Research Article

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Biofabrication of *Manilkara zapota*-assisted carbon nanoparticles: Characterization and Wound Infection Control

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ABSTRACT

Developing environmentally friendly Carbon nanoparticles (CNPs) using medicinal plants with enhanced biological activity has emerged as a strategy for the synthesize the nanomaterials for biomedical applications. This study aimed to evaluate the biogenic synthesis of carbon nanoparticles (CNPs) using *Manilkara zapota* leaf extract and their structural characteristics and biomedical potential. The synthesized nanoparticles were characterized using SEM, TEM, XRD, and FTIR analyses. Their antioxidant, anti-inflammatory inhibition, antibacterial activity, and cytotoxicity were evaluated using in vitro assays. SEM and TEM findings supported the formation of spherical nanoparticles, while XRD revealed the amorphous structure. FTIR identified the existence of functional groups such as OH, COOH, and NH, which aid in nanoparticle stabilization. The CNPs exhibited maximum antioxidant and anti-inflammatory potential at 50µg/mL, exhibited antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* with inhibition zones ranging from 9- 12 mm, and low dose-dependent cytotoxicity. The present study introduces the biosynthesized Carbon nanoparticles (CNPs) using *Manilkara zapota* leaf extract, which possess significant biomedical potential. The novelty of this study lies in the sustainable plant–mediated synthesis from *Manilkara zapota* leaf extract for Carbon nanoparticles (CNPs) with promising therapeutic potential. Further investigations are needed to evaluate their therapeutic application in wound-healing efficacy through in vitro and in vivo studies.

Keywords: Carbon nanoparticle; Scanning electron microscopy; Anti-inflammatory; Cytotoxicity.

1. INTRODUCTION

Nanotechnology has emerged as a promising field in biomedical research, offering innovative strategies for diagnosis, drug delivery, and therapeutic applications. Among the various nanoparticles, carbon nanoparticles (CNPs) including carbon dots,carbon nanotubes, graphene (Mahalakshmi *et al* 2025; Senthil, 2023) and nanodiamonds have gained significant focus due to their fluorescence properties, optical stability and biocompatibility (Manigandan and Shanmugam, 2025) These characteristics make CNPs as promising applications in diverse biomedical applications such as antioxidant, anti-inflammatory, antimicrobial, anticancer and cytotoxic effect activities(Gedda *et al.* 2023; Baskar *et al.* 2025) reported that green synthesized carbon nanoparticle (CNPs) derived from *Azadirachta indica* plant leaf extract possessed potent antioxidant activity (EC_{50}=13.87µg/mL) and a total antioxidant capacity (EC_{50}= 38.7287µg/mL) and exhibited broad spectrum antibacterial and antifungal activity(Gedda *et al.* 2023) A hydrothermal approach synthesized carbon nanoparticle (CNPs) with an average particle size of 4.17nm, which demonstrated 91.32%±0.31% through DPPH assay and potent antibacterial activity against *E. coli* (MIC:0.62 mg/mL) and *S. aureus* (MIC:0.85 mg/mL)(Zhang *et al.* 2025). Hydrothermally synthesized *Prosopis Juliflora* Carbon dots(8nm) with a quantum yield of 7.88% exhibited significant antibacterial activity against Staphylococcus aureus (MIC=1.50mg/mL), exhibited no activity against E. coli, and demonstrated excellent fluorescence for bioimaging *Caenorhabditis elegans*(Prathap *et al.* 2023). *Curcuma longa* leaf-derived carbon dots with an average diameter of 2.6±0.3nm and a negative zeta potential of- 11.3±2.7 mV exhibited complete eradication of K. pneumoniae and *S. epidermis* at 0.5mg/mL after 24 hours (Saravanan *et al.* 2021)(Kota *et al.* 2024). A study reported that heteroatom–doped carbon dots derived from medicinal plants such as exhibited 69.4% DPPH radical scavenging activity (IC_{50} =15.6µg/mL), 55.3% anti-inflammatory activity (IC_{50} =22.28µg/mL) and antibacterial activity with zones of inhibition between 10-19nm(Kota *et al.* 2024).A study demonstrates that, while compared with

conventional chemical and physical methods, the green synthesis of CNP using plant extracts provides functional surface groups and biocompatibility, showing significant potential in bioimaging and biosensing (Mondal *et al.* 2021). Nanoparticles synthesized from the plant extract contain bioactive compounds such as phenolics, flavonoids, proteins, and terpenoids, which play essential roles in capping, reduction, and stabilization of nanoparticles (Rajeshkumar and Bharath, 2017). Recently, green synthesis of nanoparticles using diverse plant parts such as fruit extracts was reported; silver nanoparticles (AgNPs) using Diospyros malabaricus fruit extract had an average size of 17.4nm and exhibited significant antibacterial activity, with an inhibition zone of 12.1±0.5nm against *S. aureus* and 13.1±0.5nm against *E. coli* at 1000µg/mL. In this study, silver nanoparticles further exhibited an IC50 of 58.63±5.74µg/mL against U87-MG cells (glioblastoma cells) (Bharadwaj et al. 2021). Elaeocarpus serratus fruit extract–derived silver nanoparticles (AgNPs) demonstrated a broad spectrum of antibacterial activity against both Gram-positive and Gram- negative bacteria, with IC_{50}=49±2.33µg/mL. (Balamurugan *et al.* 2024), seed extracts such as; green synthesized Silver nanoparticle(AgNPs) using *A. katsumadai* seeds with average size 12.6nm demonstrated potent antibacterial activity with MIC value of 20µg/mL and 40µg/mL for *E. coli* and *S. aureus* respectively (He *et al.* 2017), *Tectona grandis* seed extract is used for the synthesis for the Silver nanoparticle(AgNPs) with size ranging from 10-30nm exhibited antimicrobial activity against *E.coli* (17mm; MIC-2.0µg/mL), *S.aureus* (16mm; MIC-2.6µg/mL)*B.cereus*(12mm; MIC-5.2µg/mL) (Rautela *et al.* 2019) Iravani and Zolfaghari prepared Silver nanaoparticles (10-40nm) frombark extracts of *Pinus Eladarica* under optimized conditions of 1-6mM $AgNO_3$, 1-6mL extract , alkaline pH and elevated temperature (upto 150^0C(Iravani and Zolfaghari, 2013). A study by (Banerjee et al., 2014) synthesized silver nanoparticles (AgNPs)using leaf extracts of M. Balbsiana, A. Indica, and Ocimum tenuflorum exhibited significant antibacterial activity against Gram-positive bacteria, with inhibition zones of (16±0.0016, 14±0.02, 14±0.021 respectively (Banerjee et al., 2014).

In the present study, *Manilkara zapota* leaf extract was selected for the synthesis of CNPs. *Manilkara zapota*, also known as sapodilla or chikko belongs to the sapotaceae family, various parts of *Manilkara zapota*, such as seeds, leaves, fruits and bark, demonstrate significant antioxidant, anti-inflammatory, anticancer and antidiabetic activity (Ankli *et al.* 2002) and its contain phenolic acids, flavonoid groups, dicarboxylic acids and reducing sugars, these bioactive constituents contain carbonyl, hydroxyl and carboxy functional group which have a significant role as reducing and stabilizing agents by forming a protective layer around the nanoparticles during nanoparticle synthesis(Islam *et al.* 2020). Several methods have been developed for the synthesis of carbon nanoparticles, including physical methods such as ball milling and diffusion flame synthesis (Parveen et al. 2016); chemical methods such as chemical vapor oxidation and hydrothermal synthesis (Li et al. 2019); and microbial synthesis of nanoparticles as a biologically sustainable method. Various microorganisms can be used for the green synthesis of nanoparticles, including bacteria (Escherichia coli) (Lin et al. 2018), fungi (Agaricus bisporus, *Lentinus edodes, Pleurotus ostreatus, Flammulina velutipes, Auricularia auricula) (Gao et al. 2025), and yeast (Saccharomyces cerevisiae*) (Mirseyed *et al.* 2024). The microbial synthesis requires aseptic maintenance due to its high susceptibility to contamination and often produces low yields(Bahrulolum *et al.* 2021). The use of *Manilkara zapota* leaf extracts replaces hazardous chemical reducing and stabilizing agents and promotes an eco-friendly, low- cost synthesis process. The present study focuses on the use of *Manilkara zapota* leaf extracts for the synthesis of carbon nanoparticles, followed by the size, structure, optical properties, and crystallinity of the synthesized CNPs using characterization techniques such as SEM, TEM, XRD, FTIR, and systematic evaluation of their antioxidant, anti-inflammatory, antimicrobial, and cytotoxic activities. The findings presented in this study contribute to the existing knowledge and support further investigations by involving mammalian cell- line models and in vivo studies

2. MATERIALS AND METHODS

2.1 Synthesis of the Carbon Nanoparticle (CNPs)

The synthesis was performed in triplicate under identical experimental conditions. The leaves were thoroughly washed using tap water and then dried with distilled water and coarsely powdered. 2.5 grams of powdered *Manilkara zapota* were dissolved in 50 mL of distilled water and mixed well; then the solution was boiled at 50^0 Celsius in a heating mantle for 15-20 minutes and filtered using a muslin cloth. The extract is again kept in the heating mantle to reduce it to 5 mL and kept in the muffle furnace at 700 volts for synthesizing carbon nanoparticles using a crucible. The residues that settled in the crucible were then finally ground into a fine powder, scraped, and washed repeatedly three times with distilled water by centrifugation at 8000 rpm for 10 minutes and refrigerated in an airtight container for further investigations. (Fig:1)

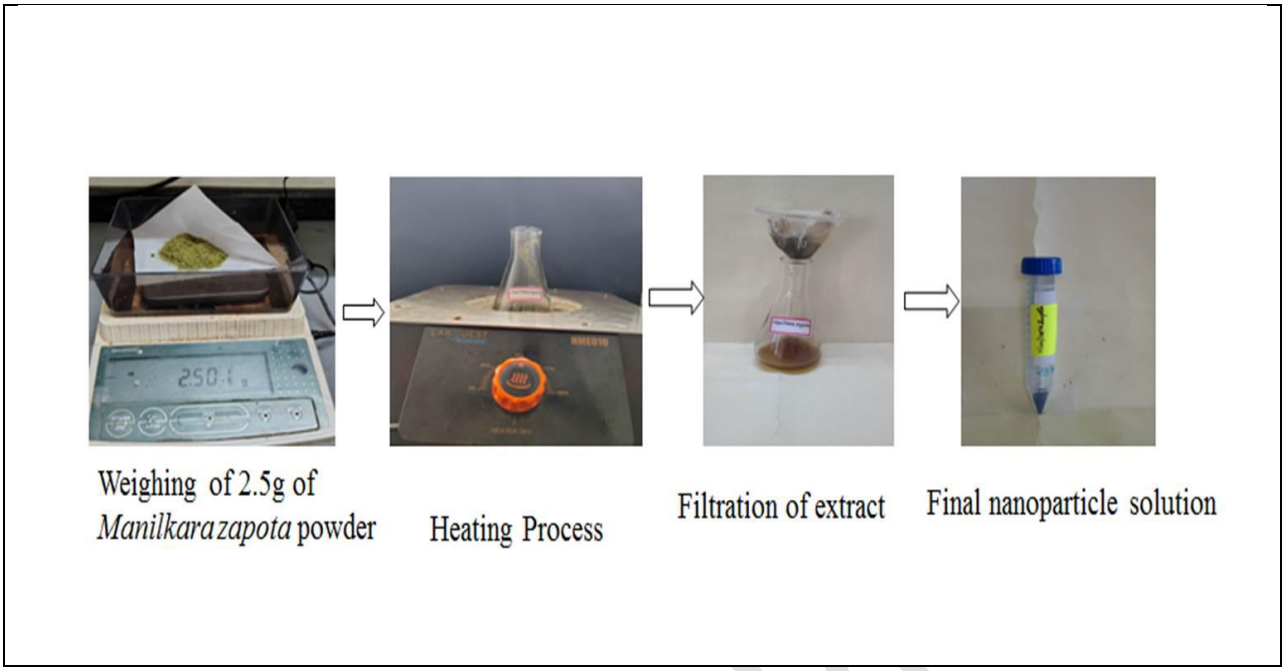

Fig. 1: Mechanistic illustration of carbon nanoparticle synthesis mediated by *Manilkara zapaota* extract

3. BIOMEDICAL APPLICATIONS

Estimation of antioxidant activity of carbon nanoparticles

3.1 H_2O_2 Assay

The hydrogen peroxide radical scavenging activity was determined for the biosynthesized carbon nanoparticles. 1 mL of different concentrations of CNP (10 µg/mL to 50 µg/mL) was added to appropriately labeled test tubes and mixed with 200 µl (0.2 mL) of hydrogen peroxide. The test tube was allowed to incubate in the dark for 10 minutes. The absorbance was measured at 532 nm.

3.2 DPPH Assay

The free radical scavenging activity of biosynthesized carbon nanoparticles was evaluated by the DPPH assay. 1 mL of biosynthesized CNPs of different concentrations (10µg/mL to 50 µg/mL) was added to appropriately labeled test tubes, mixed with1 mL of 1mM DPPH, and then the solution was allowed to incubate under dark conditions at room temperature for up to 10 minutes. After incubation, the colour change of DPPH from purple to yellow was observed, and the absorbance exhibited a peak at 517nm using a UV-Vis spectrophotometer.

Estimation of Anti-Inflammatory Activity of Carbon Nanoparticles

3.3 Bovine Serum Albumin Assay

The anti-inflammatory assays in this study followed protocols reported in previous studies (Dhanapal *et al.* 2025). BSA assay was used to determine the anti-inflammatory activity of green-synthesized CNP. 1 mL of Bovine Serum Albumin was mixed with 1 mL of different concentrations of biosynthesized CNP (10 µg/mL to 50 µg/mL), then the solution was allowed to incubate under dark conditions at room temperature for 10 minutes after it was heated using a heating mantle at 55^0C for 30 minutes. The absorbance was measured by a UV-Vis spectrophotometer at 660 nm.

3.4 Egg Albumin Assay

3 mL of egg albumin is mixed with 2.8 mL of freshly prepared 1X phosphate buffer, for the egg albumin denaturation assay. The different concentrations of green synthesized carbon nanoparticles (10 µg/mL to 50 µg/mL) were allowed to remain at ambient temperature for 10 minutes, then incubated in a water bath for 10 minutes at 55^o Celsius. The absorption was measured at 660 nm.

Estimation of cytotoxic activity of carbon nanoparticles

3.5 Brine Shrimp Lethality Assay

Brine shrimp lethality assay was used to determine the cytotoxicity of green synthesized carbon nanoparticles. 0.2 g of iodine - free salt was weighed and dissolved in 200mL of distilled water. The larvae (Nauplii) were hatched after 36 hours of incubation under continuous illumination and well–aerated conditions.

The assessment of cytotoxicity was performed using nauplii of brine shrimp as follows.

5 mL of water was filled into 6-well ELISA plates, followed by the introduction of 10 nauplii per well. Fresh dispersion of green synthesized CNP at different concentrations (10 µg/mL to 50 µg/mL) was added, and the plates were incubated for 24 hours.

After 24 hours, the ELSA plates were observed for the number of live nauplii, and the mortality rate was determined.

3.6 Antibacterial Activity

Antibacterial activity was performed using the Agar well diffusion method on Mueller – Hinton agar plates; it was sterilized at 1210C for 15- 20 min. About 100 mL of Mueller–Hinton agar was used; after the plates were allowed to solidify, they were aseptically swabbed with five clinically isolated E. coli, Pseudomonas. *Sp, E. faceium, S. aureus*. For inoculation, four wells of 9mm diameter were made in each plate using a T- shaped well cutter. The test samples were added at concentrations of (25µl, 50µl and 100µl) and the standard antibiotics were added as a control, followed by incubation at 37^0 C for 24 hours, and the antibacterial activity was determined by measuring the diameter of the inhibition zones. Antifungal activity against *C. albicans was assessed on Rose Bengal Petri* plates. After solidification, Candida albicans was swabbed, and wells were made similarly. The test samples (25µl, 50µl and 100µl) and the standard antibiotic fluconazole were added. The plates were incubated at 37^0 C for 48 hours; the zones of inhibition were measured in millimeters and conducted in triplicate for each microorganism.

Statistical Analysis

All experiments were performed in triplicate, and the results are presented as mean ± standard deviation (SD); statistical analysis was carried out using two–way ANOVA, with $p<0.0005$ regarded as statistically significant.

4. RESULTS AND DISCUSSION

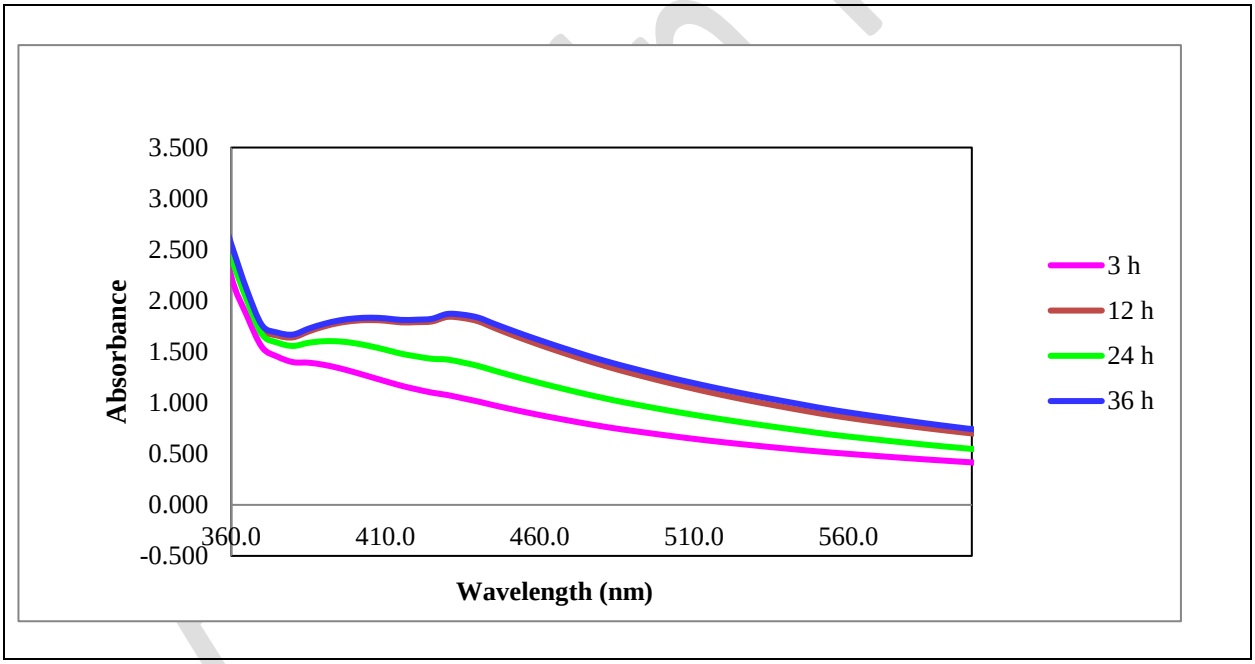

Fig. 2: UV-Vis spectroscopy of *Manilkara zapota* mediated carbon nanoparticles (CNPs)

UV-Vis spectroscopy (Fig. 2) shows the formation of *Manilkara zapota-mediated carbon nanoparticles (CNPs) at 3, 12, 24, and 36 h*. The absorption spectra showed characteristic absorption between 360 and 430 nm, confirming the formation of Carbon nanoparticles (CNPs). At 36 h, the spectrum showed increased absorbance around 420- 450 nm. This absorption profile supports the surface optical properties of the formation of Carbon nanoparticles (CNPs). Beyond 450nm the gradual decline is shown towards 650nm, which may be due to nanoparticle stabilization or the gradual depletion of the reaction components. Hence, the UV-Visible absorption pattern confirms the effective green synthesis of *Manilkara zapota-mediated carbon* nanoparticles (CNPs). Comparable absorption peaks have been reported for green-synthesized carbon nanoparticle including from *Azadirachta indica* observed a peak around 360nm (274nm)(Waseem *et al.* 2024), from *Ruba cordifolia L* carbon dots showed a UV – visible absorption peak at 332nm, corresponding to the

π*-π* transition of aromatic C=C bonds (Zhang *et al.* 2024; Dhandapani *et al* 2019).

TEM confirmed the formation of biosynthesised carbon nanoparticles under optimal conditions (Fig.3(A-C) with quasi-spherical morphology and minor aggregations. Furthermore, the SAED pattern (Fig.3(D)) showed broad diffraction rings, indicating low crystallinity and confirming the amorphous nature of the green-synthesized carbon nanoparticles. The XRD pattern was recorded over a 2θ range between $10^0$to 80^0 using Cu kα radiation. (Fig 4) The diffraction pattern showed characteristic peak at 2θ values of 28.36˚, 31.81˚, 36.49˚, 40.48˚, 52.30˚ and 66.06˚ which were indexed to the plane of (111), (200), (220), (311), (222) and (400) respectively (Fig.4). The carbon matrix is predominantly amorphous (Luo *et al.* 2022) rather than the crystalline structure, the amorphous nature may be attributed to the bioactive phytochemicals present in the *Manilkara zapota* leaf extract

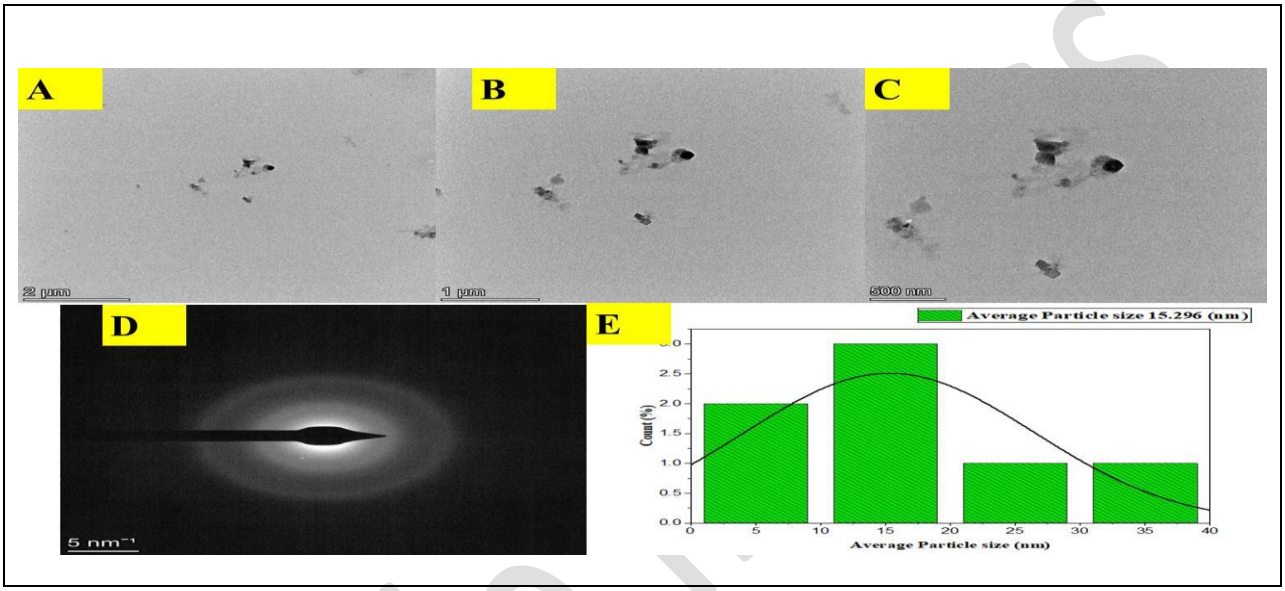

Fig. 3: (A) TEM analysis images of carbon nanoparticles using *Manilkara zapota* leaf extract at different magnifications; (B) 2 µm, 1µm (C) 500nm (D) SAED pattern and (E) Histogram image of *Manilkara zapota-mediated* carbon nanoparticles (CNPs) with an average size of 15.296nm

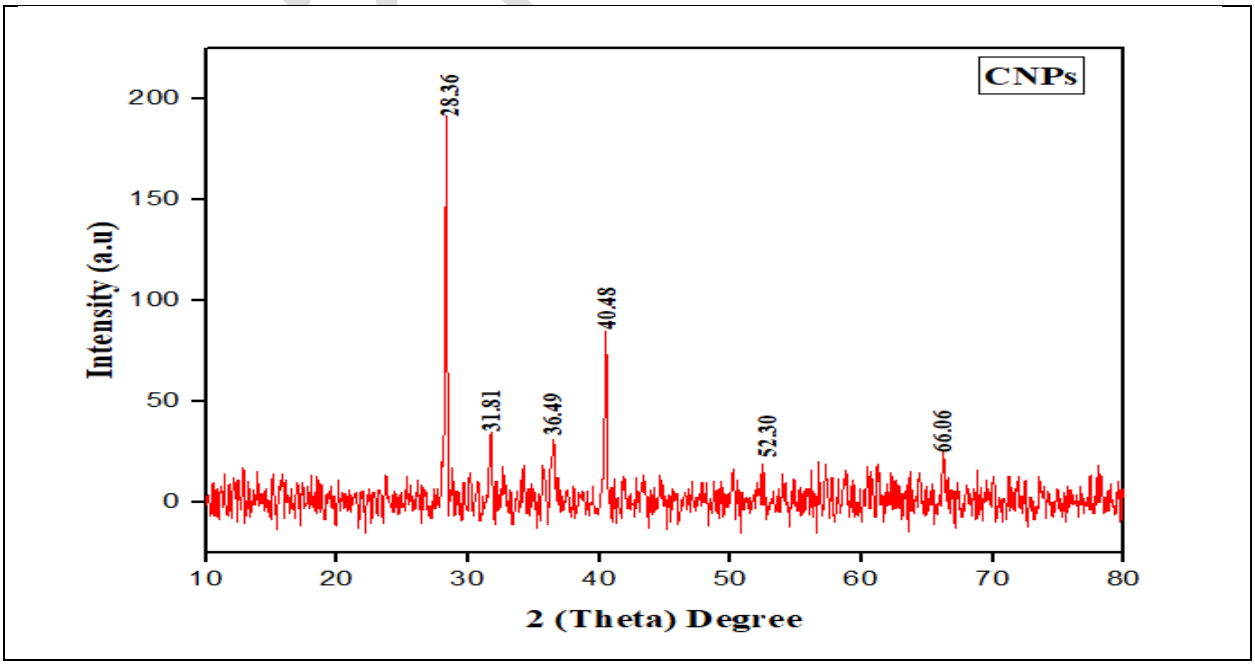

Fig 4: XRD characterization of carbon nanoparticle synthesized from *Manilkara zapota*

Similar diffraction characteristics have been reported in previous studies by Abinaya et al. 2024), who scanned the samples over a 2θ range of 10˚ to 90˚ at 40kv. A broad XRD peak was observed at 2θ=20.74˚ (JCPDS No 82-0505), with a d- spacing of 2.3nm corresponding to the (002) plane (Prathap *et al.* 2023). In another study, an XRD pattern of carbon dots prepared from medicinal plants showed a broad diffraction hump at 2θ=22˚ (Gedda *et al.* 2023).

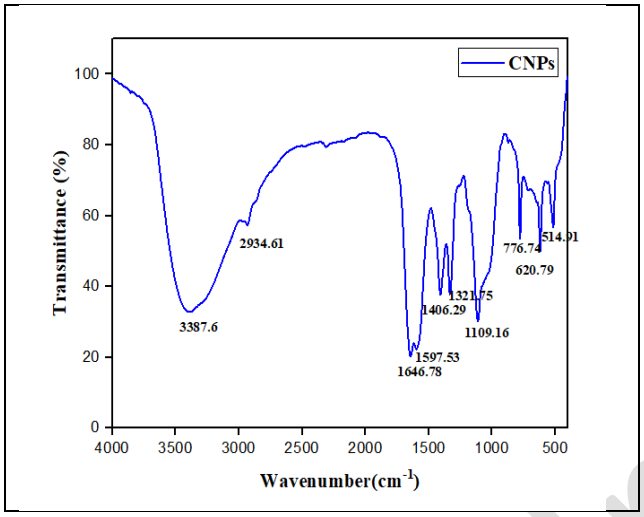

Fig. 5: FTIR characterization of carbon nanoparticles synthesized using *Manilkara zapota* leaf extract

FTIR spectrum confirmed the presence of different functional groups in *Manilkara zapota-mediated carbon nanoparticles* (CNPs) (Fig 5). The broad band at 3403.52cm^{-1} indicates O-H stretching of phenolic compounds, which act as reducing and stabilizing agents and play a role in donating electrons to facilitate nanoparticle formation. The peak at 2425.72cm^{-1} represents ≡C-H stretching in alkynes. The peak near 1627.83cm^{-1} is caused by the presence of C=C stretching (alkenes/amide groups). A band at 1383.96cm^{-1} represents C-N stretching. The band near 1117.94 cm^{-1} indicates C-O stretching of esters /ethers. Additional peaks observed at 826.36cm^{-1} and 661.74cm^{-1} correspond to C-H stretching, and the proteins and biomolecules present contributed to minimizing aggregation and improving their colloidal stability. These results confirm the presence of plant-derived biomolecules, which are responsible for the reduction as well as the stabilization of carbon nanoparticles (CNPs). These results are consistent with the results reported by (Supriya *et al.* 2025).

SEM micrographs (Fig.6) indicated that the formation of carbon nanoparticles with irregularly shaped and quasi-spherical to polygonal morphology (Fig.5; A, B) and sub-rounded to polyhedral (faceted) rather than perfectly spherical (Fig.5: C), with a small degree of aggregation in some regions, is likely due to the high surface energy of carbon nanoparticles and interactions. The average particle size was calculated as 15.296nm using ImageJ software, while the particle size distribution histogram was generated using OriginPro (2024). Comparable findings are reported on Murraya koenigii-mediated carbon nanoparticles (CNPs), which also confirmed the nanoparticle formation (Vijaya and Deepa, 2017). Vachellia nilotica-mediated carbon nanoparticles (CNPs) exhibited spherical morphology with a size ranging from 72- 127nm along with aggregation. (Vidhyarthi *et al.* 2025).

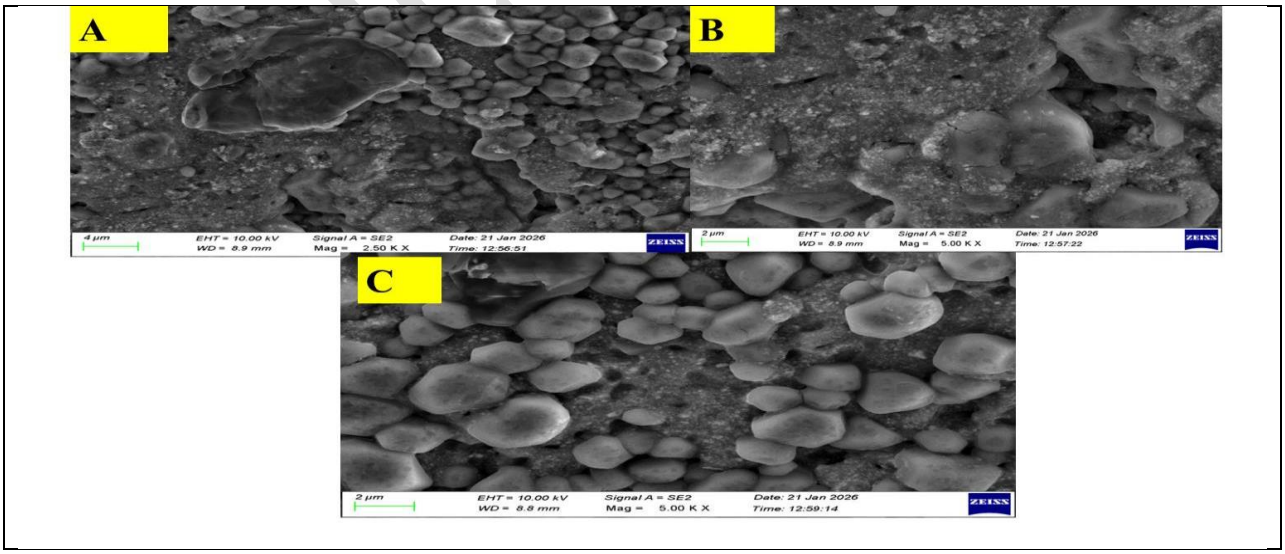

Fig. 6: SEM images of carbon nanoparticles using *Manilkara zapota* leaf extract at different magnifications; (A) 4 µm, (B) and (C) 2µm magnification

Antioxidant activity of the green-synthesized carbon nanoparticle is assessed by DPPH free radical scavenging assay and H_2O_2, FRAP assay, ABTS assay, and NO assay, compared with the standard antioxidant (Ascorbic acid). The graphical representation of DPPH assay shows the percentage of inhibition consistently increases inhibition values of 38.96±2.115, 49.28±1.11, 66.37±1.085, 76.27±1.995, 84.29±2.175, at concentrations ranging from 10-50µg/mL (Fig.7A) with an IC_{50} value of 18.929 along with the concentrations compared with the standard (Ascorbic acid) showed an IC_{50} value of 12.34668. The hydrogen peroxide scavenging assay(H_2O_2) (Fig.7B) shows the inhibition percentage of 45.96±2.17, 50.27±1.3,57.75±2.4,71.29±0875,83.45±2.02 across the same concentration range, exhibiting an IC50 value of 17.766, while the standard antioxidant displayed an IC_{50} value of 8.95. The Ferric reducing antioxidant power assay (FRAP) (Fig.7C) with inhibition values of 52.36±2.125,63.56±1.165, 68.29±2.265, 76.49±1.745, 80.27±1.035 at concentrations of 10-50µg/mL with an IC_{50} value of 3.53574, in comparison with the standard antioxidant ascorbic acid showed an IC_{50} value of -7.9351. The ABTS assay further contributed to the antioxidant potential of the Carbon nanoparticle (Fig.7D), with inhibition values of 57.65±0.175,64.81 ±2.24, 71.65±2.07,79.33 ±21.685,87.61 ±1.715. The IC_{50} value of *Manilkara zapota* CNPs was10.210, while the standard antioxidant (Ascorbic acid) showed 0.4361. The Nitric Oxide assay (NO) (Fig.7E) showed a concentration–dependent increase in antioxidant potential, with inhibition values of 53.466 ±2.215, 61.38 ±0.81, 69.15 ±1.14, 71.29 ±1.68, 76.18±0.82 with an IC_{50} value of 0.565492, while the standard showed -7.32686. Therefore, DPPH assay, H_2O_2, assay FRAP assay, ABTS assay and NO assay providing more accurate assessment of the free- radical scavenging abilities of green synthesized Carbon nanoparticles (CNPs) (Govindhraj and Shanmugam, 2025) Additionally, the green synthesized carbon nanoparticle using orange peel observed a strong antioxidant potential of 89.52% inhibition at a concentration of 4.10mg/mL(Ben *et al.* 2025). (Pant *et al.* 2023) showed that a concentration of 60µg/mL has the highest scavenging activity (Pant *et al.* 2023).

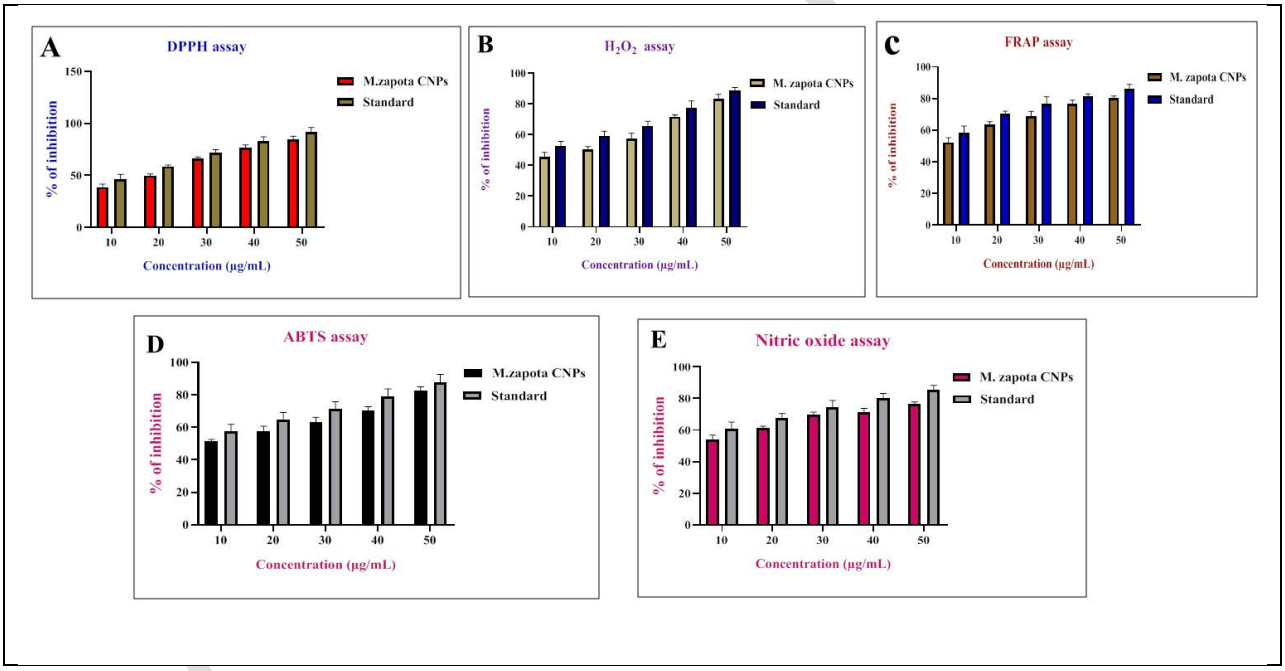

Fig. 7: Antioxidant potential of carbon nanoparticles from *Manilkara zapota* evaluated using (A) DPPH assay, (B) Hydrogen peroxide assay, (C) FRAP assay, (D) ABTS, and (E) Nitric oxide assays

The anti-inflammatory activity of green-synthesized carbon nanoparticles was assessed by Bovine Serum Albumin (BSA) and Egg Albumin assays. In Fig. 8A- C, the anti-denaturation effect of green-synthesized carbon nanoparticles was assessed using the bovine serum albumin assay, egg albumin, and MSA assay at concentrations ranging from 10µg/mL to 50µg/mL compared to the standard value. In the BSA assay exhibited a concentration- dependent inhibition values of 39.28±1.88, 51.27±0.66, 60.28±1.7, 72.37±1.82, 76.18±0.82 with IC_{50} value of 19.593 and standard (diclofenac sodium) showed an IC_{50} value of 13.29006 (Fig:.8A). EA assay exhibited the progressively inhibited inhibition values of 46.27±1.26, 51.48±0.775, 59.11±1.06, 66.49±2.175, 76.17 ±2.16, with an IC_{50} value of 16.761 and standard exhibited the an IC_{50} value of 5.426(Fig. 8B). Similiarly the MSA assay demonstrated the inhibition value of the percentage of

inhibition increases along with the concentration. The anti-inflammatory activity of *L.angustifolia mediated by carbon nanoparticles (CNPs) was observed to be dose-dependent*. (Manigandan *et al.* 2024). Demonstrated that Carbon Quantum Dots derived from *Hibiscus rosa* showed significant anti-inflammatory activity by using the Bovine Serum Albumin assay, serving as a potent inhibitor of heat - induced denaturation (Yalshetti *et al.* 2024).

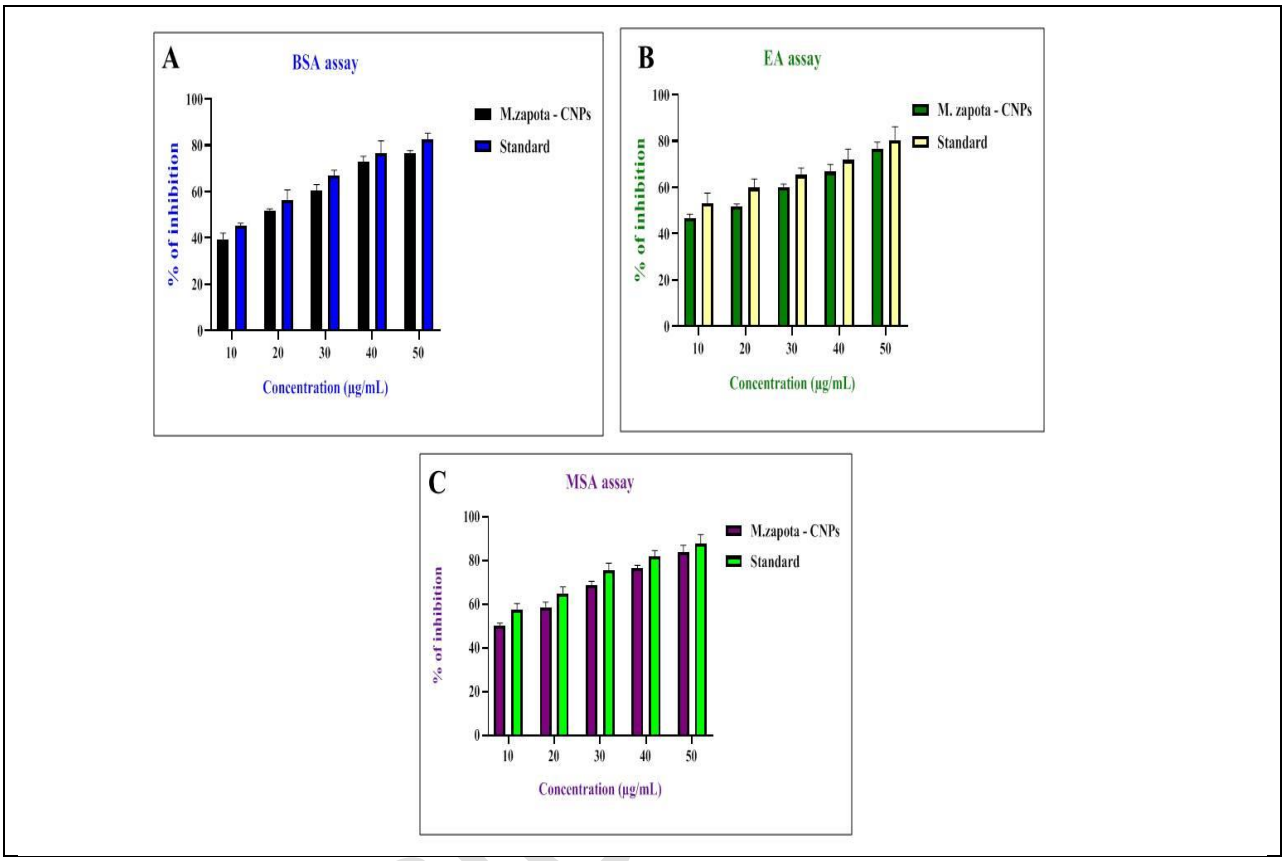

Fig. 8: Evaluation of anti-inflammatory activity of carbon nanoparticle using *Manilkara zapota* using (A) BSA assay, (B) Egg albumin assay and (C) MSA assay

The antimicrobial activity of green-synthesized carbon nanoparticles is assessed using the agar well diffusion method against selected wound-pathogenic microorganisms, including E.coli, Pseudomonas sp, E. faceium, S. aureus, and *C. albicans*. (Fig 9) The antibacterial mechanism of carbon nanoparticles initially involves interaction with the microbial cell surface, causing membrane disruption followed by reactive oxygen species (ROS) generation leading to intracellular oxidative damage (Zhu *et al.* 2023), promoting leakage of cellular contents and inhibition of metabolic processes, finally leading to microbial death(Liang *et al.* 2021). Fig. 9 shows the visual and graphical representations of the results of the antimicrobial activity of carbon nanoparticles. The antimicrobial activity of *Manilkara zapota* mediated by carbon nanoparticles progressively increases along with the concentration. Among five pathogens, *E. coli* shows the highest sensitivity, with an inhibition zone increasing from 9mm at 25µg/mL to 17mm at 100µg/mL, nearing the standard (39mm). Previous studies have demonstrated that *E. coli* showed potent susceptibility to Carbon nanoparticles (CNPs) (Prakash *et al.* 2025), while *S. aureus* showed moderate activity,12mm at 25µg/mL. In contrast, *Pseudomonas Sp*, *E. faecium*, and C. albicans showed minimal inhibition, indicating lower susceptibility. This study supports that *Manilkara zapota* carbon nanoparticles possess a strong dose - dependent antibacterial effect, primarily against *E. coli*. A prior study showed similar results of strong antibacterial potential of carbon nanoparticles from pomegranate peel using agar well diffusion against *S. aureus* and *K. pneumonie*, moderate activity against *P. aeuroginosa* compared with the standard ampicillin (Qureshi *et al.* 2021). Another study's findings show concentration-dependent antibacterial effect against Staphylococcus *aureus* and *Escherichia coli*(Calangian *et al.* 2018). A dose-dependent antibacterial activity is seen against both Gram-negative (Staphylococcus and Bacillus subtilis) and Gram-positive (*Escherichia coli* and *Pseudomonas. sp*) pathogenic bacteria (Gedda *et al.* 2023).

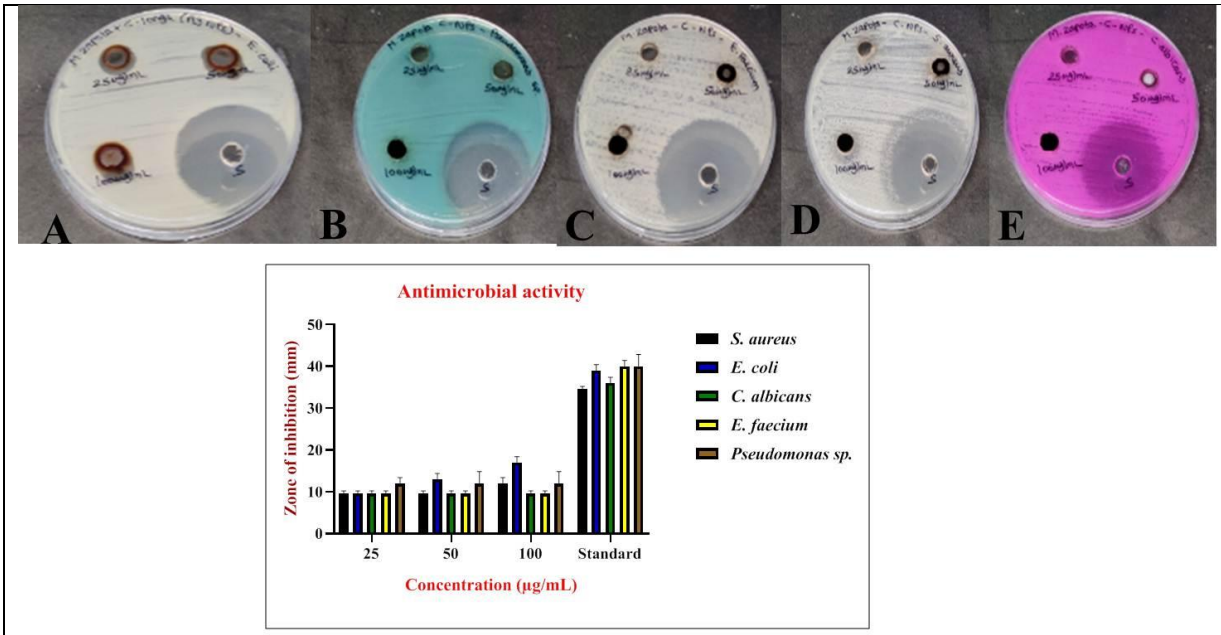

Fig. 9: Antimicrobial activity of carbon nanoparticles using *Manilkara zapota* extract against microorganisms (A) *E. coli* (B) *Pseudomonas sp*, (C) *E. faecium*, (D) *S. aureus,* and (E) *C. albicans,* and the bar diagram illustrating the comparative antimicrobial activity

The observed time-kill Curve assay demonstrated the antimicrobial effect of Manilkara zapota-mediated carbon nanoparticles, leading to decreased microbial growth over time against E. coli, E. faceium Pseudomonas sp, S. aureus, and *C. albicans*(Fig.10A-E). The initial microbial count was approximately 5.6log$_{10}$ CFU/mL for all the sample microorganisms. Among the tested concentrations, 100µg/mL a concentration–dependent reduction is observed in viable cell counts throughout the period, followed by 50µg/mL and 25µg/mL, while the control maintained minimal variation; E. faecium and S. aureus show the highest susceptibility to the carbon nanoparticle. Similarly, (Seyedjavadi et al. 2026) reported that the time-kill kinetics assay showed viable bacterial counts (log10CFU/mL) were defined as ≥3log10 CFU/ML reduction (Seyedjavadi *et al.* 2026). A previous study demonstrated a concentration- dependent bactericidal activity throughout the time-kill kinetics assay, producing a 5-log$_{10}$ CFU/mL reduction against *E. coli* within 1hour (de Sousa *et al.* 2025).

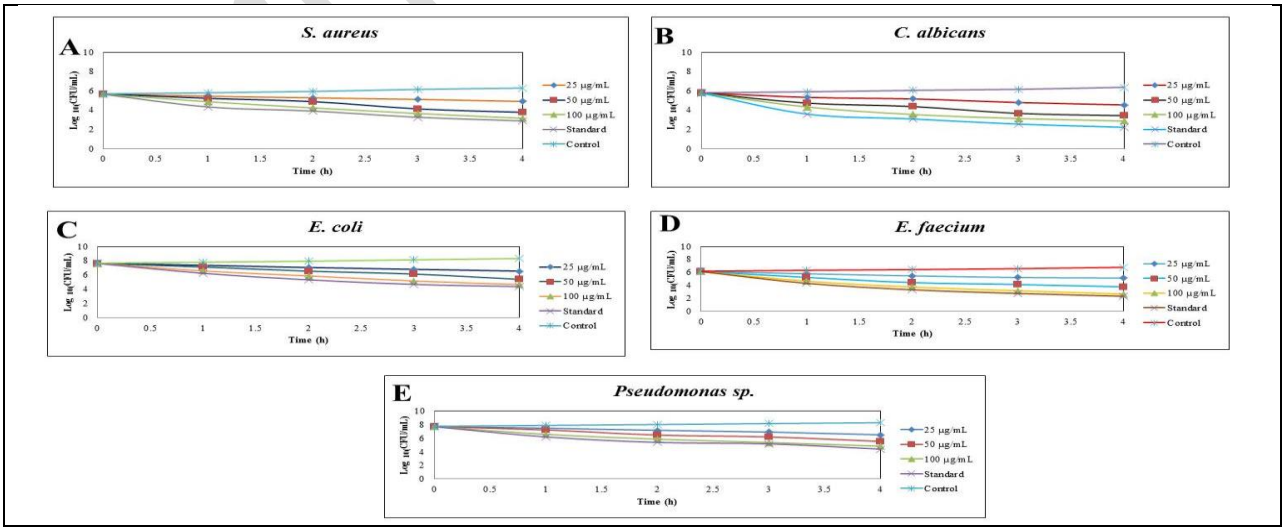

Fig. 10: Time-kill curve evaluation of microorganisms including (A) *S.aureus*, (B) *C. albicans*, (C) *E. coli*, (D) *E.facieum* and (E) *Pseudomonas sp*

The brine shrimp lethality assay (Fig 11) revealed that the cytotoxic activity of *Manilkara zapota* - mediated carbon nanoparticles showed 100% nauplii viability at all concentrations (5µg/mL to 80µg/mL) on both day 1 and day 2. Even at the higher concentrations (80µg/mL), no mortality was observed, indicating low acute toxicity towards *Artemia nauplii* under the experimental conditions. Similar findings have been reported, where low toxicity was observed in the brine shrimp lethality assay(Calangian *et al.* 2018). In a previous report, the brine shrimp lethality assay indicates that carbon dots synthesized from D-glucose showed negligible toxicity at concentrations up to 2000 rpm (Soledad-Flores and Bailón-Ruiz, 2026). No cytotoxic effects were observed for carbon nanoparticles derived from D-glucose and ethylenimine even at high concentrations of 2000 rpm (Soledad-Flores and Bailón-Ruiz,2026).

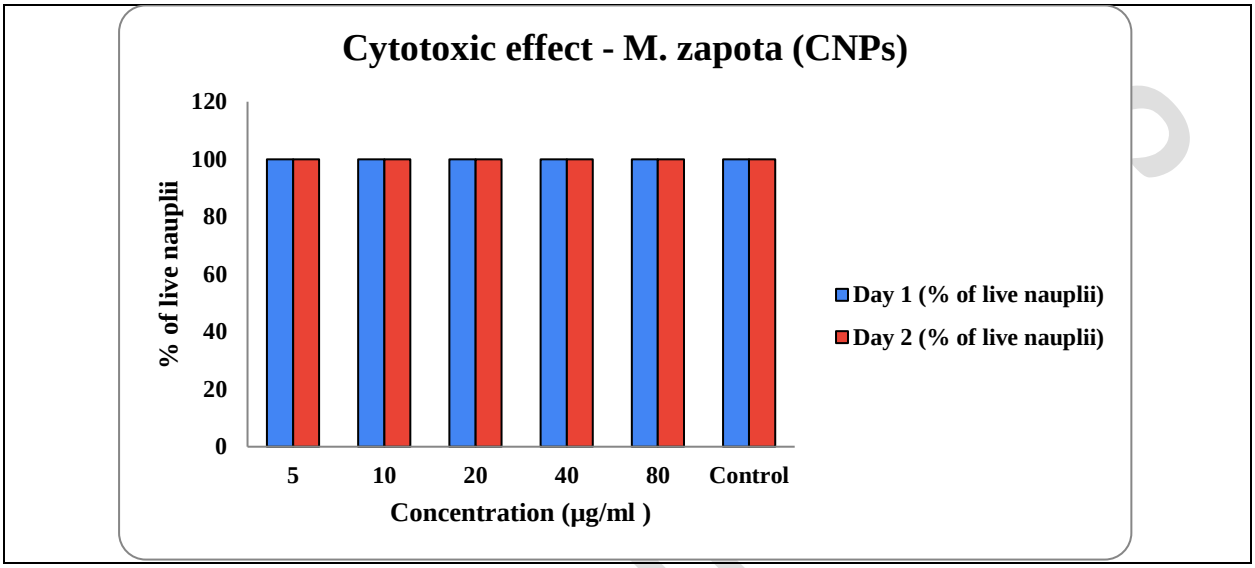

Fig. 11: Evaluation of cytotoxicity of carbon nanoparticle derived from *Manilkara zapota* using brine shrimp lethality assay

5. CONCLUSION

The present study reports the green synthesis of carbon nanoparticle (CNPs) using *Manilkara zapota* leaf extract showed significant antioxidant, anti-inflammatory and effective antibacterial activity against wound pathogenic microorganisms, and cytotoxicity studies highlights dose- dependent effects with minimal toxicity may contribute to the future therapeutic applications However, these findings are limited to the in vitro evaluation, further investigations, including minimum inhibitory concentration (MIC), minimum bactericidal concentration(MBC), mammalian cell studies, wound healing assays and in vivo studies are necessary to validate for future therapeutic applications and safety.

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AUTHOR'S CONTRIBUTION

Study framework and conceptual framework were formulated by Rajeshkumar Shanmugam. Data collection and documentation were done by Steffy Dominic. Data evaluation and validation of findings were collectively performed by Steffy Dominic, Perumal Elumalai, Rajeshkumar Shanmugam, and Nalini Jeyaprakash. The preliminary manuscript was written by Rajeshkumar Shanmugam and Steffy Dominic. All authors assessed the results and approved the finalized manuscript.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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