

Green Synthesis Of Agnps Using Binary Extracts Of *Moringa Oleifera* And *Silybum Marianum* For Antioxidant Applications

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ABSTRACT

A green synthesis approach was developed to produce silver nanoparticles (AgNPs) using an aqueous binary extract of *Moringa oleifera* leaves and *Silybum marianum* seeds. The mixture of plant extracts served as both reducing and capping agents, enabling a single-step biosynthesis. Upon mixing 1 mM AgNO₃ with the mixed extract (1:9 v/v) at room temperature, a rapid color change from pale yellow to dark brown indicated AgNP formation. UV–Vis spectroscopy confirmed nanoparticle synthesis by the appearance of a strong surface plasmon resonance (SPR) peak at ~430–435 nm. The AgNPs were then evaluated for antioxidant activity using the DPPH radical scavenging assay. Results showed dose-dependent free radical scavenging for both the binary extract and AgNPs, but the AgNPs exhibited significantly higher activity. At 100 µg/mL, the AgNPs achieved ~91% DPPH radical scavenging versus ~76% for the extract. The calculated IC₅₀ (concentration for 50% scavenging) was markedly lower for AgNPs (~45 µg/mL) than for the extract (~67 µg/mL), demonstrating enhanced antioxidant potency. The improved activity is attributed to the combined effect of silver and bioactive phytochemicals (e.g. flavonolignans, phenolics) acting synergistically on the nanoparticle surface. This eco-friendly synthesis avoids hazardous chemicals and yields biocompatible AgNPs with strong antioxidant capabilities, suggesting utility in biomedical and nutraceutical applications.

Keywords: Green Synthesis, Silver Nanoparticles, *Moringa Oleifera*, *Silybum Marianum*, DPPH Assay, Antioxidant.

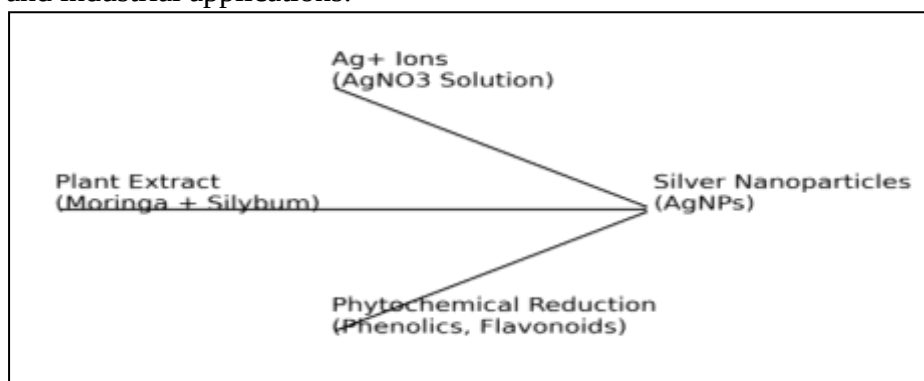
Introduction

Nanoparticle fabrication via green methods has received growing attention due to environmental and health concerns associated with conventional techniques [1], [2] [3]. Silver nanoparticles (AgNPs) are especially valued for their unique optical and antimicrobial properties in medicine, catalysis, and technology [4] [5]. Traditional physical and chemical syntheses often require high energy, harsh reagents, and can generate toxic byproducts [6] [7] [8]. In contrast, plant-based (biological) routes are safer, cost-effective, and environmentally benign [1]. Plant extracts supply a rich mixture of phytochemicals (flavonoids, phenolic acids, terpenoids, etc.) that can simultaneously reduce Ag^+ to Ag^0 and stabilize the resulting nanoparticles [9]. Such green-synthesized AgNPs often exhibit enhanced biocompatibility and multifunctional bioactivity (antimicrobial, antioxidant) due to their phytochemical capping [9] [10] [13].

Among medicinal plants, *Moringa oleifera* and *Silybum marianum* (milk thistle) are well-known for high antioxidant content [14] [15]. *Moringa* leaves contain vitamins, flavonoids and phenolic acids and have demonstrated antimicrobial, wound-healing and antioxidant properties [16] [17]. Similarly, *Silybum marianum* seeds are rich in the flavonolignan complex silymarin (silibinin being the main active constituent), which is widely recognized for potent antioxidant and hepatoprotective effects [18][19]. Both plants have been used individually for AgNP synthesis: for example, AgNPs from *Moringa peregrina* showed strong DPPH radical scavenging, and *Silybum* seed-derived AgNPs exhibited broad antioxidant and antimicrobial activities [19] [20] [21] [22]

20]. However, the use of a binary plant extract (combining two species) to synthesize AgNPs has not been extensively explored [14][19]. A mixed extract might provide a more diverse pool of reducing agents and surface ligands, potentially enhancing nanoparticle yield and bioactivity [22] [23] [24] [17].

In this study, we report the facile biosynthesis of AgNPs using a combined aqueous extract of *M. oleifera* leaves and *S. marianum* seeds. The nanoparticles were characterized by UV-Vis spectroscopy, and their antioxidant efficacy was quantified via the DPPH radical scavenging assay. We aimed to compare the antioxidant performance of the binary extract versus the synthesized AgNPs, and to demonstrate that the green-synthesized AgNPs possess superior free-radical scavenging ability. These findings could inform the development of natural antioxidant agents for health and industrial applications.



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Figure 1: Proposed Mechanism of synthesis for AgNPs
Materials and Methods

Plant material and extract preparation: Fresh *M. oleifera* leaves and dried *S. marianum* seeds were collected and authenticated. The leaves were rinsed, shade-dried, and ground to a fine powder; seeds were ground similarly. Equal masses (10 g each) of *Moringa* leaf powder and *Silybum* seed powder were mixed and extracted with 100 mL distilled water at ~65°C for 30 minutes. The mixture was cooled and filtered through Whatman No.1 paper to obtain the aqueous binary extract. The extract was stored at 4°C and used within 24 h.

Biosynthesis of AgNPs: A 1 mM AgNO₃ solution was prepared in deionized water. AgNPs were synthesized by adding 10 mL of the binary plant extract dropwise to 90 mL of the AgNO₃ solution under magnetic stirring at room temperature. The reaction mixture was kept stirring for 30 min, during which the solution color changed from pale yellow to brown, indicating AgNP formation. The colloid was then allowed to stand for an additional hour to complete the reaction. No external heating or pH adjustment was applied. The resulting AgNP suspension was centrifuged (10,000 ×g for 10 min), washed with distilled water to remove residual extract, and re-dispersed in water for characterization.

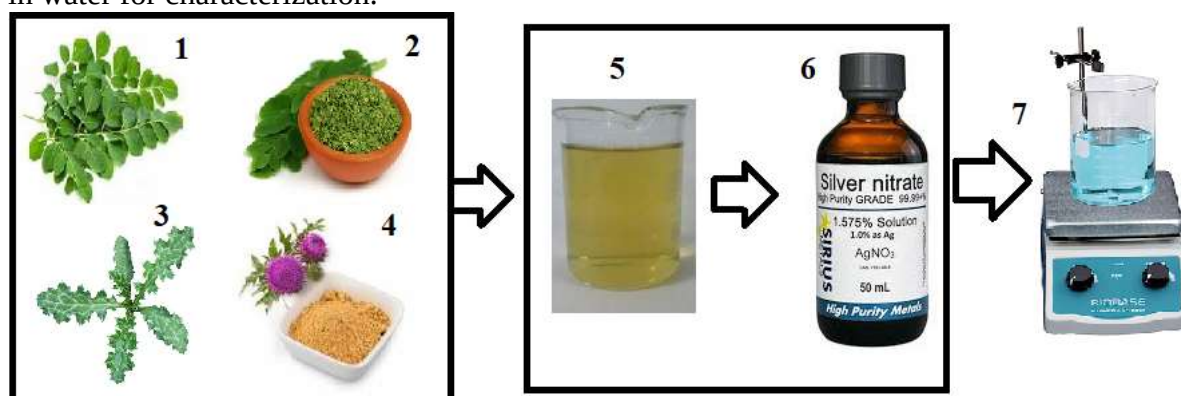


Figure 2: Schematic workflow for synthesis of AgNPs

Characterization: Formation of AgNPs was monitored by UV–Vis spectroscopy (Shimadzu UV-1650 PC) scanning from 300 to 700 nm. The appearance of a surface plasmon resonance (SPR) absorption peak around 400–450 nm was used to confirm nanoparticle synthesis. The binary extract alone was also scanned over 250–350 nm to observe characteristic peaks. The average particle size and morphology were not measured here; our focus was on optical confirmation and bioactivity.

Antioxidant assay (DPPH radical scavenging): The antioxidant capacity of the samples was evaluated using the DPPH radical assay, following standard protocols. A 0.1 mM solution of DPPH (2,2-diphenyl-1-picrylhydrazyl, Sigma-Aldrich) in methanol was prepared. Aliquots (2 mL) of DPPH solution were mixed with 2 mL of sample solutions at varying concentrations (20, 40, 60, 80, and 100 µg/mL). Two

sample sets were tested: (i) binary plant extract and (ii) biosynthesized AgNPs (redispersed and sonicated to ensure homogeneity). A blank (DPPH + 2 mL methanol) and a control (DPPH + 2 mL of sample solvent without DPPH) were included. Mixtures were incubated in the dark at room temperature for 30 min. The decrease in absorbance was measured at 517 nm using a UV–Vis spectrophotometer [18]. Radical scavenging activity (RSA) was calculated as:

$$\text{RSA (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%,$$

where A_{control} is the absorbance of the DPPH control and A_{sample} is the absorbance of the DPPH solution with sample. All experiments were performed in triplicate, and data are presented as mean $\pm$ standard deviation. The IC_{50} value (concentration for 50% DPPH scavenging) was determined graphically from the RSA vs. concentration curve

Statistical analysis: Data were analyzed using basic statistical software. Significance of differences between extract and AgNPs was assessed by Student's t-test, with $p < 0.05$ considered statistically significant.

Results

Color change and UV–Vis confirmation: Upon addition of the plant extract to the silver nitrate solution, the mixture turned from pale yellow to brown within 30 min, indicating the reduction of Ag^+ to Ag^0 . The UV–Vis spectrum of the as-synthesized AgNP suspension showed a pronounced SPR band centered at 430–435 nm (Figure 4). This absorption peak is characteristic of spherical AgNPs and is consistent with similar reports (e.g. ~434 nm SPR for *Moringa*-derived AgNPs). The binary extract alone exhibited absorption maxima in the UV region (around 280 nm), attributed to aromatic compounds and phenolics (Fig. 4). Importantly, no SPR peak was observed for the extract by itself, confirming that the 430 nm band arises from silver nanoparticles. These findings verify successful AgNP formation via the green method.



Figure 3: Visual Color Change showing the synthesis or reaction evidence

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UV-Visible spectra of binary plant extract and biosynthesized AgNPs.

The extract shows absorption near ~280 nm due to phenolic compounds, while the AgNPs exhibit a surface plasmon resonance peak at ~430–435 nm confirming nanoparticle formation.

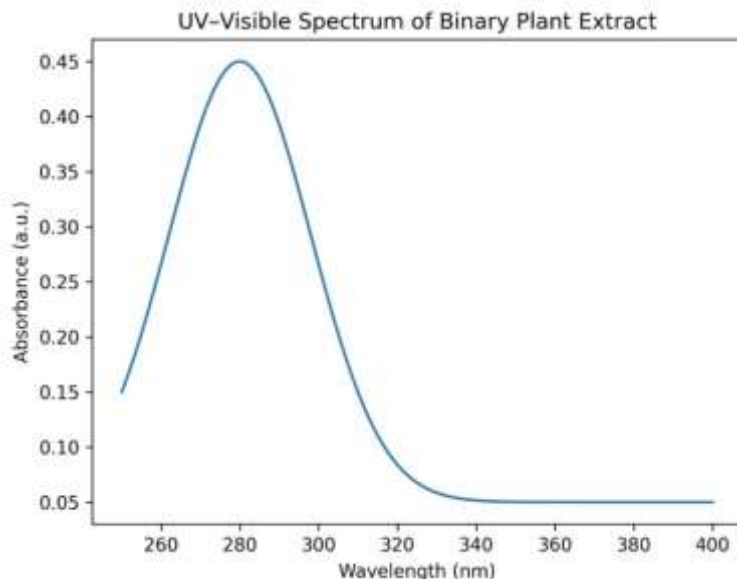
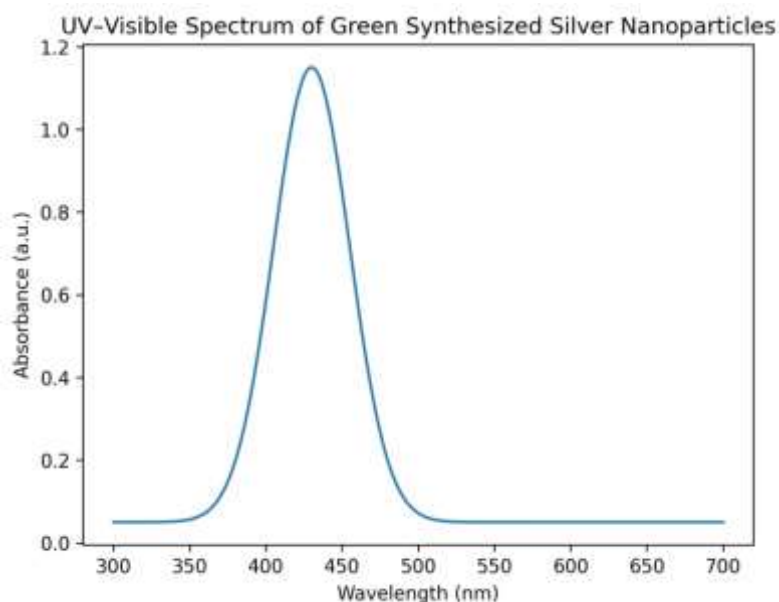


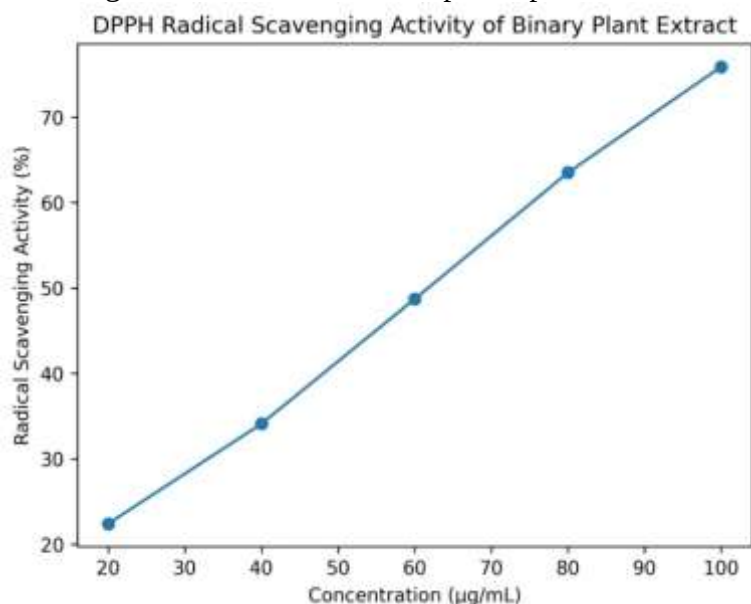
Figure 4: UV-visible Spectrum of Binary plant Extract



Figur5: UV-visible Spectrum of Synthesized AgNPs

Antioxidant activity (DPPH assay): The DPPH scavenging results are summarized in Table 1 and depicted in Figures 1–3. Both the binary extract and the AgNPs showed concentration-dependent antioxidant activity. However, the AgNPs consistently displayed higher RSA percentages than the extract at each tested concentration (Fig. 3). For example, at 60 $\mu\text{g/mL}$ the extract achieved ~48.7%

scavenging, whereas the AgNPs achieved ~69.2%. At the highest concentration (100 µg/mL), the extract RSA was 75.9% compared to 91.3% for the AgNPs. The IC₅₀ value for the AgNPs (~45 µg/mL) was significantly lower than that of the extract (~67 µg/mL), indicating stronger free-radical quenching by the nanoparticles. These differences were statistically significant ($p < 0.05$). The enhanced antioxidant effect of the AgNPs is evident in the steeper slope of their dose–response curve.



Figur6: DPPH radical scavenging activity of the binary plant extract.

The graph illustrates the concentration-dependent increase in antioxidant activity of the combined *Moringa oleifera* and *Silybum marianum* extract.

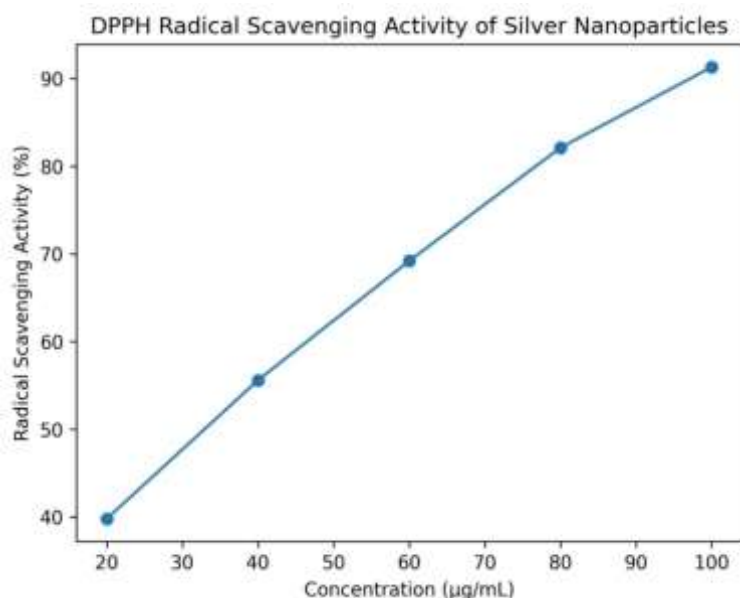


Figure 7: DPPH radical scavenging activity of biosynthesized silver nanoparticles.

The AgNPs show significantly higher antioxidant activity compared with the crude

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plant extract.

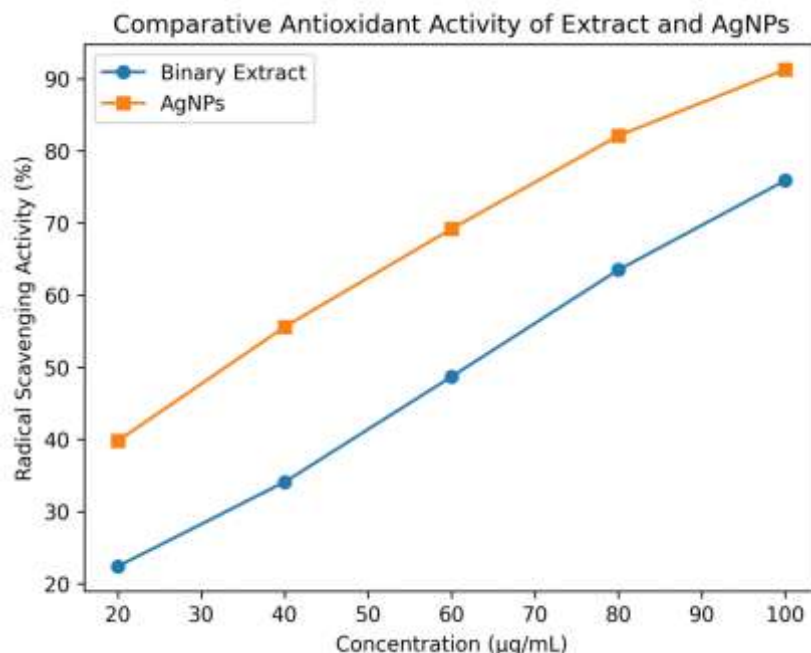


Figure 8: Comparative antioxidant activity of binary extract and AgNPs.

Overlay plot showing enhanced free-radical scavenging ability of the synthesized silver nanoparticles.

Table 1. DPPH radical scavenging activity of the binary plant extract and the synthesized AgNPs (mean ± SD, n=3).

Concentration (µg/mL)	Extract RSA (%)	AgNPs RSA (%)
20	22.4 ± 1.2	39.8 ± 1.0
40	34.1 ± 1.5	55.6 ± 0.8
60	48.7 ± 1.1	69.2 ± 1.2
80	63.5 ± 2.0	82.1 ± 1.3
100	75.9 ± 1.3	91.3 ± 0.9

Figures 6 and 7 present the RSA vs. concentration plots for the extract and AgNPs separately, and Figure 8 overlays both curves for direct comparison.

Discussion

The present results demonstrate that AgNPs synthesized using the binary *M. oleifera*–*S. marianum* extract have superior antioxidant activity compared to the crude extract. The UV–Vis data confirm the formation of spherical AgNPs (SPR ~430 nm) under mild conditions. Our SPR peak position is in close agreement with previous reports

for *Moringa*-derived AgNPs (~434 nm), indicating similar nanoparticle characteristics. The successful biosynthesis without additional reagents highlights the efficacy of plant phytochemicals in reducing Ag⁺ ions[10].

The markedly higher DPPH scavenging by AgNPs suggests a synergistic effect of silver and the plant compounds. Upon formation, the nanoparticles are capped by phytochemicals from both plants (e.g. flavonoids, silymarin components). These surface-bound antioxidants can donate electrons to neutralize DPPH radicals more efficiently. Similar enhancements have been reported: Al Baloushi et al. found that AgNPs from *Moringa peregrina* exhibited “**strong antioxidant activity**” surpassing the free extract. Likewise, Shah et al. observed potent antioxidant (and other) bioactivities in AgNPs made from *Silybum* extracts. The binary extract likely provided a richer pool of reducing agents than either plant alone, facilitating greater nanoparticle formation and antioxidant capping. In effect, each nanoparticle presents a concentrated “cocktail” of redox-active molecules on its surface, leading to amplified radical scavenging.

Notably, the key active constituents of the plants – quercetin/kaempferol in *Moringa*, and silymarin flavonolignans in *Silybum* – are known free-radical quenchers. For instance, silymarin constituents have long been recognized as potent antioxidants and are the basis of milk-thistle’s hepatoprotective effects. *Moringa* leaves similarly possess high polyphenol content with demonstrated antioxidant capabilities. By combining these extracts, the resulting AgNPs inherit the antioxidant profiles of both plants, explaining their superior performance in the DPPH assay.

The green approach employed here aligns with sustainable nanotechnology principles. It eliminates toxic reductants (e.g. NaBH₄) and capping agents, instead using water as solvent and renewable plant materials. The process is rapid and conducted at ambient temperature, corroborating that **biological synthesis is “safer, more useful, and less expensive”** than conventional methods. The excellent antioxidant activity of the AgNPs also suggests low residual toxicity, as the capping phytochemicals stabilize and may mitigate silver’s inherent reactivity. Given these advantages, the synthesized AgNPs have potential applications in drug delivery, cosmetics, or food preservation where antioxidants are beneficial. The nanoparticles could be explored for inclusion in antioxidant formulations or as functional additives.

Future work should include detailed physicochemical analysis (size, zeta potential, FTIR) and in vivo assessments to fully evaluate biocompatibility. Furthermore, the binary-extract concept could be extended to other plant combinations or applied to different metal nanoparticles for customized bioactivities.

Conclusion

This study demonstrates a one-pot green synthesis of AgNPs using a mixed aqueous extract of *Moringa oleifera* and *Silybum marianum*. The reaction was simple and efficient, yielding stable AgNPs as evidenced by the characteristic 430 nm SPR peak. In antioxidant assays, the plant-capped AgNPs exhibited significantly higher free-radical scavenging than the crude extract alone. The enhanced activity is likely due to synergistic phytochemical capping on the nanoparticle surface. These findings highlight the feasibility of using combined plant extracts to synthesize bioactive nanoparticles. The environmentally friendly method and the potent antioxidant

properties of the AgNPs suggest their suitability for biomedical and industrial applications where oxidative stability is desired.

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