the radical-scavenging profile of M. alba was characterized solely using DPPH assay, other assays are needed in order to fully characterize this profile (ABTS or FRAP; Cell-based Antioxidant Assay). Third, the composition of the metabolites present in the callus extract can vary based on culture conditions (subculture frequency, light quality, concentrations of hormones) altering both reproducibility and biological activity in nanoparticle

synthesis. Thus, the results reported here should be considered as preliminary proof-of-concept. These limitations should be filled by properly conducting the comprehensive physicochemical characterization of AgNPs, culturing callus under optimized conditions to have standard reducing and capping metabolites along with assessment of their biological activities including antimicrobial and anticancer properties. These efforts will determine whether the improved antioxidant activity observed her translates into useful biomedical applications.

We clearly state that our results are unique to M. tuberosa from the North Kalimantan region of Tanjung Palas. Metabolite profiles and the effectiveness of nanoparticle production in other populations or species may be impacted by variations in genotype, environmental factors, and culture techniques. We suggest conducting multi-site research to confirm our method's generalizability.

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