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# Phytochemical Profiling and *In Vitro* Bioactivity Testing of the Ethanolic Extract of the Seed of *Carica papaya* Integrated with Silver Nanoparticles

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**Abstract**

*Carica papaya* seeds have long been valued in traditional medicine. Although silver nanoparticles (AgNPs) boost plant extract stability and bioactivity, the hybrid of *C. papaya* ethanolic seed extract (EE) and AgNPs remains underexplored. The study aims to evaluate the biological activities (cytotoxic, antioxidant, antimicrobial and anthelmintic) of EE, both alone and in combination with AgNPs, relative to reference drugs. The study measured cytotoxicity via brine shrimp lethality bioassays (determining $LC_{50}$), assessed antioxidant capacity with DPPH scavenging ($IC_{50}$), tested antimicrobial effects on gram-positive and gram-negative strains (by inhibition zone) and evaluated anthelmintic activity using earthworms (recording paralysis and death times). EE was assessed both alone and in combination with AgNPs, and its performance was compared against reference drugs (vincristine sulfate, ascorbic acid, kanamycin and albendazole). EE displayed mild cytotoxicity ($LC_{50}$ = 89.96 µg/ml), yet AgNPs integration markedly boosted its activity ($LC_{50}$ = 66.42 µg/ml), indicating synergy; similarly, EE-AgNPs outperformed EE in DPPH scavenging ($IC_{50}$ = 25.761 vs. 41.977 µg/ml), approaching ascorbic acid's efficacy. Moreover, EE-AgNPs inhibited bacterial growth dose-dependently, being most effective against *E. coli* (but surpassed by kanamycin) and induced paralysis 31% faster and death 41% quicker than EE alone, despite albendazole showing superior activity overall. The study highlights *C. papaya* EE's therapeutic promise, especially with AgNPs, but requires further mechanistic, *in vivo* and safety studies for clinical use.

**Key words:** Silver nanoparticles, cytotoxicity, antioxidant, antimicrobial, anthelmintic.

## Introduction

Natural plant-based treatments have been widely used for centuries and persist in contemporary practice (Dias *et al.*, 2012). The WHO estimates that 80% of people globally rely on herbal medicine for basic healthcare, with approximately 21,000 plant species identified as having potential medicinal value (Akbar, 2020; Rahman *et al.*, 2022). While modern medicines provide fast relief, they also carry risks, such as side effects, affordability challenges and the emergence of resistant pathogens (WHO, 2019). In contrast, plant-derived remedies provide a safer, more affordable and sustainable alternative with fewer side effects due to their natural composition (Yuan *et al.*, 2016).

Phytochemicals exhibit synergistic therapeutic properties, offering holistic treatment for life-threatening diseases like inflammation, infections and cancer (Sarkar *et al.*, 2010). Additionally, their multi-target mechanisms reduce the likelihood of resistance, making them valuable in preventive and complementary medicine (Cragg & Newman, 2013).

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However, low bioavailability, instability and potential toxicity from unidentified compounds further limit their therapeutic reliability (Heinrich *et al.*, 2020). Incorporating nanotechnology, such as green-synthesized nanoparticles, offers significant advantages over crude plant extracts, including enhanced bioavailability, targeted drug delivery and superior therapeutic efficacy due to their high surface-area-to-volume ratio and tunable properties (Vanlalveni *et al.*, 2021). Compared to crude extracts, nanoparticles offer controlled release, lower toxicity, and better stability, enhancing their application in antimicrobial and cancer therapies (Duran *et al.*, 2016). Specifically, silver nanoparticles (AgNPs) possess unique optical and conductive properties, making them valuable in biomedicine, catalysis and nanotechnology (Liao *et al.*, 2019). Chemically synthesized AgNPs offer precise control over size, shape and surface properties, enhancing their therapeutic efficacy and reproducibility (Wei *et al.*, 2014). Moreover, AgNPs' nano-size and functionalization enhance cellular uptake and bioactivity over bulk extracts, with lower cytotoxicity and proven efficacy against resistant pathogens/tumors via reactive oxygen species (ROS) and phytochemical synergy (Iravani, 2011; Liao *et al.*, 2019).

*Carica papaya*, a widely used plant, is prized worldwide for its healing and nutritional properties in all its parts (Dotto & Abihudi, 2021). Folk medicine has commonly employed its leaves, seeds and latex to address intestinal worms, malaria and stomach ailments because of their antiparasitic properties (Owoyele *et al.*, 2008). The fruit is employed for wound healing and skin infections, while its latex exhibits anti-inflammatory effects for conditions like arthritis (Gurung & Škalko-Basnet, 2008). Additionally, papaya leaf extracts are traditionally consumed for dengue fever, attributed to their thrombocyte-boosting potential (Ahmad *et al.*, 2011). While the leaves and latex are commonly studied for their medicinal effects, the seeds are often underutilized despite their potent pharmacological potential. Reported studies suggest that papaya seeds may offer enhanced efficacy against parasites, bacteria and oxidative stress, making them a promising focus for therapeutic development (Zhou *et al.*, 2011; Mohammed *et al.*, 2014). In addition, Goku *et al*. (2020) demonstrated through *in vitro* studies using *Pheretima posthuma* that *C. papaya* seeds exhibited superior anthelmintic activity compared to other plant parts, supporting their traditional use against worm infections. In addition, Panzarini *et al.* (2014) demonstrated that its seed water extract exhibits dose-dependent antioxidant activity, suggesting its potential for oxidative stress prevention. A study by Peter *et al.* (2014) demonstrated that the ethanolic seed extract exhibited antibacterial effects, whereas the chloroform leaf extract showed no inhibition of bacterial growth.

Therefore, this study aims to investigate the phytochemical composition and evaluate the bioactive potential of *C. papaya* ethanolic seed extract (EE) integrated with silver nanoparticles (AgNPs). The research seeks to profile key secondary metabolites, such as alkaloids, flavonoids and phenolics, present in the extract and assess their synergistic effects with AgNPs on cytotoxicity, antioxidant, antimicrobial and anthelmintic activities. By combining plant-derived compounds with nanotechnology, the study intends to explore enhanced therapeutic properties, providing insights into novel biomedical applications of papaya seed extract-based nanoparticles.

## Materials and Methods

*Collection of plant material:* Seeds of *C. papaya* (Caricaceae) were collected from Tangail, Bangladesh and identified (Accession no. JUH10319) by Jahangirnagar University Herbarium, Bangladesh. After collecting 500 g of seeds, they were cleaned, washed, sun-dried for one week, ground into powder (yielding 415.75 g) and stored in an airtight container in cool, dark conditions until analysis.

*Preparation of seed extract:* Approximately 415.75 g of powdered material was soaked in 800 ml of ethanol for 14 days with occasional agitation, then coarsely filtered through cotton and further refined using Whatman (Grade 2) filter paper. The ethanol

filtrate was evaporated to dryness at room temperature, yielding a black, gummy crude extract, which was then dried and refrigerated for pharmacological analysis.

**Preparation and characterization of AgNPs**

*Synthesis of AgNPs:* AgNPs were synthesized by mixing chilled (0 °C) aqueous solutions of silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$) at a 1:3 molar ratio, following a modified Lee and Jun (2019) method. The reduction of $Ag^+$ ions immediately produced a transparent yellow solution, indicating the probability of AgNPs formation. The mixture was stirred for an hour until the stable yellow color confirmed nanoparticle stabilization without additional capping agents. Finally, the dilute AgNPs solution was concentrated 10-fold using rotary evaporation.

*Preparation of Ag-extract nanoparticles:*AgNPs were blended with a pre-prepared ethanolic extract (EE) via magnetic stirring for 30 min, after which the uncoated nanoparticles needed coating. The AgNPs-extract was coated following a modified Gopalakrishnan *et al.* (2012) method, where 2.7 g of aniline (dissolved in 300 ml water and 68 ml 6% $H_2O_2$) was gradually added to the nanoparticle solution over 30 min at room temperature. Over 23 hours, the nanoparticles became evenly coated, after which the product was isolated by filtration and dried at room temperature for pharmacological investigations.

*Characterization of AgNPs:* The formation of AgNPs was primarily confirmed through visual observation of the characteristic yellow coloration arising from surface plasmon resonance (SPR) and by UV–Vis spectroscopic analysis. The reduction of silver ions was tracked using UV-Vis spectroscopy, with absorption spectra recorded between 200 and 700 nm for analysis. The appearance of a distinct SPR absorption band in the UV–Vis spectra (200–700 nm) is widely reported as an initial and reliable indicator of silver nanoparticle formation during chemical reduction processes.

*Evaluation of phytochemical constituents:* The EE underwent phytochemical analysis using established methods (Shaikh & Patil, 2020) like Dragendorff's test (alkaloids), ferric chloride (phenolics), Molisch's test (carbohydrates), froth formation (saponins), alkaline reagent (flavonoids), etc. Color changes or precipitates confirmed the presence of these metabolites, providing initial insight into the extract's composition.

**Evaluation of pharmacological activities**

*In vitro cytotoxicity assay:* The lethality bioassay of the brine shrimp (*Macrobrachium rosenbergii*) is a simple, cost-effective cytotoxicity test where extracts are added to vials containing brine shrimp in salt water. After a day, mortality is assessed, providing a quick screening method for bioactive compounds (Padmaja *et al.*, 2002). For this study, nauplii were obtained by incubating 1.5 g of shrimp eggs in 1 l of saltwater (38 g/l) at 37°C with aeration for 24 h. A 500 µg/ml stock solution was prepared by sonicating 2 mg of the EE in 4 ml of DMSO. Eight test concentrations (80, 40, 20, 10, 5, 2.5, 1.25 and 0.625 µg/ml) were prepared in brine water and tested on nauplii, with a positive control (vincristine sulphate) included for each concentration. Furthermore, the synergistic effect of EE and AgNPs was assessed using the same concentration range. The interaction (synergistic, additive, or antagonistic) between EE and AgNPs was quantitatively evaluated by testing their 4:1 combination across established EE monotherapy concentrations and comparing the efficacy to EE alone. Each 5 ml seawater vial received 10 live nauplii via dropper, counted using a magnifying glass. After 24 hours of incubation, survivor counts were recorded, and mortality percentages were derived for each dilution.

*Antioxidant activity test:* The antioxidant potential of EE and the EE-AgNPs combination (4:1 ratio) was assessed using the reported DPPH radical scavenging method (Mishra *et al.*, 2012; Priya *et al.*, 2025), a reactive, reliable, and simple *in vitro* technique for evaluating plant extracts. A 0.1 mM ethanolic DPPH solution was prepared and stored

away from light. Test samples and ascorbic acid (AA) standard were serially diluted to concentrations of 500, 400, 200, 100, 50, 25, and 12.5 µg/ml, mixed with DPPH solution (1:1 v/v) and incubated in the dark at room temperature for 30 min. The reduction in DPPH free radicals was measured spectrophotometrically at 517 nm using a UV-Vis spectrophotometer. A control (DPPH solution without sample) and blank (ethanol without DPPH) were included for reference. The radical scavenging activity (%) was calculated using the formula (1).

Concentration = $\{(A_0-A_1)/A_0\} \times 100$ …..…… (1)

Notes: $A_0$ = Absorbance of the control,

$A_1$ = Absorbance of the test samples/standard

*Antibacterial activity test:* The agar disc diffusion method, as described by Ngamsurach & Praipipat (2022), was used to assess antimicrobial activity. Initially, sterile Mueller-Hinton agar (MHA) was poured into Petri dishes and allowed to solidify. Then, preselected bacterial suspensions of *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Klebsiella pneumoniae* were adjusted to a 0.5 McFarland standard and evenly spread across the agar surface. Aseptically, sterile filter paper discs (6 mm in diameter) soaked with the test samples (10µl), like EE at varying concentrations (75, 125, 150, and 250 mg/ml) and combinations of EE with AgNPs (4:1 ratio), were placed onto the inoculated agar. Correspondingly, a 25 mg/ml dose of sole AgNPs was employed to assess its bactericidal activity. Positive controls (30 µg Kanamycin discs) and negative controls (solvent) were run alongside the test samples. Positive controls (30 µg Kanamycin discs) and negative controls (solvent-DMSO) were run alongside the test samples. Post-incubation (37°C, 24 hours), the resulting inhibition zones were measured. Antimicrobial performance was evaluated by comparing zone sizes to those of controls.

*Anthelmintic activity assay*

The anthelmintic activity was evaluated using earthworms (*Pheretima posthuma*) following a modified protocol based on Ajaiyeoba *et al.* (2001) and Priya *et al.* (2024). For initial anthelmintic assessment, adult earthworms (8-10 cm) were selected owing to their structural similarity to human parasites and experimental convenience (Priya *et al.*, 2024). They were divided into four groups across two divisions (n = 3 worms per group): control (saline), albendazole standard, EE and EE-AgNPs combination (4:1 ratio) at two concentrations (30 and 60 mg/ml). Then the times to paralysis (no spontaneous movement) and death (no response to vigorous shaking or immersion in 50°C water) were recorded and the entire procedure lasted 130 min.

**Results and Discussion**

*Phytochemical constituents:* Qualitative assessment of EE confirmed the presence of several phytochemical groups: alkaloids, glycosides, flavonoids, saponins, phenols and tannins. The presence of alkaloids was verified by Mayer's, Fehling's and Dragendorff's reagents, but remained undetected when using Wagner's and Benedict's test methods. Moreover, multiple confirmatory tests consistently detected the presence of both flavonoids and saponins (Table 1).

*In vitro cytotoxicity assay:* In this assay, EE alone and combined with AgNPs displayed concentration-dependent cytotoxicity, while vincristine sulfate provided a benchmark for comparison. The median lethal concentration ($LC_{50}$) of EE was determined to be 89.96 µg/ml, indicating mild toxicity compared to vincristine sulfate ($LC_{50}$= 3.56 µg/ml), a known chemotherapeutic agent with potent cytotoxic effects (Table 2). Furthermore, synergistic cytotoxicity was evident in the EE-AgNP combination, resulting in a considerably lower $LC_{50}$ (66.42 µg/ml), which implies AgNPs may intensify the effects of EE's bioactive compounds (Table 2).

*Antioxidant activity test:* Using the DPPH assay with AA as reference, EE showed dose-responsive antioxidant activity ($IC_{50}$ = 41.98 µg/ml), moderately

lower than AA ($IC_{50}$ = 18.82 µg/ml), likely due to its phenolic and flavonoid content (Table 3). Moreover, incorporating AgNPs with EE led to superior antioxidant effects, as shown by the lower $IC_{50}$ of 25.76 µg/ml (Table 3).

**Table 1. Phytochemical composition of the *C. papaya* ethanolic seed extract.**

| Phytochemical group | Name of the test | ME |
|---|---|---|
| Alkaloids | a) Mayer | + |
| | b) Wagner | - |
| | c) Dragendorff | + |
| | d) Benedict's | - |
| | e) Fehling's | + |
| Glycosides | a) Legal's | + |
| Flavonoids | a) Alkaline reagent | + |
| | b) Lead acetate | + |
| Saponins | a) Foam | + |
| | b) Froth | + |
| Phenols | a) Ferric chloride | + |
| Tannins | a) Ferric chloride | + |

Note: (+) = Presence and (-) = Absence of Phytochemicals.

**Table 2. Cytotoxic effect ($LC_{50}$) of *C. papaya* ethanolic seed extract, combined AgNPs & seed extract and vincristine sulphate on brine shrimp nauplii.**

| Treatment | Concentration range (µg/ml) | Mortality (%) | $LC_{50}$ (µg/ml) | Relative toxicity |
|---|---|---|---|---|
| Ethanolic seed extract | 0.625-80 | 0-40 | 89.96 | 0.040 |
| Combined AgNPs & seed extract | 0.625-80 | 0-50 | 66.42 | 0.054 |
| Vincristine sulphate (Reference standard) | 0.625-80 | 20-100 | 3.56 | 1.00 |

**Table 3. DPPH free radical scavenging ability ($IC_{50}$ value) of *C. papaya* ethanolic seed extract, combined AgNPs & seed extract and ascorbic acid.**

| Treatment | Concentration range (µg/ml) | % of Inhibition | $IC_{50}$ (µg/ml) | Relative potency |
|---|---|---|---|---|
| Ethanolic seed extract | 12.5-500 | 41.24-85.31 | 41.98 | 0.45 |
| Combined AgNPs & seed extract | 12.5-500 | 39.92-86.25 | 25.76 | 0.73 |
| Ascorbic acid (Reference standard) | 12.5-500 | 41.24-90.02 | 18.82 | 1.00 |

*Antibacterial activity test:* EE and its AgNPs combination demonstrated notable antimicrobial activity against gram-negative (*E. coli*, *P. aeruginosa*, *K. pneumoniae*) and gram-positive (*B. subtilis*) strains, with kanamycin (30 µg) as the reference. EE's inhibition was dose-dependent, most potent against *E. coli* (18.0 mm at 250 mg/ml), then *P. aeruginosa* (17.0 mm), with reduced efficacy against *K. pneumoniae* and *B. subtilis*. Besides, enhanced antibacterial effects were observed for EE-

AgNPs, producing inhibition zones 1.2-1.3 fold greater than EE alone in both gram-negative (*E. coli*) and gram-positive (*B. subtilis*) microbes. Alone, AgNPs exhibited mild inhibitory activity against *E. coli* and *P. aeruginosa*, while no noticeable effect was observed against *K. pneumoniae*. Conversely, the AgNPs by themselves were minimally effective against *B. subtilis* (Table 4).

**Table 4. Dose-dependent antibacterial activity of *C. papaya* ethanolic seed extract and its synergistic effect with AgNPs.**

| Test organisms | Concentrations (mg/ml) | Zone of inhibition (mm) (Including disc diameter 6 mm) | | | | |
|---|---|---|---|---|---|---|
| | | EE | EE + AgNPs | AgNPs (25 mg/ml) | Kanamycin (30 µg) | DMSO (5%) |
| *Escherichia coli* | 75 | 9.0 | 9.0 | 11.0 | 24.0 | 6.0 |
| | 125 | 14.0 | 12.0 | | | |
| | 150 | 16.0 | 17.0 | | | |
| | 250 | 18.0 | 22.0 | | | |
| *Pseudomonas aeruginosa* | 75 | 6.0 | 6.0 | 10.0 | 20.0 | 6.0 |
| | 125 | 10.0 | 9.0 | | | |
| | 150 | 13.0 | 15.0 | | | |
| | 250 | 17.0 | 18.0 | | | |
| *Bacillus subtilis* | 75 | 6.0 | 7.0 | 8.0 | 23.0 | 6.0 |
| | 125 | 9.0 | 10.0 | | | |
| | 150 | 11.0 | 13.0 | | | |
| | 250 | 14.0 | 18.0 | | | |
| *Klebsiella pneumoniae* | 75 | 8.0 | 10.0 | 6.0 | 21.0 | 6.0 |
| | 125 | 11.0 | 14.0 | | | |
| | 150 | 12.0 | 15.0 | | | |
| | 250 | 16.0 | 16.0 | | | |

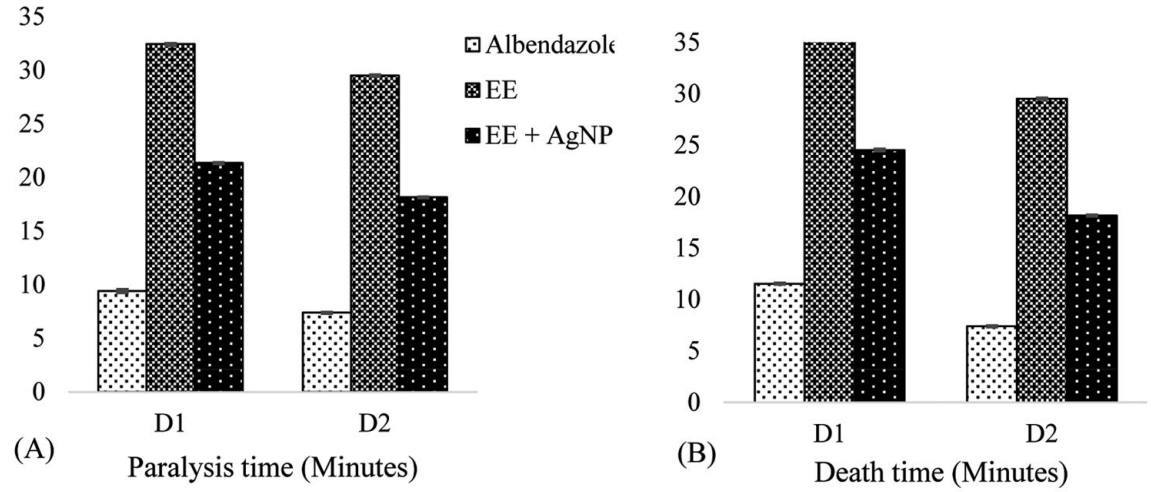


Figure 1. Comparative anthelmintic activity of ethanolic seed extract and its synergistic effect with AgNPs (A. Paralysis time; B. Death time). All values are expressed as Mean ± SEM (n=3); D=Dose; $D_1$=30, $D_2$=60 mg/ml.

*Anthelmintic activity assay:* Superior anthelmintic activity was observed for albendazole (60 mg/ml), resulting in 5.45 ± 0.020 min paralysis time and 7.38 ± 0.017 min death time. At 60 mg/ml, EE demonstrated its strongest wormicidal effects (paralysis in 25.56 ± 0.012 min; death in 29.51 ± 0.020 min), showing clear dose-dependence (Figure 1). Furthermore, a significant enhancement of anthelmintic effects was observed with EE-AgNPs, with 31% shorter paralysis times and 41% reduced death times compared to EE by itself.

This research highlights the therapeutic potential of *C. papaya* seed extract integrated with AgNPs, supporting earlier findings on its traditional medicinal uses. Previous studies have reported that AgNPs functionalized with plant extracts exhibit improved stability and biological efficacy compared to their standalone counterparts (Simon *et al.*, 2022). The broad spectrum of bioactive compounds identified in the preliminary phytochemical screening backing its ethnomedicinal efficacy against infections and oxidative stress-related disorders, warranting further study.

The cytotoxicity evaluation of plant extracts is pivotal for identifying their therapeutic potential, particularly in anticancer drug discovery. By testing toxicity in cell cultures, these studies validate the safety and efficacy of plant-derived compounds. Additionally, they provide mechanistic insights into cell death pathways, bridging ethnomedicinal use with evidence-based applications. Compared to potent vincristine sulfate ($LC_{50}$= 3.56 µg/ml), EE was weakly cytotoxic ($LC_{50}$= 89.96 µg/ml) in this study; however, combination with AgNPs enhanced its activity and lowered the $LC_{50}$ (66.42 µg/ml) (Table 2). A similar study by Khor and Wong (2014) reported an $LC_{50}$ of 374.86 µg/ml for a crude methanolic seed extract, which is over four times less potent than the $LC_{50}$ of 89.96 µg/ml observed in this study for the EE. This significant difference indicates that EE possesses substantially greater cytotoxicity. Furthermore, Husin *et al.* (2019) found that crude leaf extract was far less cytotoxic than the seed extract examined here, reinforcing the superior potency of the seed-derived material. The observed activity reflects the extract's high levels of bioactive flavonoids, alkaloids, and phenolics with known for their pro-apoptotic and anti- proliferative properties (Dumitraș & Andrei, 2022). Phytomolecules-AgNPs synergy likely increased cell toxicity (Fernandes *et al.*, 2018), as the significantly reduced $LC_{50}$ demonstrated. Such investigations spotlight potential therapeutics while affirming phytochemicals as sustainable bioactive origins.

The DPPH assay revealed that EE and EE-AgNPs scavenge free radicals, with EE showing dose-dependent moderate activity ($IC_{50}$ = 41.977 µg/ml) relative to ascorbic acid (18.821 µg/ml) (Table 3). The EE in this study demonstrated potent free radical scavenging ability ($IC_{50}$ = 41.977 µg/ml), substantially outperforming the antioxidant activities reported for similar extracts. Specifically, EE was approximately six times more potent than the ethanolic seed extract ($IC_{50}$ = 248.63 µg/ml) reported by Zhou *et al.* (2011) and nearly seven times more potent than the acetone seed extract ($IC_{50}$ = 285.77 µg/ml) described by Ang *et al.* (2012). The superior potency of the current extract (i.e. EE) is further underscored by its comparison to the leaf extracts reported by Husin *et al.* (2019), which demonstrated significantly higher $IC_{50}$ values of 216 µg/ml (methanolic) and 718 µg/ml (aqueous). Moreover, the observed boost in activity upon adding AgNPs ($IC_{50}$=25.761 µg/ml) (Table 3) implies a role in optimizing the bioavailability or delivery of the bioactive constituents in EE (Dhand *et al.*, 2015). This aligns with nanoparticle-phytochemical research, but the underlying process needs clarification. These findings highlight the potential of EE-AgNPs combinations as natural antioxidant agents for pharmaceutical or nutraceutical applications, particularly in oxidative stress-related disorders. Future research should explore their effects in cellular antioxidant models and *in vivo* systems to validate their therapeutic efficacy.

Antimicrobial testing indicated significant activity of EE and EE-AgNPs against all the tested strains (*E. coli* most susceptible), showing dose-responsive inhibition. According to Dagne *et al.* (2021), the antibacterial efficacy of the crude ethanolic seed extract against *E. coli*, *B. subtilis*, and *K. pneumoniae* was lowered at slightly higher concentrations. Although their study indicated superior potency of the ethanolic seed extract over its methanolic counterpart, the absence of a defined dose-response relationship limits the interpretability of these results. Furthermore, Rahman & Akhter (2018) reported a similar dose-dependent activity against *P. aeruginosa* (ZOI = 7.75-12.75 mm) at a comparable concentration of EE to that used in this study (ZOI = 10.0-13.0 mm). However, they observed no activity against *E. coli*. The reduced effects on *K. pneumoniae* and *B. subtilis* in this study suggest variations in cell wall composition or microbial resistance. While kanamycin was more effective (20–24 mm inhibition), EE-AgNPs approached its performance at elevated doses, particularly against *E. coli*, indicating AgNPs may intensify EE's efficacy via cellular uptake or protein interference (Hemeg, 2017). Furthermore, the inherent antibacterial activity of AgNPs suggests that AgNPs by themselves display mild antibacterial activity toward *E. coli* and *P. aeruginosa*, but not against *K. pneumoniae*, despite all being gram-negative strains. However, a minor effect was noted against the gram-positive *B. subtilis* (Table 4). These findings suggest that the antibacterial activity of AgNPs may vary depending on bacterial cell wall structure and species-specific susceptibility, with comparatively better performance against certain gram-negative organisms. Moreover, varied strain susceptibility (notably *E. coli*) also suggests structural and efflux differences (Cox & Wright, 2013), revealing EE-AgNPs' complementary antimicrobial value if future studies clarify mechanisms and optimal blends.

The present study demonstrated the superior anthelmintic efficacy of albendazole, consistent with its well-established role as a broad-spec trum antiparasitic drug that disrupts microtubule polymerization in helminths (Dayan, 2003). While slower-acting than albendazole, EE's potency suggests the presence of bioactive compounds (e.g., tannins, alkaloids) that may interfere with neuromuscular function or energy metabolism in worms (Fomum & Nsahlai, 2017). Consistent with the findings of Goku *et al.* (2020), the ethanolic seed extract demonstrated a dose-dependent anthelmintic effect. However, their reported potency at 5 mg/ml (paralysis: 7.210 ± 0.010 min; death: 9.150 ± 0.0058 min) was greater than that observed in the current study. Furthermore, seed extracts were more effective than those from stem bark or leaves (Goku *et al.*, 2020; Obeng *et al.*, 2022). In Red Sokoto goats, an aqueous *C. papaya* seed extract demonstrated anthelmintic efficacy comparable to thiabendazole and was more effective than a powdery form (Ameen *et al.*, 2018). However, the treatment lacked a dose-response relationship. The anthelmintic efficacy and safety of the seeds, attributed to their complex of bioactive compounds like alkaloids and saponins, provides a scientific basis for their traditional preference over the leaves and stem bark (Okeniyi *et al.*, 2007; Garcia-Bustos *et al.*, 2019). Furthermore, in the current study, combining EE with AgNPs boosted anti-parasitic effects, resulting in 31% shorter paralysis and 41% faster death compared to EE alone. This enhancement might be attributed to: AgNPs independently disrupting parasitic redox balance (via ROS generation), while EE compounds inhibit critical enzymes (e.g., acetylcholinesterase), collectively accelerating paralysis (Zhang *et al.*, 2016); AgNPs potentially enhancing the solubility and tissue penetration of EE's bioactive compounds; and their ability to physically adhere to the worm's cuticle, increasing permeability to phytochemicals (Awlqadr *et al.*, 2025).

The study reveals potential in *C. papaya* EE and AgNPs combinations, but has constraints. The *in vitro* models may not fully replicate *in vivo* biological complexity, particularly for antimicrobial and anthelmintic applications. The mechanisms behind AgNPs' enhancement of EE's efficacy, such as improved solubility, cellular uptake, or redox modulation, remain hypothetical without direct

molecular evidence. Cytotoxicity and antioxidant assays, though informative, lack validation in animal or clinical models, raising questions about safety and therapeutic relevance. Additionally, variations in microbial susceptibility (e.g., stronger effects on *E. coli* than *K. pneumoniae*) suggest strain-specific interactions that warrant deeper mechanistic study. Future work should include *in vivo* studies with specific isolated bioactive compounds, dose optimization of AgNPs, and long-term toxicity assessments to translate these findings into practical applications.

This study outlines a green synthesis route for AgNPs, with formation confirmed through UV-Vis spectroscopy and visual observation. However, the lack of advanced techniques, such as Fourier-Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Transmission Electron Microscopy (TEM), limits the depth of mechanistic insight into their characteristics, stability and synthetic mechanisms of AgNPs. The current experimental focus was on nanoparticle synthesis and subsequent application rather than comprehensive physicochemical characterization. The future studies will include detailed particle size and morphology analyses using advanced techniques to further validate and complement the current findings. Nonetheless, the development of this simple, cost-effective protocol represents a significant contribution, providing a foundational methodology that facilitates future collaborative research and initial bioactivity screening.

## Conclusion

The findings validate the medicinal value of *C. papaya* seed extract-AgNPs combinations through improved performance across various biological activity assessments. The synergistic effects of EE-AgNPs, marked by improved efficacy compared to EE alone, highlight the promise of plant-nanoparticle combinations in drug development. However, the reliance on *in vitro* models and incomplete mechanistic understanding of AgNPs' role underscores the need for further research.

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## Conflict of interest

No conflicts of interest are reported by the authors.

## Authors' contributions

**Ratna Sarkar:** Conceptualization, Methodology, Investigation, Writing–original draft; **Abu Zobayed**: Methodology, data curation, formal analysis, writing–review & editing; **Mahfuza Akter:** methodology, investigation, visualization; **Sayema Arefin:** Validation, formal analysis, writing–review & editing; **Israt Jahan Ira:** Conceptualization, methodology, resources, supervision, project administration, funding acquisition.

## Data availability statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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