GMR
The Original

BIOGENIC SYNTHESIS OF SELENIUM CHITOSAN NANOCOMPOSITES THROUGH *MELIA DUBIA* EXTRACT AND BIOMEDICAL APPLICATIONS

Jonikha Sree Saravana Kumar[1], Dhanyaa Muthukumaran[2], Bhakkiya Rani R[3], Rajeshkumar Shanmugam[4*], Alagendran Subbarayalu[5], Arjun Pandian[6]

[1]Nanobiomedicine Lab, Department of Anatomy, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai - 602105, Tamil Nadu, India.
[2]Nanobiomedicine Lab, Department of Anatomy, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai - 602105, Tamil Nadu, India.
[3]Nanobiomedicine Lab, Department of Anatomy, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai - 602105, Tamil Nadu, India.
[4*]Nanobiomedicine Lab, Department of Anatomy, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai - 602105, Tamil Nadu, India, Email: rajeshkumars.sdc@saveetha.com
[5]Clinical Neuroscience and Phytomedicine Laboratory, Department of Research, Saveetha College of Nursing (SCON), Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Thandalam, Chennai – 602105, Tamil Nadu, India.
[6]Tissue Culture, Natural Products & Microbial Biotechnology Research Laboratory, Saveetha Institute of Basic Medical Sciences (SIBMS), Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Thandalam, Chennai 602 105, Tamil Nadu, India.

ABSTRACT
This research examines the eco-friendly synthesis of selenium nanoparticles (SeNPs) using Melia stem extract and their integration into chitosan-based nanocomposites (NCs) to combat bacteria and biofilms. The antioxidant activity was evaluated through various *in vitro* assays DPPH, H_2O_2, FRAP, ABTS, and nitric oxide all showed potent, dose-dependent free radical scavenging effects. The nanocomposites were characterized and evaluated to combat typical wound-causing microbes including *E. coli, S. aureus, Pseudomonas* sp., *E. faecalis* and *E. faecium*. The antimicrobial potential effective in tackling these common wound pathogens was evaluated through the well diffusion technique, time-dependent bactericidal analysis, and biofilm inhibition studies. The results revealed dose-dependent antimicrobial effects, with *E. faecium* and *E. faecalis* exhibiting the highest sensitivity at 100 µg/mL. Additionally, the SeNPs-chitosan NCs effectively inhibited biofilm formation, especially at 100 µg/mL. These findings suggest that *M. dubia*-mediated SeNCs could be sustainable, biocompatible alternatives to conventional antimicrobials for wound healing applications. Cytotoxic effect assessed with the brine shrimp lethality test indicated high survival rates, suggesting low toxicity. Incorporating the SeNPs into the chitosan nanocomposite improved their dispersion and biocompatibility. Zebrafish embryo tests demonstrated excellent safety at the highest concentrations, 80% in viability and hatching and no notable morphological abnormalities. *M. dubia*-derived SeNPs incorporated within chitosan nanocomposites present a sustainable platform with strong antioxidant properties and high biocompatibility, highlighting their potential for future use in drug delivery, tissue regeneration, and therapeutic applications, under SDG we targeted 3; Good Health Well-being, 14; Life on Land.

KEYWORDS: Antioxidant, Biocompatibility, Cytotoxicity, *Melia dubia*, SeNCs, Wound pathogens

1. INTRODUCTION

Nanotechnology, the study of nanoscale technology, is categorized within the broader scope of scientific advancement, where technology plays a vital role in driving innovation and interdisciplinary research [1]. Materials with properties distinctively different from their bulk and molecular counterparts are called nanoparticles [2]. Based on the specific purpose or intended location, nanoparticles can be introduced through ingestion, direct application to the affected area, skin-based administration, or circulation through the bloodstream using clinically approved techniques [3]. Biological synthesis of nanoparticles is by processes mediated by plants or microorganisms as an eco-friendly alternative to the dangerous physical and chemical synthesis methods, which are expensive, energy-intensive, and potentially [4].

SeNPs have a strong therapeutic effect; they improve ROS production dose-dependently [5]. SeNP scavenge free radicals efficiently *in vivo* and *in vitro* [6]. Free radicals are reactive molecules produced both through natural metabolic activities within the body and from exposure to environmental pollutants. They attack the healthy cells' DNA and cause them to deteriorate. Antioxidants are compounds that protect cells against the deteriorating effects of reactive oxygen species [7]. Numerous studies have shown that SeNPs exhibit remarkable antioxidant activities both *in vitro* and *in vivo* by improving the activation of selenoenzymes, which neutralize free radicals within the body [8]. The low toxicity and high biocompatibility are demonstrated by selenium nanoparticles (SeNPs), which have been gaining the scientific community's attention for their potential implementation as therapeutic and theragnostic agents [9]. Chitosan, a deacetylated derivative of chitin known for its natural antimicrobial properties. It can be sourced from crustacean shells (crabs, shrimp, and crayfish) through chemical or microbiological processes and produced by certain fungi [10]. Chitin is

recognized as nontoxic, biodegradable, edible, biocompatible, antioxidative, antimicrobial, anti-oncogenic, and thermally stable; it also possesses a nanofibrous and porous surface [11]. Chitosan is produced by extensively deacetylating chitin using strong alkaline conditions [12].

Melia dubia Cav. (Meliaceae) Also known as Malabar neem is a multipurpose tree found in tropical and native to subtropical regions. *M. dubia* is mainly grown for its therapeutic and industrial importance [13]. Its bioactive compounds have been reported to exhibit a range of activities, including insecticidal, antifeedant, antitumor, anti-inflammatory, antioxidant, antibacterial, and antifungal effects. The antioxidant properties and healing effects on skin wounds are attributed to the extract's high flavonoid content, which enhances nitric oxide radical scavenging and promotes faster epithelialisation during wound healing [14].

This study examines the biological activity of *M. dubia* stem extract combined with selenium nanoparticles embedded in a chitosan nanocomposite, focusing on their cytotoxic potential and effects on embryonic development.

2. MATERIALS AND METHODS

Preparation of *Melia dubia* stem extract:

The *M. dubia* stems were collected from the Nanoherbal garden at Saveetha Medical College. After that, the stem portions were carefully washed with running water to remove surface impurities, then chopped into smaller pieces and dried in the shade for about 3–4 days. After the shade-dried stem pieces of *M. dubia* were ready, they were pulverized into a fine powder. Later, 1 g of the *M. dubia* stem powder was ground well, and it was mixed with 100 mL of purified water. The mixture was warmed for roughly 15–20 minutes at 50°C, and then the boiled blend was passed through muslin cloth for filtration. The filtrate was collected and kept aside (Fig. 1 a – g)

Preparation of *M. dubia* – Se NPs:

For the synthesis of selenium nanoparticles, we prepared a 30 mM solution of sodium selenite, using 50 mL of distilled water. Then an equal volume of 50 mL of *M. dubia* stem extract was poured into it, more or less right away. The mixture was then set on a magnetic stirrer for Homogeneous dispersion. After that, the absorbance values were obtained using UV–Visible spectroscopic analysis. Once the synthesis was done, the selenium nanoparticle suspension was centrifuged at 8000 rpm for 10 minutes. After spinning, the nanoparticle pellet was gathered carefully [15].

Preparation of Chitosan Nanocomposite:

A mixture nanocomposite was formulated by dissolving 0.5 g of chitosan in 1 mL of 1% glacial acetic acid, followed by dilution with 49 mL of distilled water. The mixed solution was placed in the magnetic stirrer at 450 rpm for 24 hours to make a clear chitosan solution. 1 mL SeNPs synthesized using *M. dubia* were then added to this chitosan solution (4 mL), which was stirred continuously on a magnetic stirrer for 3–4 hours. Upon adding the SeNCs, a light brown nanocomposite formed. The mixture was again stirred on the magnetic stirrer for one day.

Antimicrobial activity:

Agar well diffusion method

From the previous research articles of the antibacterial potential was performed employing the agar well diffusion assay for selenium nanocomposites (SeNCs) produced from *M. dubia* was tested against the wound pathogens, including *E. faecium, E. coli, S. aureus, E. faecalis,* and *Pseudomonas* sp. [16, 17]

Antibiofilm assay:

From the previously mentioned methods, the antibiofilm assay was evaluated for the *M. dubia*–Se NCs, and it shows the effectiveness of biofilm eradication efficacy. The concentrations of SeNCs taken were 25, 50, and 100 µg/mL, and the antibiotic used is amoxicillin, which acts as the control. This method enabled the evaluation of the values of SeNCs required to effectively inhibit bacterial growth. By monitoring the impact over time, this method provided valuable insights into the antimicrobial effectiveness of SeNCs and their potential as alternative agents against wound pathogens [18-20]

Time kill kinetics assay

From the previously mentioned articles of [20,21], a time-kill curve assay was conducted to assess bactericidal activity and the concentration-dependent relationship between *M. dubia*-mediated selenium nanocomposite and the overall growth rate of *Pseudomonas* sp., *E. faecium, E. faecalis, E. coli,* and *S. aureus* over consistent time intervals. An antibiotic (such as amoxicillin) was used as a reference standard.

Antioxidant activity

DPPH radical scavenging assay

From the previously mentioned articles of [22], the antioxidant activity of *M. dubia*- SeNCs is analyzed using the DPPH assay (2,2-diphenyl-1-picrylhydrazyl). Various concentrations (10 - 50 µg/mL) of selenium nanocomposites mediated by *M. dubia* were utilized for the DPPH assay. Methanol served as the blank control.

Hydrogen peroxide radical scavenging assay

In previously published research articles by [26], the ability of the biosynthesized CS-SeNCs to scavenge H_2O_2 was evaluated. The H_2O_2 assay used ascorbic acid as the standard control. It was tested against different concentrations of CS-SeNCs, ranging from 10 to 50 µg/mL.

FRAP Assay
Based on previous research articles of [23-25], the FRAP (Ferric Reducing Antioxidant Power) assay was tested against various concentrations from 10 - 50 µg/mL of CS-SeNCs. Ascorbic acid served as the standard.

ABTS
From the previously mentioned articles of [26], the ABTS assay was tested for *M. dubia*-mediated SeNPs and chitosan nanocomposite at various concentrations of 10 - 50 µg/mL. Ascorbic acid served as the standard antioxidant.

Nitric oxide radical scavenging assay
From the previously mentioned articles of [26], the nitric oxide radical inhibition assay was tested for *M. dubia*-mediated SeNCs at various concentrations at 10 - 50 µg/mL, and ascorbic acid was used as the standard.

Cytotoxic effect:
Brine shrimp lethality assay
From the previously mentioned articles of [26], the brine shrimp lethality assay for *M. dubia*-mediated SeNCs was tested against concentrations at 5, 10, 20, 40, and 80 µg/mL.

Zebrafish embryonic toxicology evaluation of Se NCs:
From the previously mentioned articles of [26], the zebrafish toxicology of *M. dubia*-mediated SeNCs is tested for concentrations at 5, 10, 20, 40, and 80 µg/mL.

Zebrafish embryo evaluation
From the previously mentioned articles of [26], the zebrafish embryos were exposed to various SeNPs concentrations of 5, 10, 20, 40, and 80 µg/mL at 24 to 96 hours post-fertilization (hpf).

RESULTS AND DISCUSSION
Visual observation
The formation of selenium nanocomposites can be confirmed by the colour change from light orange to reddish rust orange (Fig. 1e). UV-visible spectra have shown a narrow peak at 355 nm (Figure 1h), supporting the formation of SeNPs. Initial confirmation of selenium nanoparticle formation was achieved through UV spectrophotometry.

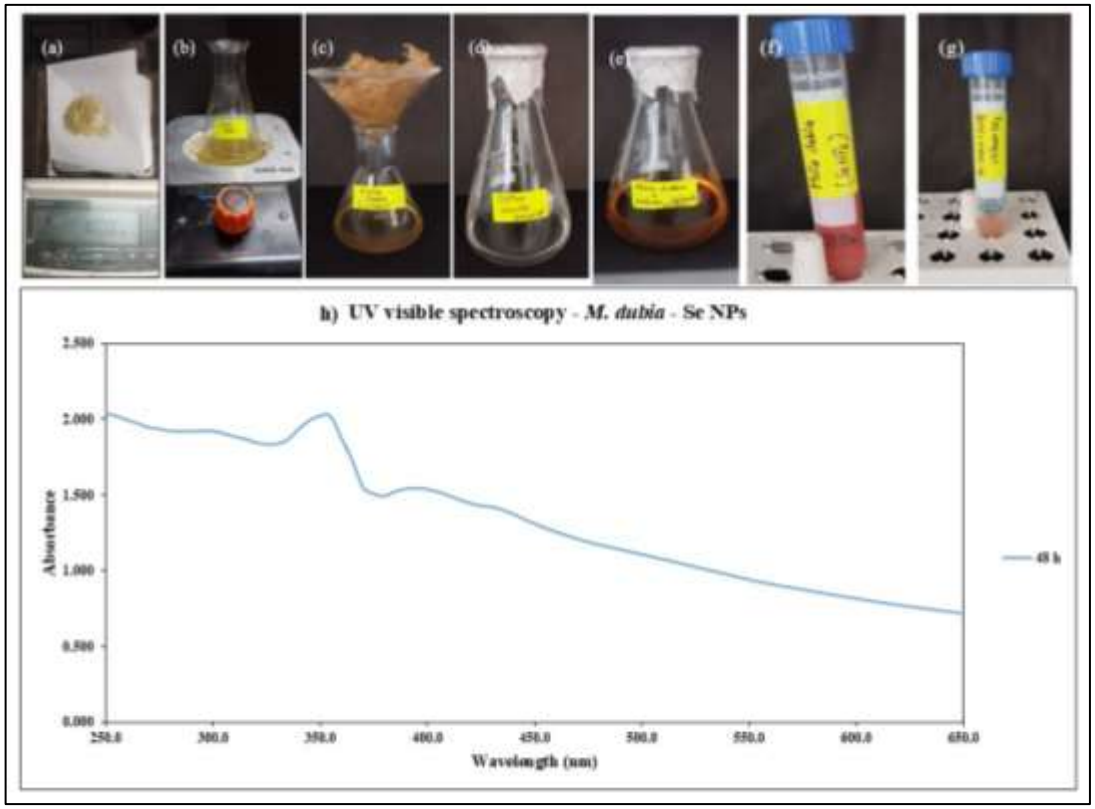

Figure 1: (a -g) Preparation of *M. dubia* stem – Se nanoparticles and chitosan nanocomposite h) Graphical representation of UV vis spectroscopy of *M. dubia* – Se NPs

Antimicrobial activity

The selenium nanocomposite synthesized from *M. dubia* exhibits a good zone of inhibition, as shown in Figure 2. The antibacterial activity was conducted against *Pseudomonas* sp., *E. coli, E. faecium, E. faecalis,* and *S. aureus*. The concentrations of SeNPs taken were 25, 50, and 100 µg/mL. The smallest zone of inhibition was observed with *E. coli* and *Pseudomonas* sp. in 100 µg/mL, which is 12 mm. The medium range of inhibition was observed with *E. faecalis* and *S. aureus*, which was 13 mm. The largest inhibition zone was produced by *E. faecium*, which is 14 mm, suggesting strong antimicrobial efficacy.

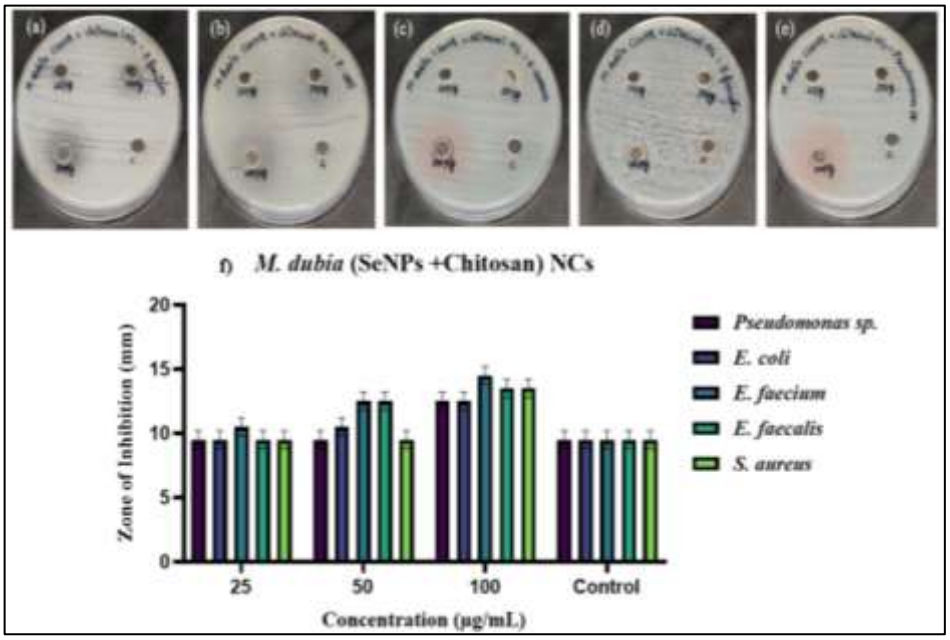

Figure 2: Graphical representation of agar well diffusion method using *M. dubia* – Se NCs against wound pathogens

Time kill kinetics curve assay

At 0 to 4 hours, the standard antimicrobial agent shows stronger activity than SeNCs at the tested concentrations. SeNCs from *M. dubia* demonstrate broad-spectrum antibacterial activity with varying effectiveness across different bacterial species. The bactericidal activity was evaluated using the time-kill curve assay of SeNCs. At 25 µg/mL, the concentration shows minimal effect against all bacteria. At a 100 µg/mL concentration of SeNC, there is a progressive reduction in the bacterial viability over time of the SeNCs. (Figure 3)

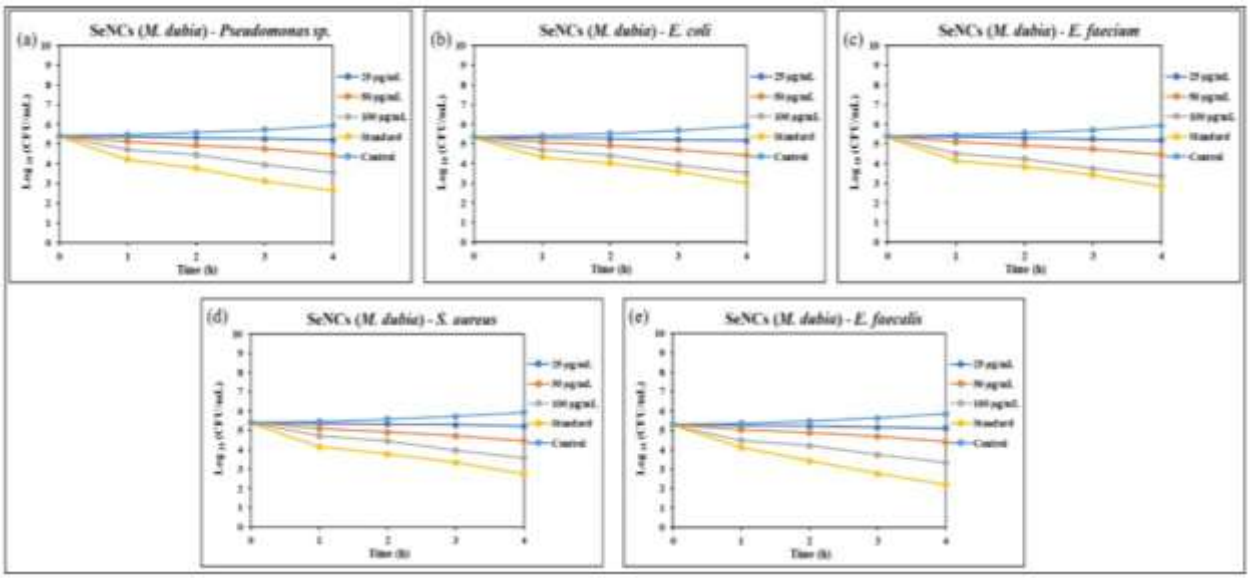

Figure 3: Graphical representation of time kills curve kinetics assay against wound pathogens a) *Pseudomonas* sp. b) *E. coli* c) *E. faecium* d) *S. aureus* e) *E. faecalis*

Antibiofilm activity

This graph demonstrates that SeNPs + Chitosan nanocomposites have antibiofilm activity against multiple bacterial species, including both Gram-positive (*S. aureus, E. faecalis, E. faecium*) and Gram-negative (*E. coli, Pseudomonas* sp.) bacteria, with relatively consistent efficacy across different concentrations. At a concentration of 100 µg/mL, it exhibits the lowest biofilm mass, indicating the highest antibacterial activity for most bacterial species tested. The control condition shows the highest biofilm mass, indicating the lowest antibacterial activity. (Figure 4).

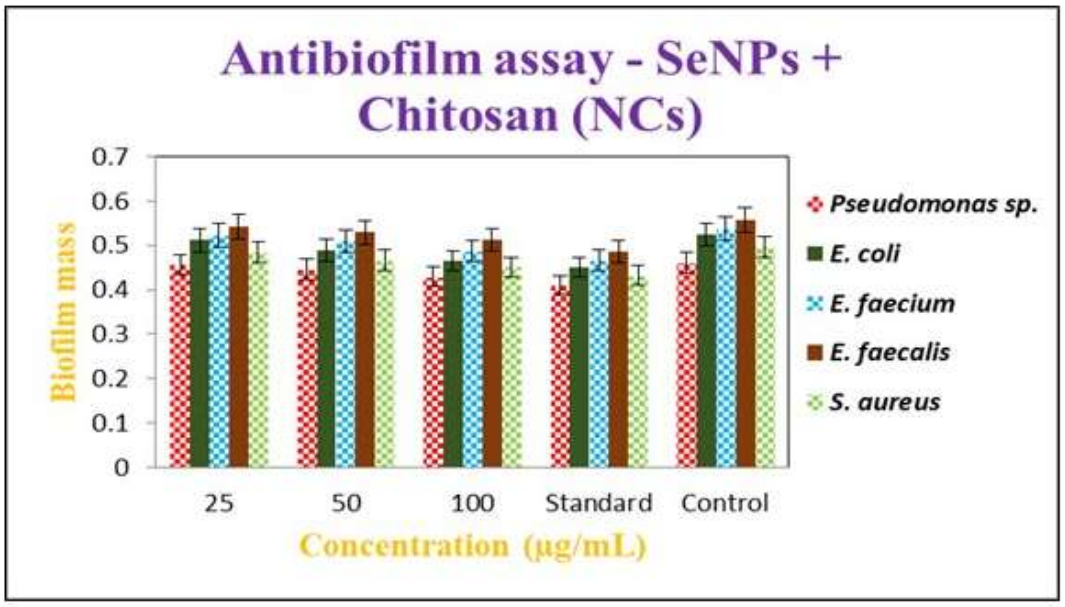

Figure 4: Graphical representation of Antibiofilm activity using *M. dubia* – Se NCs against wound pathogens

Antioxidant activity

DPPH assay

The antioxidant activity of SeNCs, synthesized using *M. dubia*, was assessed via the DPPH assay. This showed a clear dose-dependent kind of increase in antioxidant effectiveness. The SeNCs showed activity, reaching a highest inhibition of 85% at 50 µg/mL, compared with about 38% inhibition when only 10 µg/mL was used. When compared with the usual antioxidant ascorbic acid, which gave 94% inhibition (Figure 3a), the formulation still suggested a strong antioxidant potential (Figure 5 a).

H_2O_2 assay

The results seemed to show a slow but steady rise in scavenging action as the concentration went up, and it hit a notable 81% at 50 µg/mL, while 10 µg/mL was only 44%. For context, ascorbic acid (as the control) gave a scavenging rate of 88.97% at 50 µg/mL (Figure 3b). So overall, these findings indicate the antioxidant ability of the formulation mediated SeNPs8.(Figure 5 b)

FRAP assay

The SeNCs seemed to show a clear performance upgrade, and it peaked at about 81% when the dosage was 50 µL, while it began around 51% at 10 µL. This formulation also displayed strong reduction behaviour from the testing; basically, SeNCs gave better outcomes than ascorbic acid, which is the usual antioxidant benchmark, and that one stayed at 85% of its capacity (Figure 5 c).

ABTS assay

The ABTS assay looks at how antioxidants can stop the ABTS radical cation from remaining, and the SeNCs scientists made from M. dubia showed a sort of full effectiveness in that test. The SeNCs had a noticeable boost in performance, because they got their top stopping strength of 80% when researchers added 50 µg/mL, yet at 10 µg/mL they only showed 51% effectiveness. The SeNCs present strong free radical scavenging capability too, since the assay outcomes were better than ascorbic acid, which reached 87% inhibition (Figure 5 d).

Nitric oxide assay

For the nitric oxide assay, it is used to gauge how well antioxidant compounds can remove and stop nitric oxide (NO) radicals. In the test, the SeNCs seemed to perform better, they showed 79% inhibition at 50 - 10 µg/mL they were lower with 53% inhibition. Overall, the SeNCs show solid free radical scavenging ability, because they reached 85% inhibition, and this value goes past ascorbic acid, which is the reference antioxidant (Figure 5 e).

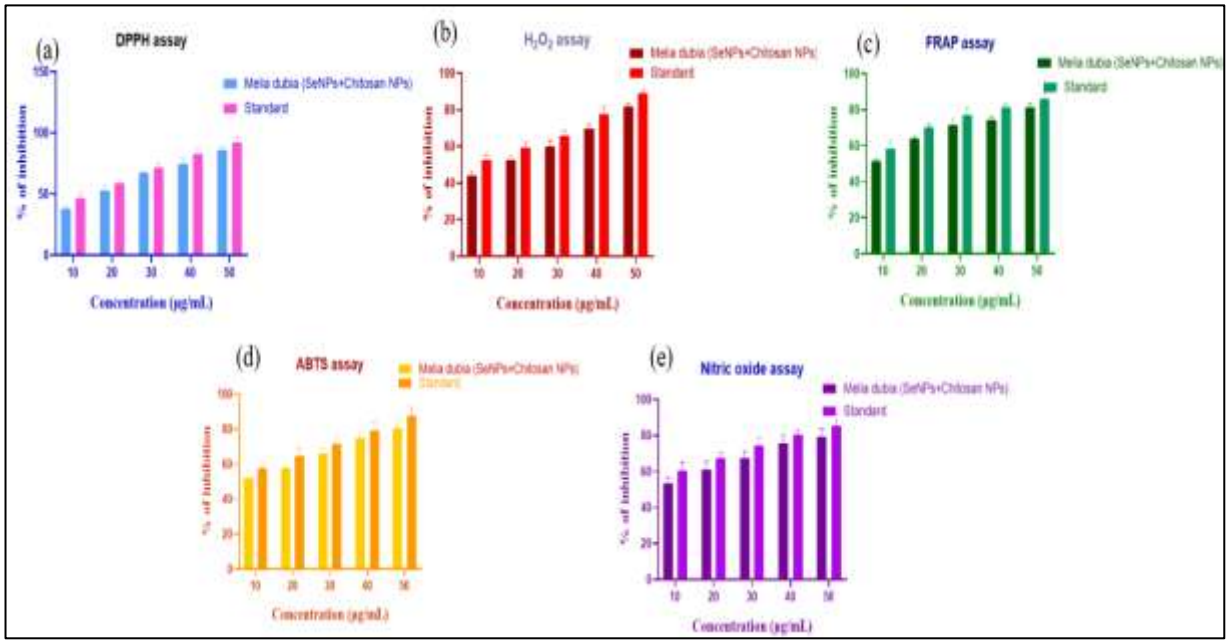

Figure 5: Graphical representation of antioxidant activity of *M. dubia*-SeNCs a) DPPH, b) H2O2 assay C) FRAP assay D) ABTS assay E) NO assay

Cytotoxic effect

Brine shrimp lethality assay

M. dubia - SeNCs show low toxic effects when tested with chitosan nanocomposite, because the chitosan nanocomposite shows high survival rates. In general, the brine shrimp have 100% survival at all concentrations on day 1, but by day 2, there is slight mortality at 80 µg/mL, so you get 90% live nauplii. Overall, the work seems to validate that *M. dubia* SeNPs combined with Chitosan NCs provide high biocompatibility, which is an essential requirement for their use in biomedical applications (Figure 6).

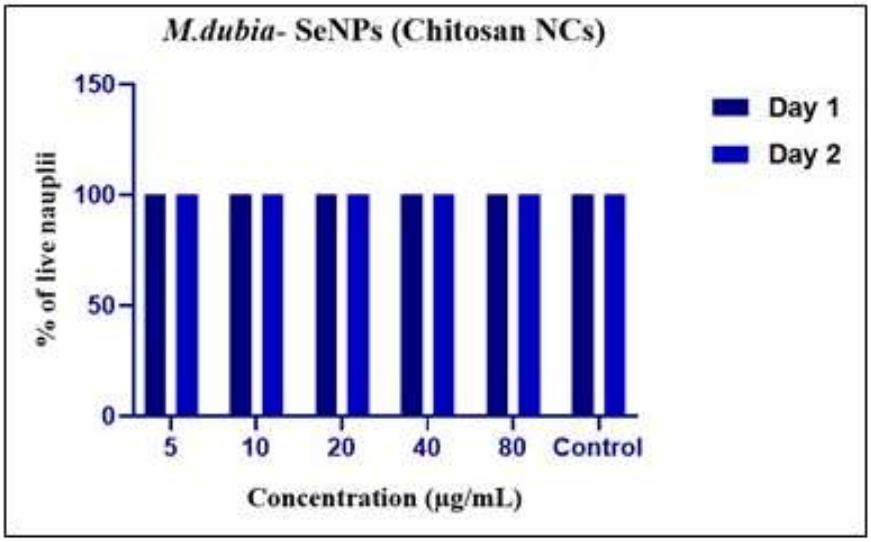

Figure 6 : Graphical representation of cytotoxic effect of brine shrimp lethality assay using *M. dubia* Se NCs

7.2 Zebrafish Embryo Toxic Effect

Viability rate:

Zebrafish eggs experience decreasing survival rates when researchers increase SeNPs. The tested concentrations of 5, 10, 20 and 40 µg/mL demonstrate that the synthesized nanoparticles show excellent biocompatibility because their viability remains almost at 100%. The 80 µg/mL concentration shows a minor decrease in cell viability, which drops to about 80%, while the control group maintains almost complete viability (Figure 7 d).

Hatching rate:

The hatching rate of zebrafish eggs diminishes as the concentration of SeNPs increases. At concentrations of 5, 10, 20, and 40 µg/mL, the hatching success remains nearly 100%, indicating no adverse effect on embryo development at these doses. However, at 80 µg/mL, the hatching rate slightly drops to around 80%, suggesting a slight inhibitory effect on hatching at higher concentrations, while the control group continues to exhibit a viability rate close to 100%. The SeNPs (Chitosan NCs) appear non-toxic at lower concentrations but may have some mild effect at higher doses (80 µg/mL) (Figure 7 e).

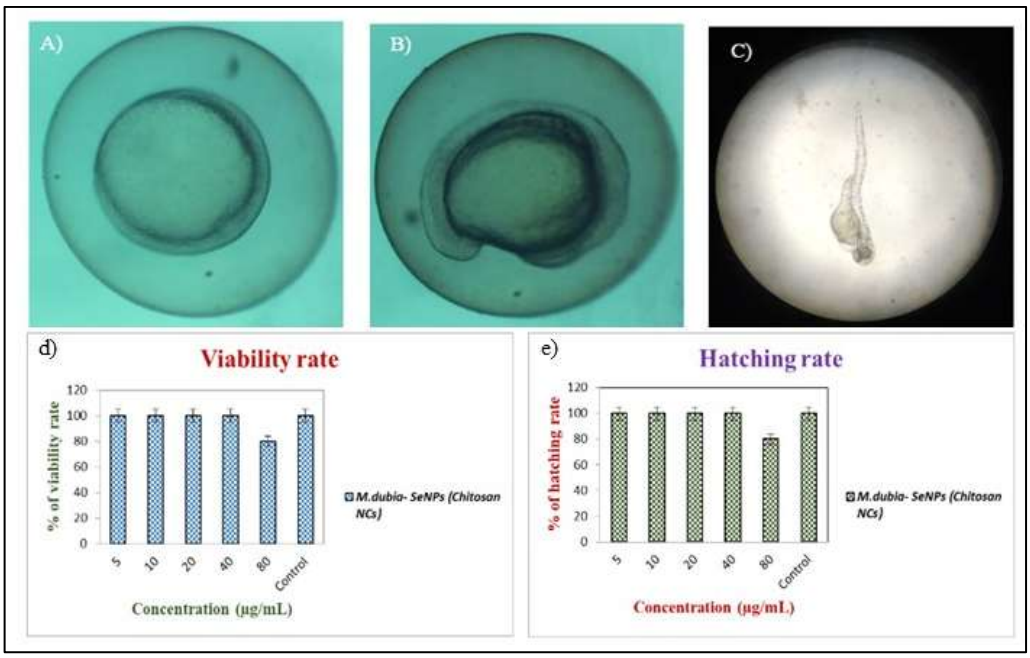

Figure 7 (a – c): day 1, day 2, day 3 images of zebrafish embryos, d) e) viability and hatching rate of zebrafish embryonic toxic effect using M. dubia Se NCs

Untreated embryos exhibited a 100% hatching rate. At 20 and 40 µg/mL of the extract, embryos exhibited a 100% hatching rate, with no significant difference between the two concentrations. Additionally, at lower concentrations such as 5 and 10 µg/mL, there were no notable malfunctions up to 72 hours post-fertilization (hpf). Notably, zebrafish embryos exposed to selenium nanoparticles synthesized using *M. dubia* showed no observable abnormalities.

DISCUSSION

The research confirms the eco-friendly synthesis of SeNPs using *M. dubia* stem extract with a UV-Vis absorption peak at 355 nm. The *Aloe vera* leaf extract mediated Se NPs was synthesized showed UV vis absorption at 350 nm, which is similar to M. dubia -Se NPs [27].

These SeNPs showed significant antibacterial and biofilm-forming resistance when incorporated into nanocomposites with chitosan. The antibacterial activity was measured using the agar well diffusion assay, in which *E. faecium* was the most sensitive [28]. The time-kill assays demonstrated a concentration-dependent bactericidal effect, with the most susceptible being *E. faecalis* and the most resistant *Pseudomonas* sp. [29-33] The SeNPs - chitosan nanocomposite achieved complete biofilm inhibition when tested at a concentration of 100 µg/mL. The SeNCs demonstrated exceptional antioxidant properties through DPPH, ABTS, FRAP, H_2O_2, and nitric oxide scavenging tests, which showed their ability to neutralise free radicals [34-35]. The DPPH test results showed that Aloe vera and Se NPs produced 67% and 57% antioxidant activity, respectively. The ABTS assay showed that *Aloe vera* and Se NPs exhibited 73% and 54% antioxidant activity, respectively [36]. The FRAP assay of aloe vera and Se NPs showed similar inhibition. The research demonstrates *M. dubia* -Se NPs produce similar antioxidant effects [37]. The SeNCs confirmed excellent antioxidant performance across multiple tests while showing minimal harmful effects to living organisms, according to their high survival rates during brine shrimp and zebrafish embryo testing. The toxicological testing showed that the nanocomposites remain safe when used at lower concentrations because only slight developmental toxicity occurred at the maximum tested concentration of 80 µg/mL [38-40]. The brine shrimp assays showed that the tested substances had minimal toxic effects. *A. linearis* – Se NPs exhibited 70% viability at the highest concentration. Zebrafish embryotoxicity testing showed that lower concentrations produced high viability and hatching rates, which demonstrated no developmental toxicity in the study [41,42].

CONCLUSION

The research establishes an eco-friendly method for selenium nanoparticle production through the use of *M. dubia* stem extract to create chitosan nanocomposites. The SeNCs confirmed powerful antimicrobial properties against wound pathogens, together with protective effects against oxidative stress in multiple tests, while showing minimal harmful effects on cells. The *M. dubia*-derived SeNCs exhibit both biological activity and biocompatibility, which makes them

suitable for use in biomedical applications that require antioxidant protection. The therapeutic potential of these substances should be studied through *in vivo* experiments in upcoming research, under SDG we targeted 3; Good Health Well-being, 14; Life on Land.

Acknowledgement
We would like to thank Saveetha Institute of Medical and Technical Sciences, Chennai, for the support and encouragement.

Conflicts of interest
The authors declare no conflicts of interest.

Funding source
The authors received no funding support for this research work.

REFERENCES
1. Nasrollahzadeh, M., Sajadi, S. M., Sajjadi, M., & Issaabadi, Z. (2019). An introduction to nanotechnology. In Interface science and technology (Vol. 28, pp. 1-27). Elsevier.
2. Biswas P, Wu CY. Nanoparticles and the environment. Journal of the air & waste management association. 2005 Jun 1;55(6):708-46.
3. Anselmo AC, Mitragotri S. Nanoparticles in the clinic. Bioengineering & translational medicine. 2016 Mar;1(1):10-29.
4. Joudeh, N., Linke, D. Nanoparticle classification, physicochemical properties, characterization, and applications: a comprehensive review for biologists. J Nanobiotechnol 20, 262 (2022). https://doi.org/10.1186/s12951-022-01477-8.
5. Venugopal S. Therapeutic potential of selenium nanoparticles. Frontiers in Nanotechnology. 2022 Oct 24;4:1042338.
6. Shoeibi, S., Mozdziak, P. & Golkar-Narenji, A. Biogenesis of Selenium Nanoparticles Using Green Chemistry. Top Curr Chem (Z) 375, 88 (2017). https://doi.org/10.1007/s41061-017-0176-x.
7. Wang YY, Qiu WY, Sun L, Ding ZC, Yan JK. Preparation, characterization, and antioxidant capacities of selenium nanoparticles stabilized using polysaccharide–protein complexes from *Corbicula fluminea*. Food bioscience. 2018 Dec 1;26:177-84.
8. Chen N, Yao P, Zhang W, Zhang Y, Xin N, Wei H, Zhang T, Zhao C. Selenium nanoparticles: Enhanced nutrition and beyond. Critical reviews in food science and nutrition. 2023 Dec 20;63(33):12360-71.
9. Ferro C, Florindo HF, Santos HA. Selenium nanoparticles for biomedical applications: From development and characterization to therapeutics. Advanced healthcare materials. 2021 Aug;10(16):2100598.
10. Hasan S, Boddu VM, Viswanath DS, Ghosh TK. Chitin and Chitosan. Science and Engineering; Springer Nature Switzerland: Cham, Switzerland. 2022.
11. Kaya M, Mujtaba M, Ehrlich H, Salaberria AM, Baran T, Amemiya CT, Galli R, Akyuz L, Sargin I, Labidi J. On chemistry of γ-chitin. Carbohydrate polymers. 2017 Nov 15;176:177-86.
12. Muthu M, Gopal J, Chun S, Devadoss AJ, Hasan N, Sivanesan I. Crustacean waste-derived chitosan: Antioxidant properties and future perspective. Antioxidants. 2021 Feb 3;10(2):228.
13. Shah SN, Wani TA, Ram B, Koul M, Awasthi P, Rajput DS, Reddy GR. An efficient protocol for in vitro organogenesis and antioxidant studies in *Melia dubia* Cav. African Journal of Biotechnology. 2016 Jun 6;15(19):768-75.
14. Trung HT, Purnomo KA, Yu SY, Yang ZJ, Hu HC, Hwang TL, Tuan NN, Tu LN, Duc DX, Quang LD, Backlund A. Anti-inflammatory and Antiphytopathogenic Fungal Activity of 2, 3-seco-Tirucallane Triterpenoids Meliadubins A and B from *Melia dubia* Cav. Barks with ChemGPS-NP and *In Silico* Prediction. ACS omega. 2023,8(40):37116-27.
15. Azhagu Madhavan S., Arjun P., Antihyperglycemic activity of polyphenolic metabolites and biosynthesized silver nanoparticles from Pedalium murex: Characterization and application of antioxidant and uropathogenic antimicrobial activities. Microbial Pathogenesis. 2025. 205: 107620.
16. Azhagu Madhavan S., Arjun P. Identification and characterization of silver nanoparticles from *Erythrina indica* and its antioxidant and Uropathogenic antimicrobial properties. Microbial Pathogenesis, 2024. 190: 106635.
17. Pradeep Manigandan, Akshay J Kumar, Aleyamma punnose Kovoor, Rajeshkumar Shanmugam. Antioxidant Activity of Honey-Derived Carbon Nanoparticles Incorporated Herbal Formulation Mediated Silver Nanocomposite against Wound Pathogens. Nanotechnology Perceptions, 20, S8(2024)608–617.
18. Manigandan P, Shanmugam R. Green preparation of fluorescence carbon nanoparticles using honey and its biomedical applications- An *in vitro* study. Journal of Drug Delivery Science and Technology. 2025 Apr 14:106919.
19. Dinesh G, Lakshmi Thangavelu, S Rajeshkumar, Pradeep Manigandan Green Synthesis of Silver Nanoparticles Using Coriandrum sativum Extract and Their Antioxidant, Anti-inflammatory and Antibacterial Activities Against UTI Pathogens Texila International Journal of Public Health, Special Issue-2024 DOI: 10.21522/TIJPH.2013.SE.24.05.Art037.
20. Rajeshkumar, S., Malarkodi, C., Roy, A., Munusamy, T., Kumar, A., Setyawan, H.Y., Sharma, K. and Verma, R., 2024. Bacterial assisted preparation of cadmium sulfide/polyvinyl alcohol nanocomposites and its biological applications. Environmental Nanotechnology, Monitoring & Management, p.101030.
21. Aleyamma punnose Kovoor, Arjun Krishnan G, Pradeep Manigandan, Rajeshkumar Shanmugam, Lakshmi Thangavelu. Green Synthesis of Silver Nanoparticles from *Aegle Marmelos* and *Cissus quadrangularis* Leaf Extract and Evaluation of Its AntiInflammatory and Antioxidant Effect. Nanotechnology Perceptions 20 No. S7 (2024) 276–289.

22. Dathan PC, Nallaswamy D, Rajeshkumar S, Joseph S, Ismail S, Rashid N. Evaluation of Anti-inflammatory, Antioxidant and Antimicrobial Activity of Pomegranate Peel Extract: An In-vitro Study. Journal of Clinical & Diagnostic Research. 2024, 1;18(6): ZC01-ZC08. DOI: 10.7860/JCDR/2024/69878.19463.
23. Joseph S, Nallaswamy D, Rajeshkumar S, Dathan P, Rasheed N, Jose L, Jacob J, Munuswamy T. Exploring the Impact of Green Tea Extract-Enhanced TiO2, ZnO Nanocomposite: Insights into Cytotoxicity via MTT Assay, Cell Morphology, and Zebrafish Embryonic Study. Trends in Biomaterials and Artificial Organs. 2024;38(3):147-52.
24. Dhave Prasath V M, Prabhakaran A K, Rajeshkumar Shanmugam, Dhanyaa Muthukumaran. Evaluation of Zebrafish Embryonic Toxicology using *Mimosa Pudica*-Assisted Selenium Nanoparticles. Nanotechnology Perceptions 20 No. S7 (2024) 484–489.
25. Sasarom M, Wanachantararak P, Chaijareenont P, Okonogi S. Biosynthesis of copper oxide nanoparticles using *Caesalpinia sappan* extract: *In vitro* evaluation of antifungal and antibiofilm activities against *Candida albicans*. Drug Discoveries & Therapeutics. 2023 Aug 31;17(4):238-47.
26. Munusamy T, Shanmugam R. Green synthesis of copper oxide nanoparticles synthesized by *Terminalia chebula* dried fruit extract: characterization and antibacterial action. Cureus. 2023 Dec 7;15(12).
27. Vyas J, Rana SH. Antioxidant activity and biogenic synthesis of selenium nanoparticles using the leaf extract of Aloe vera. Int. J. Curr. Pharm. Res. 2017;9(147):10-22159.
28. Elango R, Gunasekaran V, Sathishkumar P. Eradication of ampicillin-resistant *Enterococcus faecalis* in root canal infections using tannic acid: A tooth model investigation. Archives of Oral Biology. 2026 May 27:106638.
29. Thirumurugan, A., Padmanaban, M., Kumar, D. S., Govindharaju, R., Padmavathy, S., Karthikeyan, V., ... & Karthik, S. (2026). Phytochemical and antioxidant properties of Kedrostis foetidissima and its larvicidal potential against mosquitoes. South African Journal of Botany.
30. Ayyadurai, T., Mohan, P. K., Annamalai, A., Kamaraj, C., & Waheeb, M. Q. (2026). Biogenic silver nanoparticles synthesis from Guilandina bonduc L. seed kernel extract with antibacterial, antioxidant and anticancer potential against ovarian Cancer cells. Results in Chemistry, 103246.
31. Jancyhelan, J., Thirumurugan, A., Alagumanian, S., & Vignesh, K. (2026). Eco-friendly biosynthesis of silver nanoparticles using marine algal extract and their biomedical applications. Results in Chemistry, 103606.
32. Anbazhagan, V., Natrajan, A., Arul, J. et al. Strategic development of a buffalo farming framework and optimization of the foraging behaviour using mathematical models. Trop Anim Health Prod 58, 424 (2026). https://doi.org/10.1007/s11250-026-05211-6
33. Ayyadurai, T., Ravi, S., & Annamalai, A. (2026). Steroidogenic Enzyme Dysregulation in Polycystic Ovary Syndrome: Mechanistic Insights and Emerging Therapeutic Strategies. The Journal of Steroid Biochemistry and Molecular Biology, 107088.
34. Manobharathi G, Raghu S, Krishnan A. Antimicrobial efficacy of modified *gutta-percha* for obturation–A systematic review. Journal of Conservative Dentistry and Endodontics. 2025 Jan 1;28(1):2-9.
35. Sathishkumar P, Khan F. Leveraging bacteria-inspired nanomaterials for targeted controlling biofilm and virulence properties of *Pseudomonas aeruginosa*. Microbial Pathogenesis. 2024 Dec 1;197:107103.
36. Dhabian SZ, Jasim RS. Antioxidant, cytotoxic, and antihemolytic activity of greenly synthesized selenium nanoparticles using *Elettaria cardamomum* extract. J. Nanostruct. 2023;13:76-85.
37. Giriprasad M, Mariraj I, Rajeshkumar S, Pradeep M, Santhoshkumar J. Evaluation of antidiabetic and anti-inflammatory action of selenium nanoparticles mediated through aspalathus linearis-An *in vitro* study. The Medical journal of Malaysia. 2025 Jan;80(Suppl 1):29-36.
38. Ayyadurai, T., Moola, A.K., Mohan, P.K., Thiruppathi, S.K., Shanmugam, A. and Bollipo Diana, R.K., 2022. Ameliorative effects of Guilandina bonduc L. aqueous seed extract on letrozole induced polycystic ovary syndrome in female wistar albino rats. Advances in Traditional Medicine, 22(4), pp.885-903.
39. Jeyaram, M., Jeyaram, Y., Ayyadurai, T., Gurusamy, M. (2026). Exploration of Streptomycetes as Biocontrol Agents Against Plant Pathogens. In: Kaur, T., Kumar, H., Manhas, R.K. (eds) Streptomycetes: Biological Candidates for Sustainable Agriculture. Springer, Singapore. https://doi.org/10.1007/978-981-95-5803-2_6
40. Raman S, Kasirajan S, Chinnapandi B, Karthikeyan K, Pandian A, Chandrasekaran K, Al-Ansari MM, Sugumar V, Srinivasan P. Luminescent Biogenic Selenium Nanoparticles From *Indigofera aspalathoides* Vahl ex DC: A Novel Hepatoprotective Strategy for Enhancing Liver Health. Luminescence. 2025 Feb;40(2):e70101.
41. Baskar G, Palaniyandi T, Al-Mutiry M, Alshammari N, Saeed M. Biocatalytic Green Synthesis of Iron Oxide Nanoparticles Using Red Seaweed *Portieria hornemannii* for Biomedical Applications. Biocatalysis and Agricultural Biotechnology. 2025 Oct 24:103831.
42. Ayyanan, R., Hariharan, M., Balaji, M., Vimal, S. 2025. Enhanced Antidiabetic and Antioxidant Potential of Green-Synthesized Selenium Nanoparticles using *Momordica cymbalaria*. TPM–Testing, Psychometrics, Methodology in Applied Psychology, 32(S2 (2025), 204-211.