

A Preliminary Study on the Green Synthesis, Characterization, In Vitro Antioxidant, Anti-Inflammatory, And Cytotoxic Properties of Silver Nanoparticles Using Both Aqueous and Ethanol Leaf Extracts of *Annona Muricata* (Soursop)

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ABSTRACT

The use of nanoparticles across various fields has garnered significant attention due to their unique properties and diverse applications. This preliminary study explored the green synthesis of silver nanoparticles (AgNPs) using aqueous and ethanol extracts of *Annona muricata* (soursop) leaves as reducing and stabilizing agents. The AgNPs synthesized using leaf extracts were characterized by FTIR, SEM-EDX, TEM, and DLS analyses. The FTIR spectra indicated the presence of phytochemicals acting primary as reducing and capping agents. SEM analysis revealed predominantly spherical to quasi-spherical nanoparticles with sizes ranging from ~20-70 nm, while EDX confirmed the elemental presence of silver (Ag). TEM showed mostly spherical nanoparticles with sizes ranging from ~3-30 nm (AgNPs synthesized from 5 mL ethanol extract) to ~10-40 nm (AgNPs synthesized from 3 mL aqueous extract). DLS analysis indicated moderate polydispersity and negative zeta potentials (-21.7 mV to -29.4 mV), suggesting good colloidal stability, with ethanol-derived AgNPs exhibiting superior properties. The AgNPs displayed significant, concentration-dependent in vitro bioactivities. Those synthesized with 5 mL ethanol leaf extract exhibited the highest ferric reducing antioxidant power (FRAP: 324 µg AAE/mL), DPPH radical scavenging (64.29%), and ABTS⁺ scavenging activity (73.9%). They also demonstrated potent anti-inflammatory activity (78.82% BSA denaturation inhibition at 50 µg/mL), surpassing the standard drug diclofenac sodium, along with notable cytotoxicity in the brine shrimp lethality assay (LC₅₀: 32.5 µg/mL). These findings highlight the effectiveness of *A. muricata* leaf extracts, especially ethanol extract, for

the eco-friendly synthesis of AgNPs, yielding nanoparticles with promising antioxidant, anti-inflammatory, and cytotoxic potential.

Keywords: Green synthesis, *Annona muricata*, silver nanoparticles, antioxidant activity, anti-inflammatory activity, cytotoxicity, leaf extract, characterization

INTRODUCTION

Nanoparticles (NPs) are materials with at least one characteristic dimension in the 1–100 nm range. Depending on their morphology, NPs are classified as zero-, one-, two-, or three-dimensional (0D–3D) structures. Nanotechnology focuses on the precise manipulation and control of matter at the nanoscale (10^{-9} m) to engineer materials with unique physicochemical properties and advanced functionalities [1-5].

The biological method of nanoparticle synthesis using plant extracts (green syntheses) is widely regarded as an eco-friendly and superior alternative to chemical and physical methods [6]. Unlike the physical and chemical methods that require high energy and chemicals, respectively, biological agents utilize naturally occurring phytochemicals, proteins, and enzymes for the formation and potential capping of nano-hybrids [7]. The synthesis of silver nanoparticles (AgNPs) through a biological approach is known for its simplicity, reliability, non-toxicity, and environmental friendliness [8]. These green-synthesized AgNPs are biocompatible and safe for various therapeutic applications, leading to their incorporation in household products such as antimicrobial agents, textiles, wound dressings, and antiseptic sprays [9]. The versatility of AgNPs in drug delivery, diagnostics, and disease treatment has sparked a surge in scientific research [6]. These nanoparticles have demonstrated antibacterial [10], anti-diabetic [11], anti-inflammatory [11], antioxidative [12], and antiproliferative properties [13], making them valuable assets in combating

various diseases. Plant biogenic-induced synthesis of nanoparticles is particularly noteworthy for its anti-cancer capabilities, minimal side effects, and numerous positive effects in diseased states [14]. Studies have linked the anti-cancer properties of AgNPs to their ability to induce oxidative stress, mitochondrial dysfunction, DNA damage, and ultimately apoptosis [15]. Numerous compounds derived from plants have shown efficacy against a wide range of human ailments. Plant extracts from species such as *Desmodium gangeticum* (L.) [16], *Catharanthus roseus* [17], *Reinwardtia indica* [18], *Justicia carnea* [19], *Camellia sinensis* [20], and *Shorea roxburghii* [21] have been utilized in the synthesis of AgNPs with promising biomedical applications. This growing body of research underscores the potential of plant-based nanoparticle synthesis in advancing healthcare and addressing various medical challenges. *Annona muricata*, a species of *Annona* in the custard apple tree family *Annonaceae*, is known for its edible fruit [22]. Commonly referred to as "Soursop" due to its slightly acidic taste when ripe, *Annona muricata* is native to the Caribbean and Central America but is now widely cultivated, and in some regions, has become invasive in tropical and subtropical climates worldwide, including India [23]. Various parts of the *A. muricata* plant have been used in traditional medicine to treat a range of ailments. The seeds are used to combat worms, while the fruits are used to alleviate arthritis and fever. The leaves are utilized to address hyperglycemia, inflammation, spasms, and cancer. Additionally, the plant's juice and leaves, and branch infusions have been employed as sedatives and

treatments for respiratory illnesses, malaria, gastrointestinal issues, and liver, heart, and kidney problems [24].

Furthermore, the leaves, bark, and roots of *A. muricata* have long been utilized for their various medicinal properties. These include antihypertensive, antidiabetic, anti-inflammatory, smooth muscle relaxant, sedative, and antispasmodic effects [25]. Rich in secondary metabolites such as saponins, alkaloids, flavonoids, terpenoids, annonaceous acetogenins, megastigmanes, cyclopeptides, and essential oils, *A. muricata* offers a variety of potential biomedical applications [26]. While there have been numerous publications on the successful synthesis of silver nanoparticles from various extracts of *A. muricata* parts, the plant's well-known therapeutic potential has not been fully explored in the combined synthesis and characterization of silver nanoparticles using aqueous and ethanolic leaf and root extracts of *A. muricata*. Furthermore, there is a need for more research on the antioxidant, anti-inflammatory, and cytotoxic potentials of green-synthesized AgNPs. As such, this study aims to synthesize and characterize silver nanoparticles using the aqueous and ethanolic leaf and root extracts of *A. muricata*, and also to examine the antioxidant, anti-inflammatory, and cytotoxic potentials of the green-synthesized AgNPs.

MATERIALS AND METHODS

2.1 Preparation of plant extracts

The fresh leaves of *A. muricata* were procured from a local herbal market, thoroughly washed, air-dried, and finely ground into a homogeneous powder. The powdered plant materials were subsequently used for the preparation of aqueous and ethanol extracts, as well as for the synthesis of silver nanoparticles. Extracts were prepared using the decoction method. Briefly, 25 g of powdered leaves were added separately into 250 mL of distilled water and ethanol (1:10 w/v) in individual beakers. The mixtures were heated to 95°C and maintained at this temperature for

20 minutes. After cooling to room temperature, the extracts were filtered through Whatman No. 1 filter paper and stored at 4°C for subsequent use [27].

2.2 Synthesis of silver nanoparticles

The reducing and capping potential of aqueous and ethanol leaf extracts of *A. muricata* in the synthesis of silver nanoparticles (AgNPs) from silver nitrate (AgNO_3) was evaluated. A 10^{-3} mM aqueous AgNO_3 solution was prepared by dissolving 0.17 g of AgNO_3 in 1 L of distilled water. Subsequently, 3.0 mL and 5.0 mL of each leaf extract were separately introduced into 10 mL aliquots of the AgNO_3 solution in different beakers at room temperature. The mixtures were then stored in a dark chamber to minimize photochemical interference. Within 15 minutes, a visible color transition from green to yellow or brown was observed in the leaf extracts, confirming the formation of AgNPs [19, 28].

2.3 Characterization of silver nanoparticles

The formation of silver nanoparticles was initially confirmed by visual observation of a color change from green to yellow and brown, indicating surface plasmon resonance. UV-visible spectroscopy monitored the bioreduction of Ag^+ ions within the 300-700 nm range. Functional groups responsible for reduction and capping were identified using Fourier transform infrared (FTIR) spectroscopy, with samples prepared as KBr pellets. Morphology and elemental composition were assessed through scanning electron microscopy coupled with energy-dispersive X-ray (SEM-EDX) spectroscopy. Particle size and shape were determined via transmission electron microscopy (TEM), with samples prepared on carbon-coated copper grids. Hydrodynamic size distribution and zeta potential measurements were performed using dynamic light scattering (DLS).

2.4 Ferric reducing antioxidant potential (FRAP) assay

The FRAP assay of AgNPs synthesized from *A. muricata* extracts were assessed following standard method [29]. The FRAP reagent was prepared by

mixing 25 mL of acetate buffer (30 mM, pH 3.6) with 2.5 mL of TPTZ solution (10 mM) and 2.5 mL of ferric chloride solution (20 mM). This mixture was incubated at 37 °C for 15 minutes before use. Ascorbic acid (vitamin C) was used as the standard, and a calibration curve was generated using concentrations ranging from 50 to 500 µg/L in water. For the assay, 150 µL of plant extract (0.1 mg/mL in methanol) or standard solution was added to 2.85 mL of the FRAP reagent in test tubes. The resulting mixture was incubated in the dark for 30 minutes, and absorbance was measured at 593 nm. A blank sample contained methanol in place of the plant extracts. Results were expressed as micrograms of ascorbic acid equivalents (AAE) per millilitre, as previously reported [19]

2.5 DPPH free radical scavenging activity

The DPPH radical scavenging activity of the extracts was determined using standard procedure [30], with slight modifications [31]. A stock solution of DPPH was prepared by dissolving 24 mg of DPPH in 100 mL of methanol and stored under refrigeration. A working solution was obtained by diluting the stock with methanol to achieve an absorbance of approximately 0.98 ± 0.02 at 517 nm [32]. In the assay, 3 mL of the working DPPH solution was mixed with 100 µL of plant extract (1 mg/mL) or standard solution. Absorbance was recorded at 517 nm over 30 minutes. The percentage of radical scavenging activity was calculated using the formula:

% Antioxidant activity = $[(Ac - As)/Ac] \times 100$, where Ac and As represent the absorbances of the control and the sample, respectively. In the control setup, methanol replaced the plant extract.

2.6 ABTS⁺ radical scavenging activity

The ABTS⁺ radical scavenging activity was measured using standard method [33], with modifications [34]. The working solution of ABTS⁺ was prepared by reacting 9.5 mL of ABTS solution (7 mM) with 245 µL of potassium persulfate (100 mM) and adjusting the final volume to 10 mL with distilled water. This mixture was kept in the dark at room temperature for

18 hours. It was then diluted with potassium phosphate buffer (0.1 M, pH 7.4) to achieve an absorbance of 0.70 ± 0.02 at 734 nm. Serial dilutions of the plant extracts (50-1250 µg/mL in methanol) were prepared. For the assay, 10 µL of each dilution was mixed with 2.99 mL of the ABTS working solution, and absorbance was measured at 734 nm. Antioxidant activity was expressed as a percentage, calculated using the formula:

% Antioxidant activity = $[(Ac - As)/Ac] \times 100$, with Ac and As denoting the absorbances of the control and sample, respectively. The control contained methanol in place of the sample.

2.7 Anti-inflammatory potential of the synthesized AgNPs

The anti-inflammatory activity of the synthesized AgNPs was evaluated using the Bovine Serum Albumin (BSA) denaturation assay [35]. A reaction mixture consisting of 0.45 mL of BSA and 0.05 mL of AgNPs (50 µg/mL) was prepared, and the pH was adjusted to 6.3. The samples were kept at room temperature for 10 minutes and subsequently incubated at 55 °C in a water bath for 30 minutes. Diclofenac sodium served as the standard drug, while the control group received dimethyl sulfoxide (DMSO). Absorbance was measured at 660 nm using a spectrophotometer. The percentage inhibition of protein denaturation was calculated using the equation:

% Inhibition = $[(\text{Absorbance of control} - \text{Absorbance of sample}) / \text{Absorbance of control}] \times 100$.

2.8 Cytotoxicity potential of the synthesized AgNPs using brine shrimp lethality assay

The brine shrimp lethality assay was employed to evaluate the cytotoxic effects of the synthesized AgNPs, following standard procedure [36]. Two extracts dilutions (500 µL, 50 µL, and 5 µL) were added to sets of five graduated tubes, and each tube received 10 brine shrimp larvae using a Pasteur pipette. Marine salt solution was added to achieve final concentrations of 1000 µg/mL, 100 µg/mL, and

10 µg/mL, respectively. Each concentration was tested in five replicates, along with five control tubes containing no extract. After a 24-hour exposure period at room temperature, the number of surviving larvae was counted. The percentage mortality at each concentration was calculated, and if mortality occurred in the control group, the results were corrected using Gakuya's formula [37]:
 Corrected mortality (%) = [(Test mortality – Control mortality) / Control mortality] × 100.

2.9 Statistical analysis

The experimental data were analyzed using appropriate statistical methods. Spectral data were processed and visualized using OriginPro v9.1, while statistical analyses and additional graphical

representations were performed using SPSS Statistics v26.0. For comparative analysis between sample groups, one-way analysis of variance (ANOVA) was applied, followed by Tukey's post hoc test to determine significant differences. A threshold of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The successful green synthesis of silver nanoparticles (AgNPs) using both aqueous and ethanol leaf extracts of *Annona muricata* was confirmed through multiple characterization techniques. The successful formation of nanoparticles, evidenced by a distinct color change and UV-Vis absorbance, was observed with the aqueous and ethanol leaf extracts.



Figure 1: Visual indicator of the reduction process confirms the formation of AgNPs (left to right).

The visible color change observed upon mixing the extracts with silver nitrate (AgNO_3), transitioning to yellow and brown (Figure 1), is a classic indicator of AgNP formation, attributed to surface plasmon resonance (SPR) [38]. This visual evidence was corroborated by UV-Vis spectroscopy, which revealed

distinct SPR absorption peaks centered around 420 nm for all synthesized AgNPs (Figures 2-5). The presence of these peaks confirms the reduction of silver ions (Ag^+) to silver atoms (Ag^0) by phytochemicals present in the *A. muricata* extracts [6, 19].

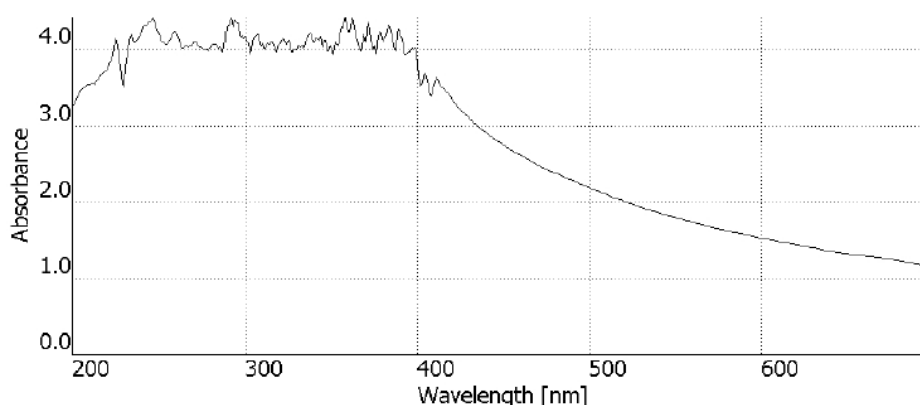


Figure 2: UV-Visible spectrum of AgNPs from 3 mL aqueous leaf extract showing absorbance at 420 nm

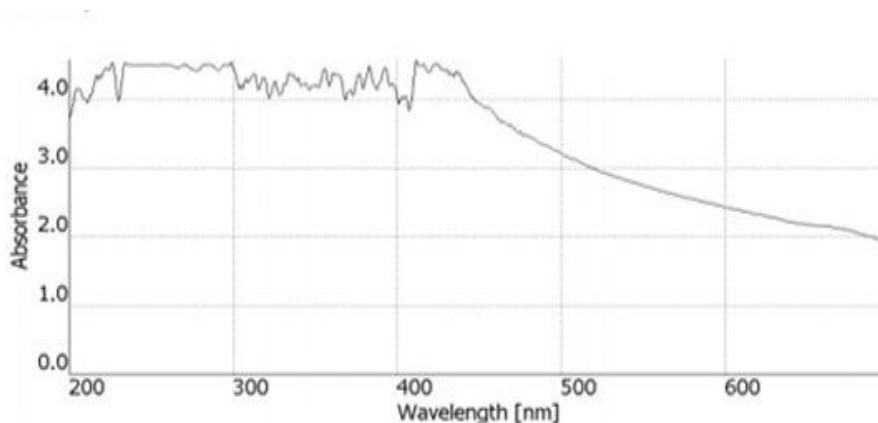


Figure 3: UV-Visible spectrum of AgNPs from 5 mL aqueous leaf extract showing absorbance at 420 nm

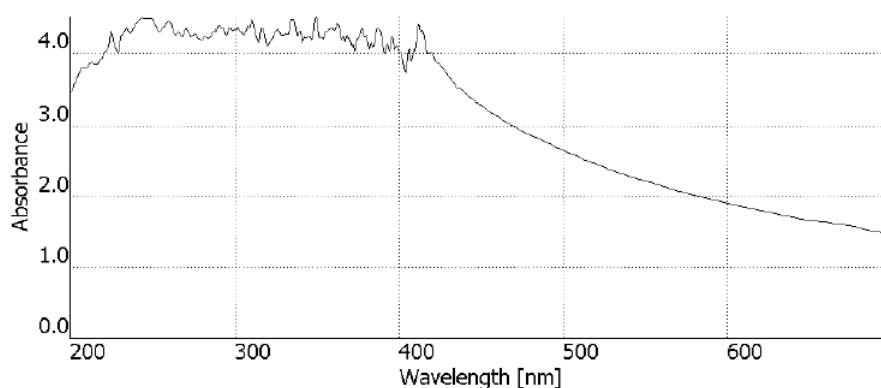


Figure 4: UV-Visible spectrum of AgNPs from 3 mL ethanol leaf extract showing absorbance at 420 nm

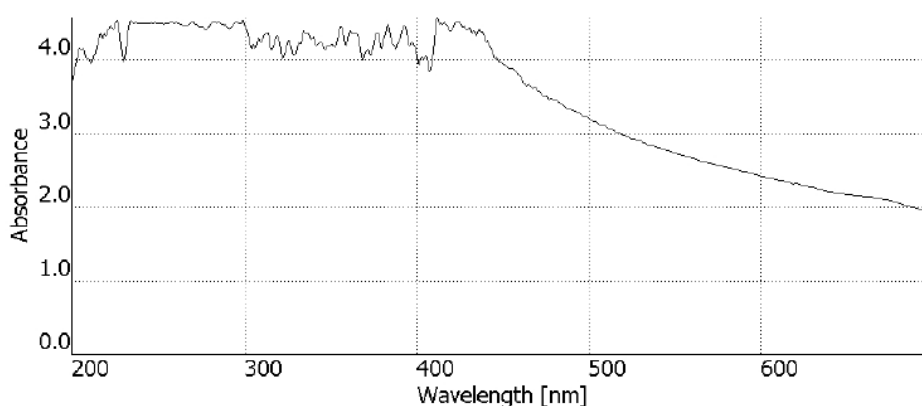


Figure 5: UV-Visible spectrum of AgNPs from 5 mL ethanol leaf extract showing absorbance at 420 nm

The Fourier transform infrared (FTIR) spectroscopy provided crucial insights into the biomolecules responsible for Ag⁺ reduction and stabilization (Figure 6 to 11). Peaks identified in the crude extract (Figure 6) and associated with functional groups like O-H

stretching (3338.55 cm^{-1} , alcohols/phenols), N-H stretching (3338.55 cm^{-1} , amines), C=O stretching (1633 cm^{-1} , carboxylic acids), and C=C stretching (1637.61 cm^{-1} , alkenes) are shown in Table 1.

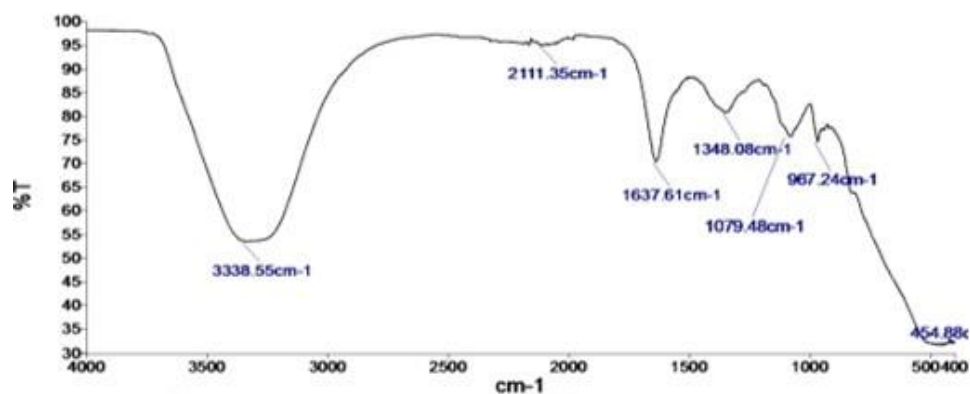


Figure 6: FTIR spectrum of crude extract of *A. muricata*

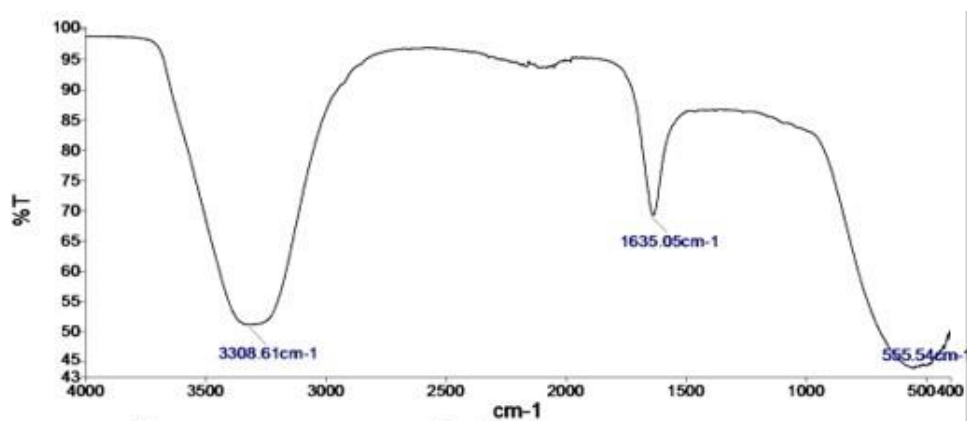


Figure 7: FTIR spectrum of silver nitrate (AgNO_3) solution

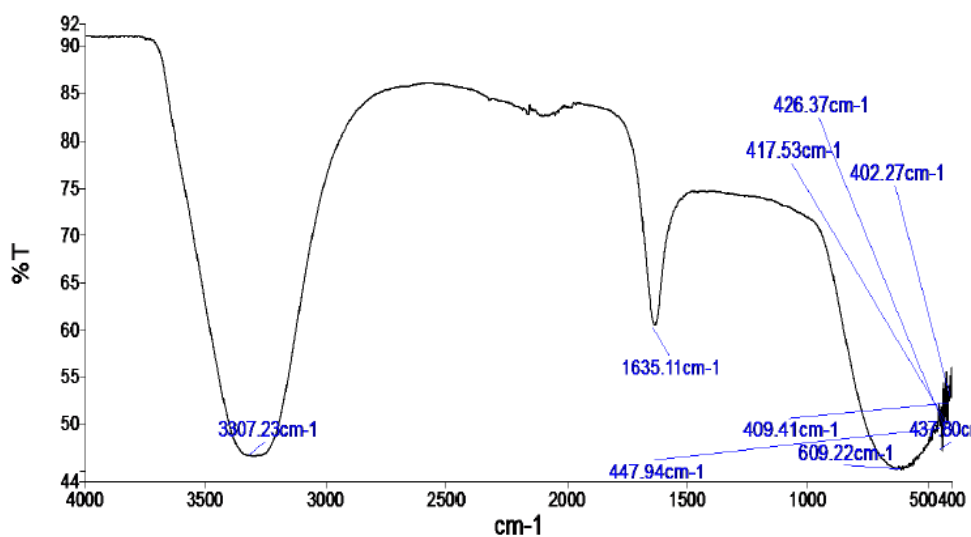


Figure 8: FTIR spectrum of AgNPs from 3 mL aqueous leaf extract

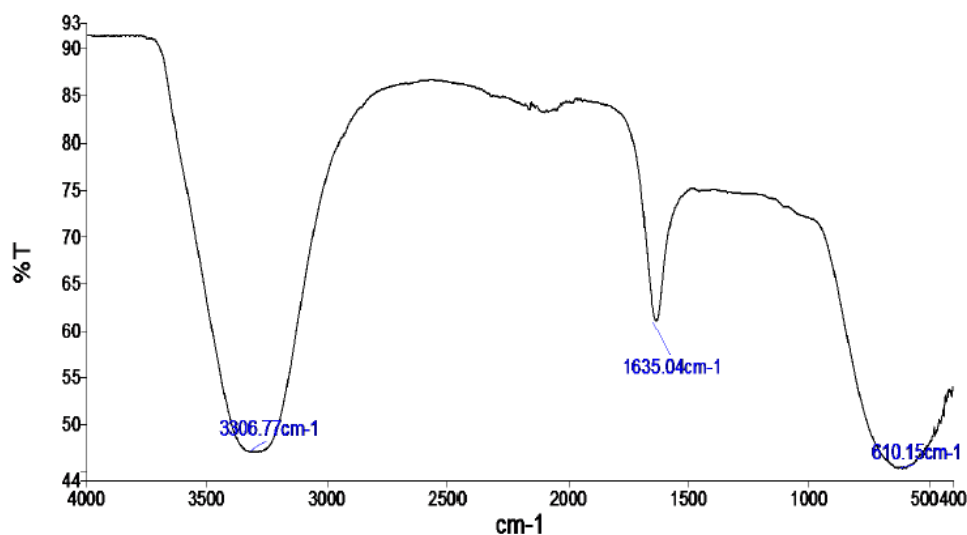


Figure 9: FTIR spectrum of AgNPs from 5 mL aqueous leaf extract

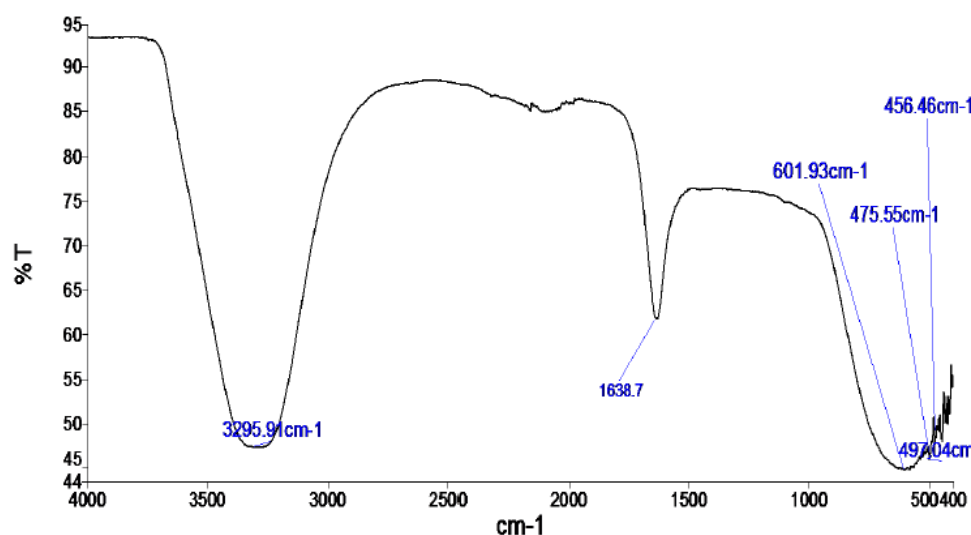


Figure 10: FTIR spectrum of AgNPs from 3 mL ethanol leaf extract

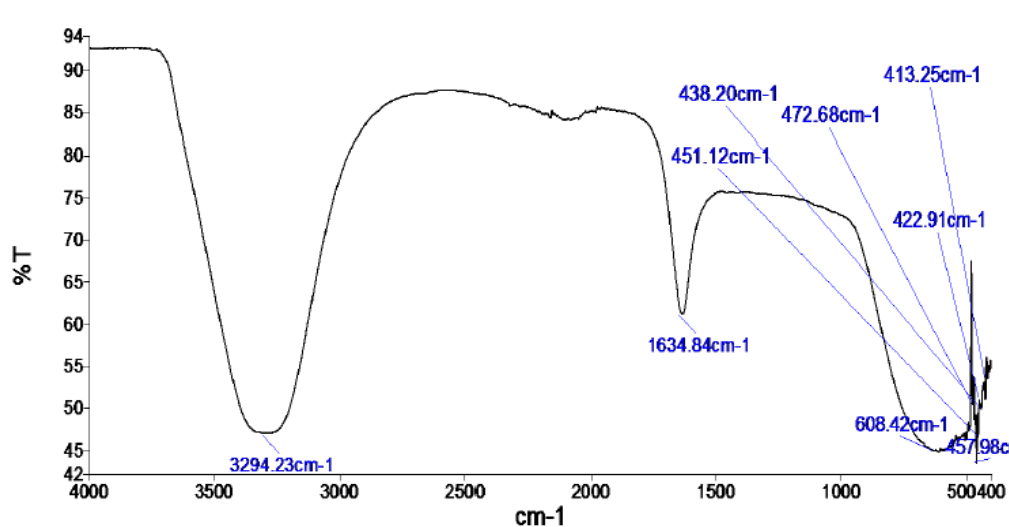


Figure 11: FTIR spectrum of AgNPs from 5 mL ethanol leaf extract.

Table 1. FTIR spectrum interpretation of identified functional groups

S/N	Wavelength cm ⁻¹	Compound class	Group	References
1	1079.48	Aliphatic amines	C-O stretching of primary alcohol	[4]
2	1633	Carboxylic acid	C=O stretching	
3	1637.61	Mono-substituted alkene	C=C stretching of alkene	[4]
4	3000	Alcohol	aromatic C=C-H bond	[1].
5	3338.55	Amine Hydroxyl	-OH stretching of alcohol. Hydroxyl groups of polyphenolic compounds.	[2] [5]

The persistence or shift of these peaks in the synthesized AgNPs (Figures 8-11) suggests that phytochemicals in the extracts act as primary reducing and capping agents, stabilizing the nanoparticles and preventing aggregation. This aligns with the established role of plant secondary metabolites in the green synthesis mechanism [6, 25]. The SEM analysis (Figures 12-15) revealed that both solvent type and extract volume significantly influenced the morphology, dispersion, and size distribution of the synthesized silver nanoparticles. In general, all samples exhibited predominantly spherical to quasi-spherical nanoparticle morphologies; however, their surface textures and dispersion varied significantly. AgNPs produced with 5 mL ethanol extract (Figure 15) were the most well-dispersed and least agglomerated, with particle sizes ranging from about 20-50 nm, suggesting effective capping due to a higher concentration of phytochemicals. In contrast, those synthesized with 5 mL aqueous and 3 mL ethanol extracts (Figures 13 and 14, respectively) exhibited moderate aggregation with slightly larger particle sizes (about 30-60 nm), while the 3 mL aqueous extract sample (Figure 12) showed the highest degree of agglomeration and particle sizes ranging from about 20-70 nm. These findings align well with established literature on solvent and concentration effects on phytosynthesized AgNPs.

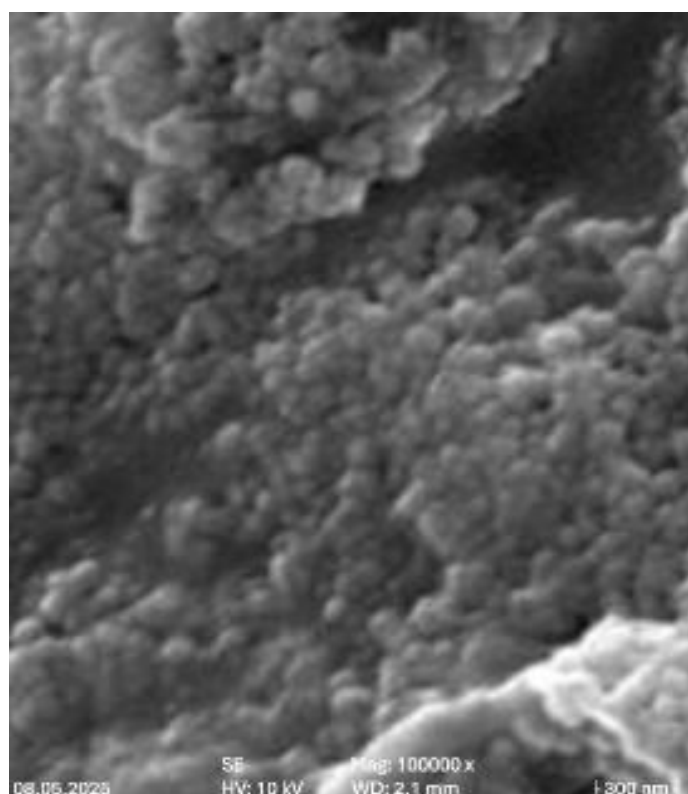


Figure 12: SEM image of AgNPs synthesized using 3 mL aqueous leaf extract of *A. muricata*

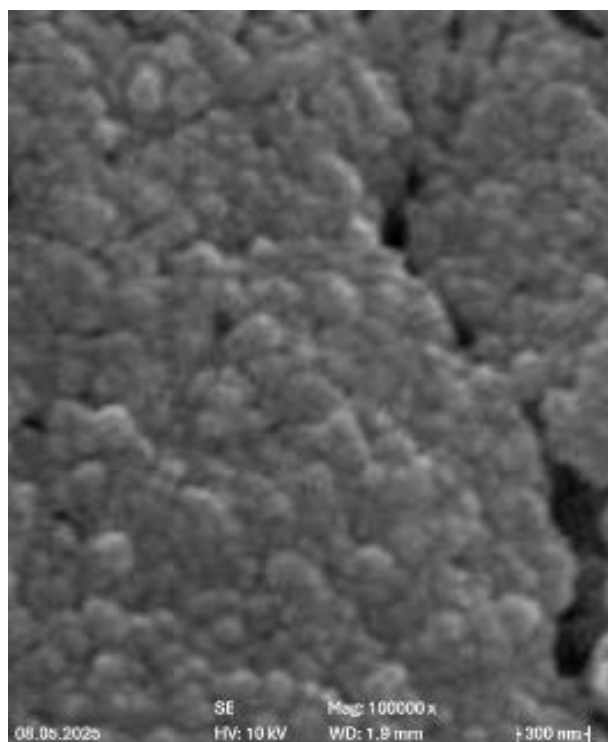


Figure 13: SEM image of AgNPs synthesized using 5 mL aqueous leaf extract of *A. muricata*

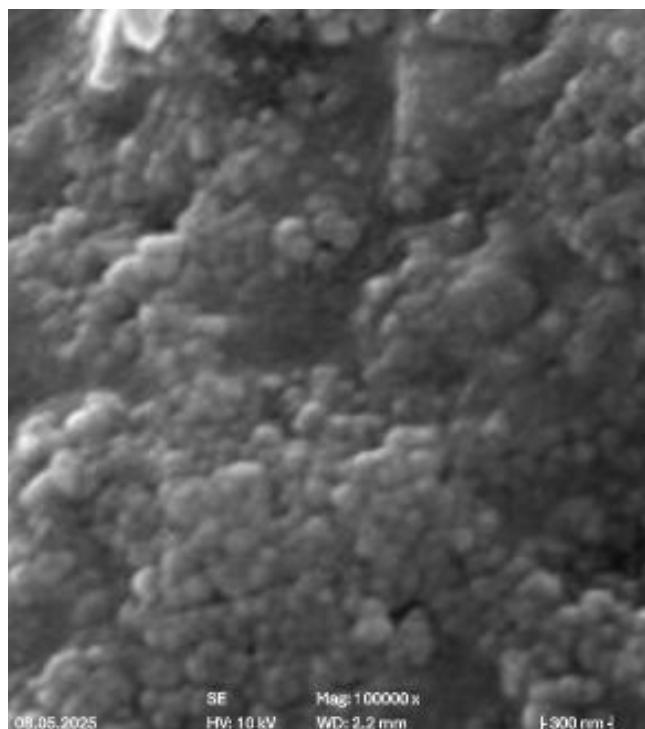


Figure 14: SEM image of AgNPs synthesized using 3 mL ethanol leaf extract of *A. muricata*

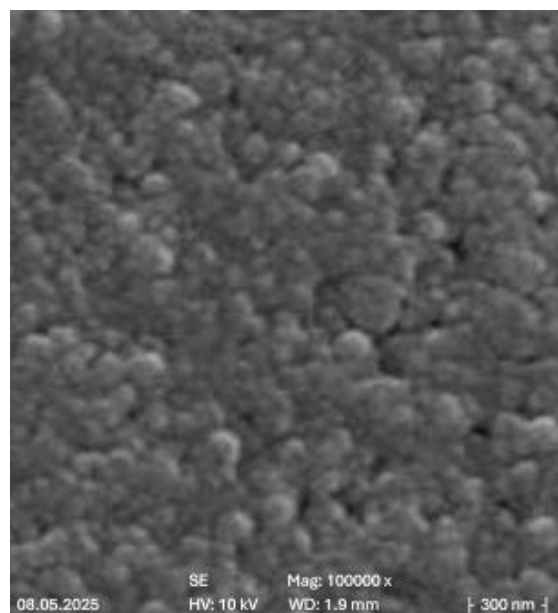


Figure 15: SEM image of AgNPs synthesized using 5 mL ethanol leaf extract of *A. muricata*

The EDX spectra shown in Figures 16 - 19 displayed a prominent silver (Ag) peak at 3 keV, indicating the successful synthesis of silver nanoparticles [39]. The characteristic absorption observed between 2 - 4 keV is consistent with the presence of metallic nanoparticles, attributed to surface plasmon resonance (SPR). Additional elements (not shown) detected are likely associated with phytochemicals, enzymes or proteins naturally occurring in the leaf tissue of *A. muricata* [16].

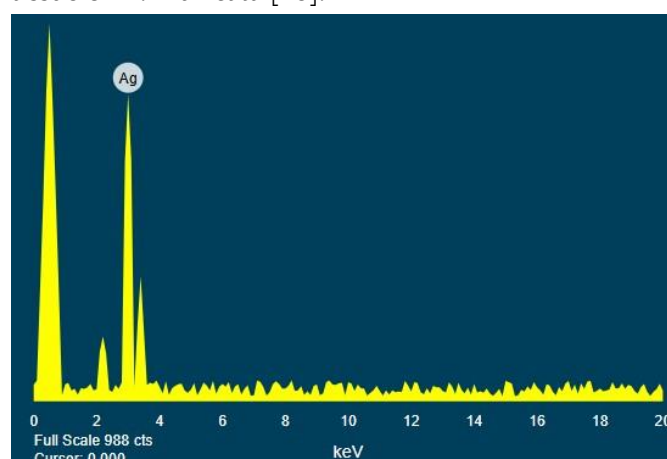


Figure 16: EDX spectrum of AgNPs synthesized from 3 mL aqueous leaf extract of *A. muricata*

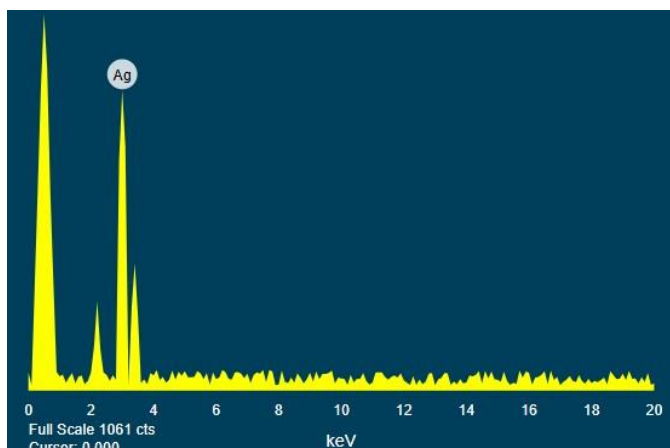


Figure 17: EDX spectrum of AgNPs synthesized from 5 mL aqueous leaf extract of *A. muricata*

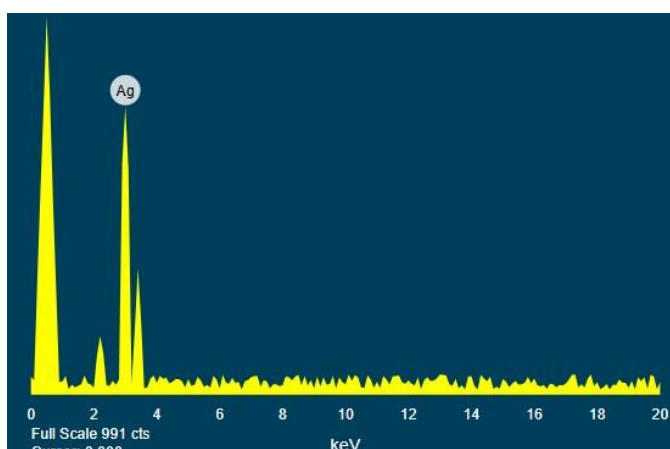


Figure 18: EDX spectrum of AgNPs synthesized from 3 mL ethanol leaf extracts of *A. muricata*

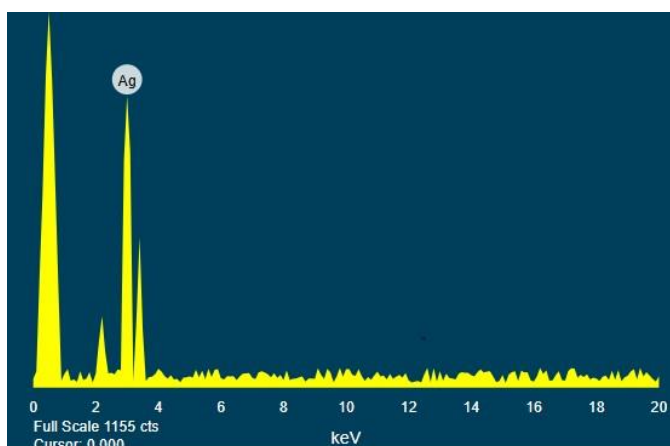


Figure 19: EDX spectrum of AgNPs synthesized from 5 mL ethanol leaf extracts of *A. muricata*

TEM analysis revealed significant morphological differences in AgNPs synthesized using varying *A.*

muricata leaf extracts (Figures 20-23). Nanoparticles ranged from about 3-40 nm, with size and dispersion directly influenced by the extract solvent and volume. AgNPs from 3 mL aqueous extract (Figure 20) were predominantly spherical (~10-40 nm) but exhibited dense agglomeration, suggesting insufficient capping phytochemicals for stabilization.

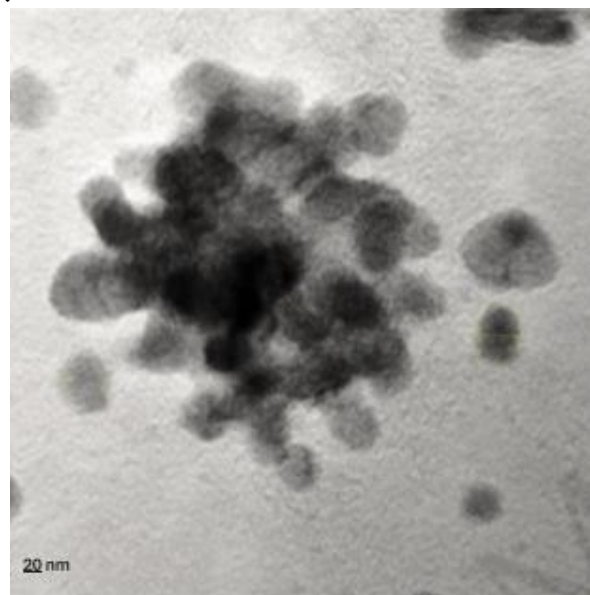


Figure 20: TEM image of AgNPs synthesized using 3 mL aqueous leaf extracts of *A. muricata*

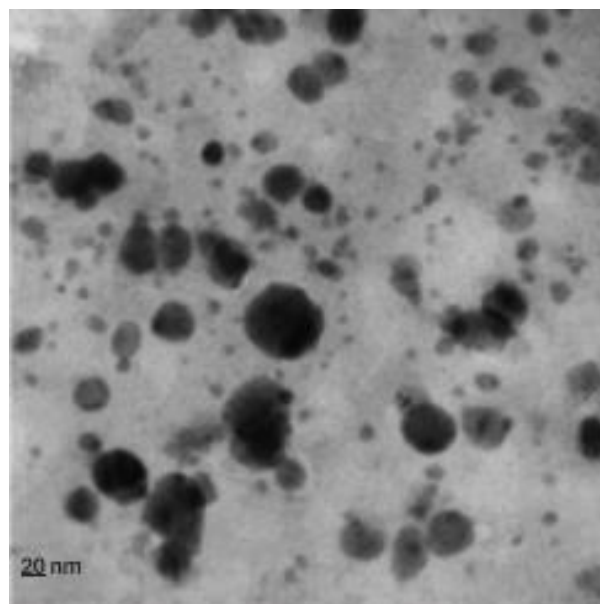


Figure 21: TEM image of AgNPs synthesized using 5 mL aqueous leaf extracts of *A. muricata*

Similarly, 3 mL ethanol extract (Figure 22) produced spherical particles (~10-30 nm) with moderate clustering. In contrast, 5 mL aqueous extract (Figure 21) yielded more monodisperse spherical nanoparticles (~5-40 nm) with reduced aggregation, attributed to enhanced stabilization from higher phytochemical concentrations in the polar aqueous medium. The smallest and most homogeneous nanoparticles (~3-30 nm) were achieved with 5 mL ethanol extract (Figure 23), though minor agglomeration persisted. This combination of ultra-small size and improved dispersion aligns with the role of ethanol in efficiently extracting non-polar reductants (e.g. terpenoids, flavonoids) that promote rapid nucleation and colloidal stability [25]. The inverse correlation between extract volume and particle size, as evidenced by smaller nanoparticles at higher volumes (5 mL), confirms accelerated nucleation kinetics under increased reductant availability, consistent with green synthesis principles [19, 35].

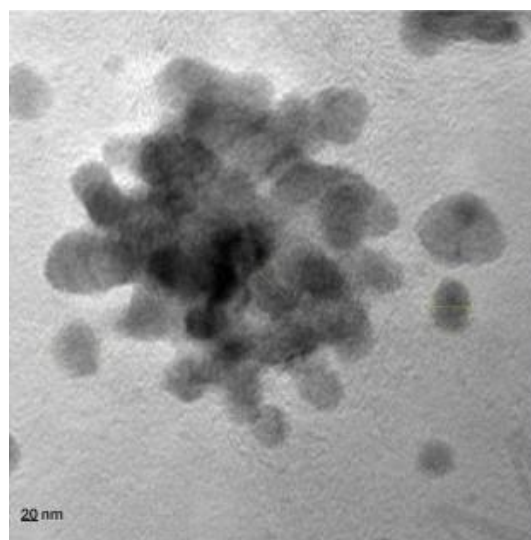


Figure 22: TEM image of AgNPs synthesized using 3 mL ethanol leaf extracts of *A. muricata*

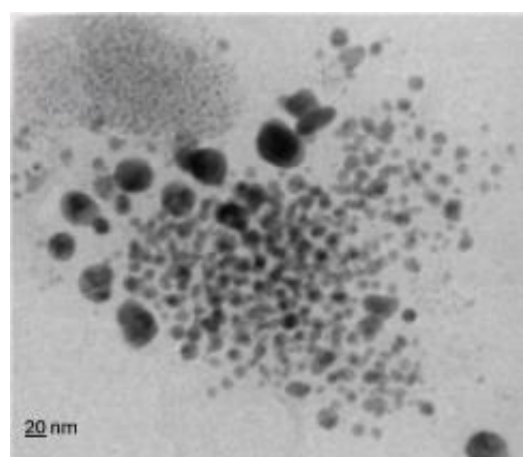


Figure 23: TEM image of AgNPs synthesized using 5 mL ethanol leaf extracts of *A. muricata*

Table 2: Dynamic light scattering (DLS) analysis of synthesized AgNPs

Sample ID	Extract type	Extract volume (mL)	Average particle size (nm)	Polydispersity Index (PDI)	Zeta potential (mV)	Remarks
AgNP-ALE-3	Aqueous	3	58.2 ± 4.3	0.352	-21.7 ± 1.5	Moderately polydisperse
AgNP-ALE-5	Aqueous	5	43.7 ± 2.9	0.265	-24.8 ± 1.2	Improved dispersion and stability
AgNP-ELE-3	Ethanolic	3	51.4 ± 3.5	0.301	-26.1 ± 1.8	Smaller size, better stability
AgNP-ELE-5	Ethanolic	5	39.9 ± 2.2	0.218	-29.4 ± 1.3	Most uniform and stable

Dynamic light scattering (DLS) results (Table 2) generally supported the size trends observed by SEM and TEM but reported larger average hydrodynamic diameters (39.9-58.2 nm) to TEM, and Polydispersity Index (PDI) values above 0.2, indicating moderate polydispersity and the presence of agglomerates or biomolecular coronas in solution. This variation in AgNPs size can also be attributed to the fact that DLS measures the hydrodynamic diameter, which includes not only the nanoparticle core but also the surrounding hydration layer and any capping or stabilizing agents [40]. As a result, the measured size does not represent the actual core size of the AgNPs. The increasingly negative zeta potential values (ranging from -21.7 mV to -29.4 mV) correlated with higher extract volumes and ethanol use, indicating improved electrostatic stabilization and colloidal stability in these samples, particularly in AgNP-ELE-5 [35]. A zeta potential value of -29.4 mV suggests a high concentration of negative ions on the surface of the particles. This, in turn, implies a strong repulsive force among the particles due to the high negative charge, which reduces the likelihood of aggregation and enhances nanoparticle stability [41].

The synthesized AgNPs exhibited significant concentration-dependent antioxidant activity across all assays. The results of the FRAP assay (Figure 24) demonstrated the ability of the AgNPs to reduce Fe^{3+} to Fe^{2+} , similar to the standard ascorbic acid, with activities ranging from 224 to 324 $\mu\text{g AAE/mL}$. AgNPs synthesized using 5 mL of ethanol leaf extract (AgNP-ELE-5) exhibited the highest activity, supporting the notion that ethanol extraction often yields higher concentrations of antioxidant phytochemicals such as flavonoids and phenolics compared to aqueous extraction [25]. Similarly, in the DPPH radical scavenging assay (Figure 25), all AgNPs showed substantial scavenging activity comparable to the standard, ascorbic acid, ranging from 48.98% to 64.29% at 1 mg/mL. AgNP-ELE-5 again performed the best

(64.29%), comparable to many plant extracts reported for DPPH scavenging [19].

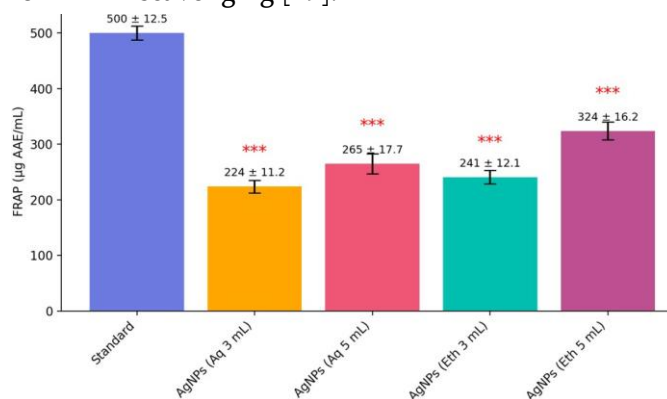


Figure 24: FRAP activity of synthesized AgNPs

NB: Aqueous leaf extract (Aq.), ethanolic leaf extract (Eth); the red asterisks confirm every extract is statistically different from the standard ($p < 0.001$).

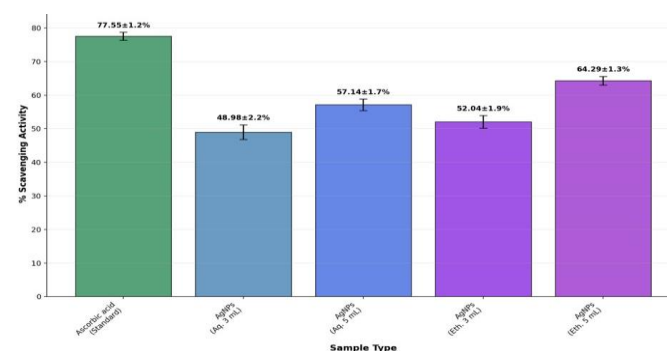


Figure 25: Percentage DPPH free radical scavenging activity of synthesized AgNPs

NB: Aqueous leaf extract (Aq.), ethanolic leaf extract (Eth).

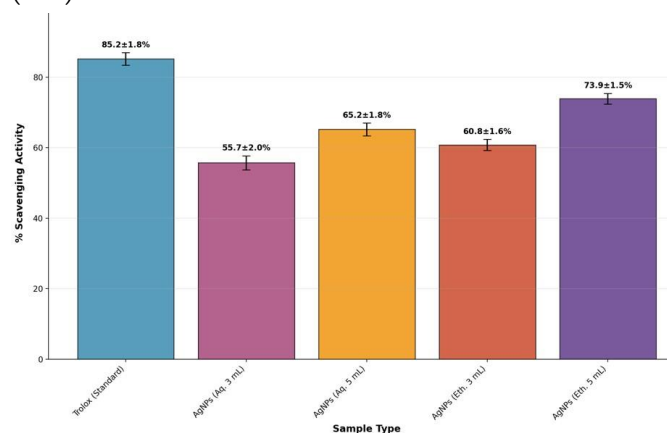


Figure 26: Percentage ABTS $\cdot^+$ radical scavenging activity of synthesized AgNPs

NB: Aqueous leaf extract (Aq.), ethanolic leaf extract (Eth).

The results of the ABTS^{•+} radical scavenging assay (Figure 26) further confirmed the antioxidant potential of the synthesized AgNPs, as they exhibited similar activity to the standard (Trolox), with scavenging activities ranging from 55.7% to 73.9% at 1000 µg/mL. This consistently superior performance of nanoparticles synthesized using the ethanol extract, particularly at the higher volume (5 mL), strongly suggests that the enhanced extraction efficiency of ethanol for key antioxidant phytochemicals in *A. muricata* leaves translates directly into AgNPs with superior radical scavenging capabilities, potentially due to synergistic effects between the silver core and the capping phytochemicals [6].

The anti-inflammatory potential, assessed via inhibition of Bovine serum albumin (BSA) denaturation (Figure 27), was also significant and extract-dependent. All AgNPs samples inhibited denaturation by 67-79% at 50 µg/mL. Notably, AgNPs from ethanol extracts of 3 mL and 5 mL (ELE-3: 76.47%, ELE-5: 78.82%) outperformed those from aqueous extracts of 3 mL and 5 mL (ALE-3: 67.06%, ALE-5: 70.59%) and even surpassed the standard diclofenac sodium (74.12%). This superior efficacy of the ethanol-derived AgNPs further underscores the importance of the phytochemical profile carried over from the extract into the nanoparticle capping layer. The ability to inhibit protein denaturation is a recognized mechanism for mitigating inflammation, suggesting potential for these AgNPs in anti-inflammatory applications [35].

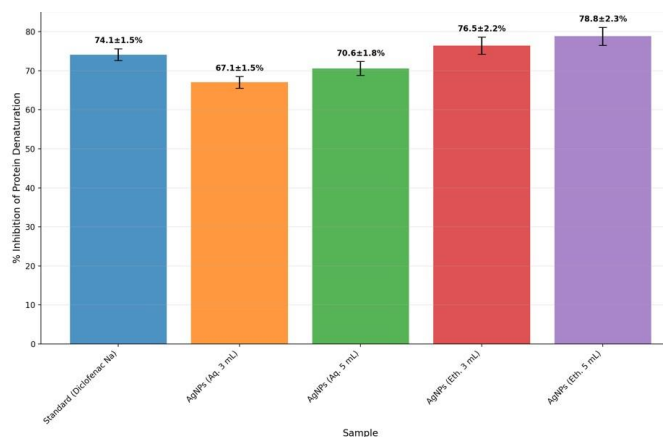


Figure 27: Anti-inflammatory potential of the synthesized AgNPs

NB: Aqueous leaf extract (Aq.), ethanol leaf extract (Eth).

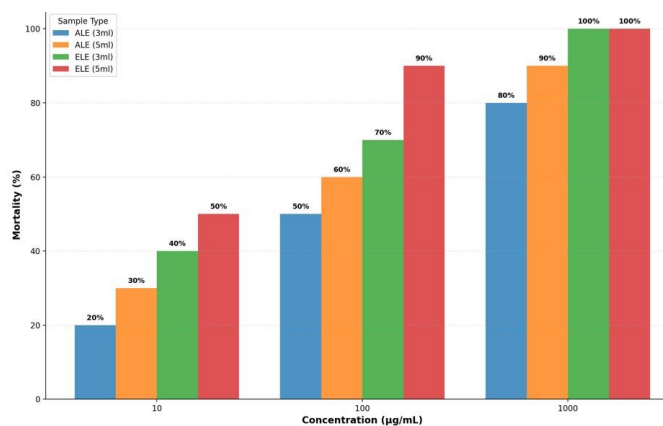


Figure 28a: Percentage mortality of the synthesized AgNPs at 10, 100, and 1000 µg/mL

NB: Aqueous leaf extract (ALE), ethanol leaf extract (ELE).

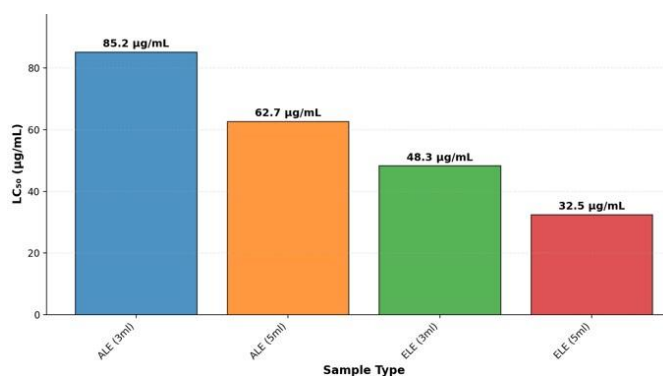


Figure 28b: LC₅₀ values of the synthesized AgNPs

NB: Aqueous leaf extract (ALE), ethanolic leaf extract (ELE).

The brine shrimp lethality assay (Figures 28a and b) revealed promising cytotoxic potential of the synthesized AgNPs. Mortality increased dose-dependently (10-1000 µg/mL) for all samples. The calculated LC₅₀ values (concentration lethal to 50% of shrimp) showed a clear trend: AgNP-ELE-5 (32.5 µg/mL) < AgNP-ELE-3 (48.3 µg/mL) < AgNP-ALE-5 (62.7 µg/mL) < AgNP-ALE-3 (85.2 µg/mL). This indicates that AgNPs synthesized with ethanol extract, particularly at 5 mL volume, possessed the highest toxicity (Figure 28b). The significantly lower LC₅₀ value for AgNP-ELE-5 compared to the others suggests substantially enhanced cytotoxic potency. While the brine shrimp assay is a preliminary indicator of cytotoxicity, the results strongly suggest that these AgNPs, especially those from ethanol leaf extract, warrant further investigation for potential anticancer applications, as AgNPs are known to induce cytotoxicity through mechanisms like oxidative stress and mitochondrial dysfunction in cancer cells [6, 25].

The correlation between well-dispersed, minimally agglomerated nanoparticles (as shown by SEM, with AgNP-ELE-5 exhibiting a smaller size of ~20-50 nm, and by TEM, showing ~3-30 nm), better stability (DLS indicated the highest zeta potential for AgNP-ELE-5), higher antioxidant activity, and increased cytotoxicity further supports the critical role of synthesis parameters (specifically extract type and volume) in determining the biological efficacy of the resulting AgNPs.

CONCLUSION

This preliminary study successfully demonstrates the efficient green synthesis of AgNPs using *A. muricata* leaf extracts. Comprehensive characterization confirmed nanoparticle formation, identified key phytochemicals involved, and revealed size and stability characteristics influenced by the extract type

and volume. Crucially, the synthesized AgNPs, particularly those derived from ethanol extract at a higher volume (5 mL), exhibited potent antioxidant, anti-inflammatory, and cytotoxic activities *in vitro*. These enhanced bioactivities are likely attributable to the superior extraction of bioactive phytochemicals by ethanol and their subsequent role in nanoparticle capping and stabilization. The findings highlight the significant impact of synthesis parameters on the functional properties of biosynthesized AgNPs and strongly support the potential of *A. muricata*-derived AgNPs, especially from ethanol extracts, for applications in biomedicine, particularly as antioxidant, anti-inflammatory, and anticancer agents, meriting further *in vivo* and mechanistic studies.

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