Anti-Cancer Effects Of Avocado Seed Extract Bio-Assisted Selenium Nanoparticles In Breast Cancer Cells – An *In Vitro* Study

Rupa Devi R[1], Aswin Sundar R[2], GeethaRV[1], ParameswariRoyapuram Parthasarathy[3*]
[1]Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai - 600 077, Tamilnadu, India.
[2]Department of Orthopaedics, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Thandalam, Chennai, Tamil Nadu, India.
[3]Centre for Global Health, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Thandalam, Chennai, Tamil Nadu, India.
***Corresponding Author**
parameswarirp.smc@saveetha.com

Cite this paper as: Rupa Devi R, Aswin Sundar R, GeethaRV, ParameswariRoyapuram Parthasarathy (2024) Anti-Cancer Effects Of Avocado Seed Extract Bio-Assisted Selenium Nanoparticles In Breast Cancer Cells – An In Vitro Study. *Frontiers in Health Informatics, 13* (3), 8400-8406

ABSTRACT

Selenium nanoparticles were reported for its good biocompatibility and low toxicity. Avocado seed triggers the immune system, reduces premature aging of the skin and prevents cancer. Avocado seeds have shown to possess bactericidal and fungicidal properties. It means that they are able to neutralize different harmful microorganisms. Therefore, in the present study we investigated the effect of avocado seed mediated selenium nanoparticles on MCF-7 breast cancer cells. Cytotoxic effect of the avocado seed selenium nanoparticle was assessed by MTT assay at varying concentrations of 25-150µg/ml. Morphological changes were observed using the Phase contrast microscope. The apoptotic effect of selenium nanoparticles synthesized from avocado seed extract was evaluated by ethidium bromide/acridine orange dual staining assay. The results demonstrated that the selenium nanoparticles were found to be cytotoxic towards MCF-7 breast cancer cells with a maximum inhibition observed at 150µg/ml. IC_{50} was found to be 75µg/ml. Morphological changes were observed in selenium nanoparticles treated cells when compared to untreated control. Further, the dual staining assay showed induction of apoptosis exhibited as yellowish green fluorescent cells in comparison to the control group that appeared green fluorescence. Therefore, it is concluded that selenium nanoparticles synthesized using avocado seed extract have significant anti proliferative and apoptotic effect against MCF-7 breast cancer cells.

Keywords: *selenium nanoparticles, avocado seed, green synthesis, anti-cancer activity*

INTRODUCTION

Selenium (Se) is an essential trace element, primarily acquired through the diet in the form of selenium-containing amino acids [1]. Active selenium compounds exist in various forms, including selenoproteins, selenium salts, and nanoparticles, with their chemical structure playing a vital role in determining their beneficial effects [2].Selenoproteins play a crucial role in human health, primarily due to their antioxidant properties, and are linked to various beneficial effects such as anti-inflammatory and antiviral activities.Selenium has emerged as a promising agent in cancer prevention and treatment [3]. Clinical trials have highlighted its anticancer properties, with the most significant benefits of Se supplementation observed in colorectal, lung, and prostate cancers [4].However, it has a narrow margin of safety, with toxic effects occurring at intake levels exceeding the recommended daily range of 30-90µg [1].To mitigate the toxicity associated with soluble selenium, selenium nanoparticles (Se-NPs) have been developed and assessed for their anticancer potential [5]. Both free and encapsulated Se-NPs have demonstrated efficacy in reducing cancer cell proliferation *in vitro* [6 - 8]. Moreover, Se-NPs have shown promising results *in vivo*, exhibiting effectiveness and good tolerance, allowing Se to be utilized at doses that would otherwise be toxic in its soluble form [9].

Breast cancer is the most frequently diagnosed cancer among women globally. In 2020, approximately 2.3 million new cases were reported, with the disease causing 685,000 deaths, representing one in six cancer-related fatalities [10].Genetic mutations, particularly in the BRCA1 and BRCA2 genes, play a crucial role in increasing disease susceptibility, while molecular pathways like PI3K/AKT/mTOR are key drivers of tumorigenesis.Therapeutic strategies such as radiotherapy,chemotherapy, surgery, targeted therapyand endocrine therapy are well-established and widely used in breast cancer treatment [11]. However, the adverse effects associated with these conventional approaches have hindered their broader clinical application [12]. Therefore, it is essential to develop safer and more effective individualized treatment strategies for breast cancer.The use of nanoparticles in breast cancer therapy has gained substantial attention due to their potential to enhance drug delivery and improve therapeutic outcomes [13].

Nanoparticles can be synthesized in various method including chemical, physical and green synthesis methods. Nevertheless, the green synthesis method of nanoparticles prepared using different parts of the plants, fruits and microbes has obtained significance as most of the physical and chemical methods of nanoparticles synthesis were found to be toxic and cost – expensive [14].Therefore, the primary objective of this study is to swiftly synthesize selenium nanoparticles (SeNPs) using aqueous extracts from avocado seeds and evaluate their potential efficacy against human breast cancer cells (MCF-7).

MATERIALS AND METHODS

Preparation of Avocado seed extract

The avocado seeds were thoroughly rinsed with deionized water to remove any impurities and then dried at 50°C to eliminate moisture. Once dried, the seeds were finely ground using a blender to produce a powder. Five grams of this powder were then mixed with 500 milliliters of deionized water, forming a brown extract solution. The mixture was heated at 60°C for 30 minutes with continuous stirring to ensure proper extraction.

Synthesis of avocado seed extract mediated selenium nanoparticles

In a typical preparation, 5 mL of avocado seed extract is first diluted with 45 mL of double-distilled water (DDW), followed by the addition of 20 mL of a 40 mM selenous acid solution. The mixture is then stirred at 37 °C for 24 hours, allowing a visible color change to occur. After the reaction, the product is purified by repeated centrifugation at 10,000 rpm for 10 minutes, with several washes using DDW to eliminate any impurities. Finally, the resulting pellet is freeze-dried for two days before being used for further analysis.

Cell line study

Reagents

DMEM (Dulbecco's Modified Eagle Medium), Phosphate Buffered Saline (PBS), Trypsin-EDTA, Fetal bovine serum (FBS), were purchased from Gibco, Canada. Acridine orange (AO), ethidium bromide (EtBr), Dimethyl sulfoxide (DMSO), [3-(4,5-dimethythiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT), DAPI, AO/EtBr were purchased from Sigma Chemical Pvt Ltd, USA. All other chemicals used were extra pure of molecular grade and were purchased from SRL, India.

Cell line maintenance

Estrogen dependent (MCF-7) breast cancer cell lines were obtained from the NCCS, Pune. The cells were grown in T25 culture flasks containing DMEM supplemented with 10% FBS and 1% antibiotics. Cells were maintained at 37◦C in a humidified atmosphere containing 5% CO2. Upon reaching confluency, the cells were trypsinized and passaged.

Cell viability (MTT) assay

The effect of the avocado seed mediated Se-NPs was assessed by MTT assay. The assay is based on the reduction of soluble yellow tetrazolium salt to insoluble purple formazan crystals by metabolically active cells.

MCF-7 cells were plated in 96 well plates at a concentration of 5x104 cells/well. 24h after plating, cells were washed twice with 100µl of serum-free medium and starved by incubating the cells in serum-free medium for 3 hours at 37ºC. After starvation, the cells were treated with different concentrations of the synthesized nanoparticles for 24h. At the end of 20h, the medium from the control and treatment group was discarded and 100µl of MTT containing DMEM (0.5mg/ml) was added to each well. After 4h of incubation at 37ºC in the CO2 incubator, the MTT containing medium was discarded and the cells were washed with 1x PBS. The formazan crystals formed were dissolved in dimethyl sulfoxide (100µl) and the intensity of the color developed was measured using a Micro ELISA plate reader at 570 nm. The number of viable cells was expressed as the percentage of control cells cultured in serum-free medium. Cell viability in the control medium without any treatment was represented as 100%. The cell viability is calculated using the formula: % cell viability = [A_{570nm} of treated cells/A_{570nm} of control cells]×100.

Morphology study

Based on MTT assay we selected optimal dose by linear regression analysis for further studies. Analysis of cell morphology changes was observed using a phase contrast microscope. 2×10^5 cells were seeded in 6-well plates and treated with the highest concentration of 150µg/ml of nanoparticles for 24h. At the end of the incubation period, the medium was removed and cells were washed once with a phosphate buffer saline (PBS pH 7.4). The plates were observed under a phase contrast microscope for morphological changes.

Determination of mode of cell death by acridine orange (AO)/ethidium bromide (EtBr) dual staining

The apoptotic effect of avocado seed-Se nanoparticles in MCF-7 cell death was determined by AO/EtBr dual staining. The cells were treated with 150µg/ml of the synthesized nanoparticle for 24h and then the cells were harvested, washed with ice-cold PBS. The pellets were resuspended in 5µl of acridine orange (1mg/mL) and 5µl of EtBr (1mg/mL). The induction of apoptosis in the cells were then observed in stained cells using an inverted fluorescence microscope.

Statistical analysis

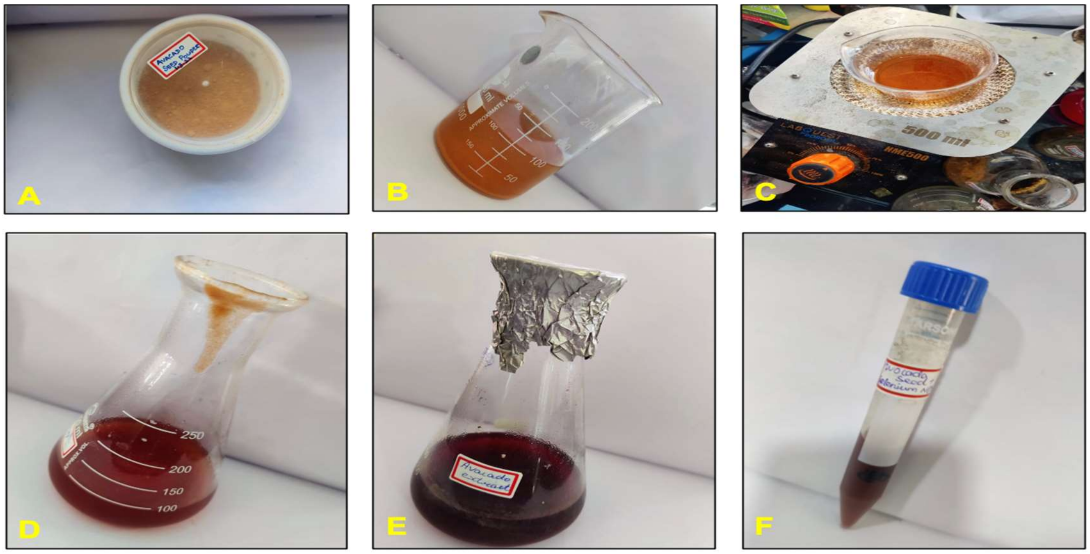
All data obtained were analyzed by One way ANOVA followed by Student's t-test using SPSS software. Data were represented as Mean ± SEM for triplicates. The level of statistical significance was set at $p<0.05$.

RESULTS

UV-Visible spectra analysis of synthesized Se-NPs

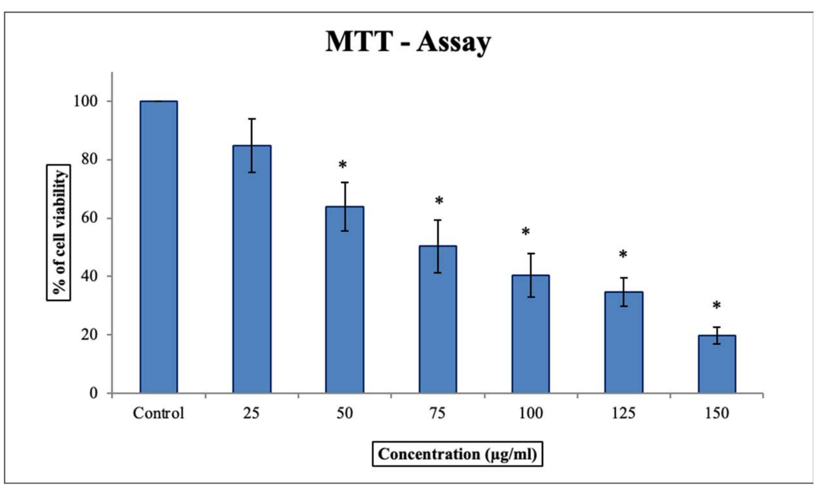
The Se-NPs was successfully synthesized from avocado seed extract. The changes of color yellow to ruby red indicated the synthesis of Se-NPs and was substantiated by UV-Visible spectrum with its peak absorption spectrum observed at 277.5nm.

Figure 1: Preparation of avocado seed extract mediated synthesis of selenium nanoparticles



Effect of Se-NPs on cell viability of MCF-7 breast cancer cells

The MTT assay results revealed a dose-dependent cytotoxic effect of Se-NPs on MCF-7 breast cancer cells after 24 hours of treatment. As the concentration of Se-NPs increased from 25 to 150 µg/mL, there was a progressive decline in cell viability. At the lowest concentration of 25 µg/mL, 84.74% of the cells remained viable, while a significant reduction was observed at higher concentrations, with only 19.69% cell viability at 150 µg/mL. The half-maximal inhibitory concentration (IC50) was determined to be 75 µg/mL, indicating that Se-NPs effectively reduced the viability of MCF-7 cells at this concentration. These results suggest a strong cytotoxic potential of Se-NPs against breast cancer cells, with increasing nanoparticle concentrations significantly inhibiting cell survival.



Graph 1: The bar graph showing the cytotoxic effect of the avocado seed selenium nanoparticles in MCF-7 breast cancer cells using MTT assay.

Effect of Se-NPs on morphology of breast cancer cells

The treatment of breast cancer cells with Se-NPs at a concentration of 75 µg/mL for 24 hours resulted in significant morphological changes indicative of cell death. Observable alterations included cell shrinkage, membrane blebbing, and the rounding of cells, which are characteristic features of apoptosis. These findings suggest that Se-NPs induce cytotoxic effects at this concentration, leading to the disruption of normal cell morphology and eventual cell death.

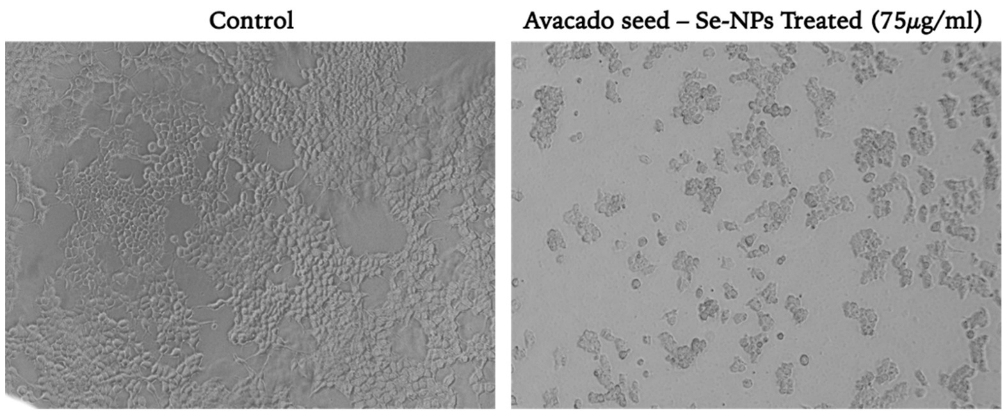


Figure 1: Representative images showing the effect of synthesized Se-NPs on MCF-7 cells after 24h incubation. Magnification, 10x.

Effect of Se-NPs on apoptosis induction in MCF-7 cells

The impact of selenium nanoparticles (Se-NPs) on apoptosis induction in MCF-7 cells was assessed by treating the cells with Se-NPs at a concentration of 75 µg/mL for 24 hours. The assessment of apoptosis was performed using the EtBr/AO (ethidium bromide/acridine orange) staining method, which differentiates between viable, early apoptotic, and late apoptotic cells based on their fluorescence. In the treated cell population, apoptosis was evident, with the staining results highlighting significant induction of cell death. Viable cells, which exhibited green fluorescence, were visibly present but less numerous compared to the control. Early apoptotic cells, characterized by yellowish-green fluorescence, were clearly distinguished, indicating an initial phase of apoptosis. This observation suggests that the Se-NPs effectively induced early apoptosis at the specified concentration.

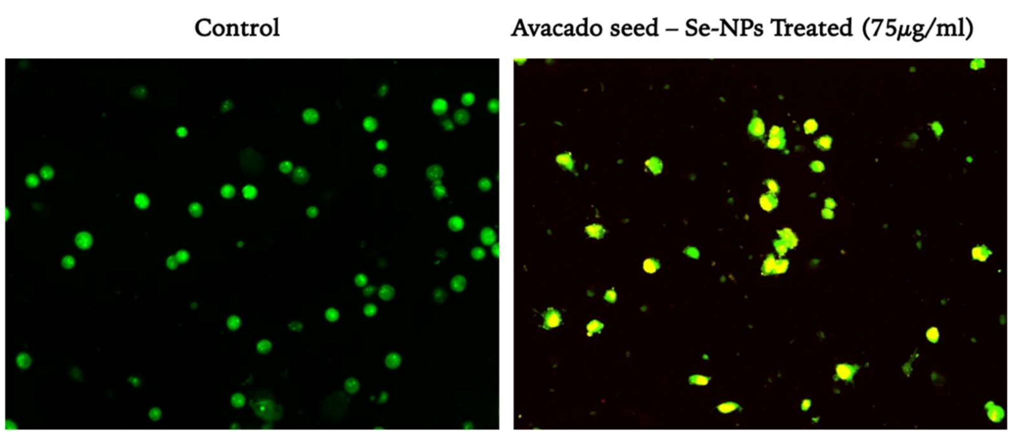


Figure 2: Representative images showing the apoptotic effect of synthesized nanoparticle cells following 24h treatment in MCF – 7 breast cancer cells, magnification 20x. Viable cells appear green in fluorescence and early apoptotic cells appear yellowish green on staining with acridine orange and ethidium bromide stain

DISCUSSION

In this study, we evaluated the cytotoxic and apoptotic effects of selenium nanoparticles (Se-NPs) on MCF-7 breast cancer cells. The MTT assay results revealed a dose-dependent cytotoxic effect of Se-NPs, with a significant reduction in cell viability as the concentration increased. At 25 µg/mL, cell viability was 84.74%, whereas at 150 µg/mL, it dropped to 19.69%. The IC_{50} value of 75 µg/mL indicates that Se-NPs are potent in reducing cell viability, suggesting a strong cytotoxic potential against MCF-7 cells. These results align with previous studies demonstrating the anticancer potential of Se-NPs. For instance, Cittrarasu et al. (2021) reported the green synthesis of Se-NPs and their cytotoxic effects on various cancer cell lines, underscoring the general efficacy of Se-NPs in inducing cell death [15]. Similarly, Spyridopoulou et al. (2021) highlighted the apoptotic and immunogenic cell death markers induced by biogenic Se-NPs in colon cancer cells, reinforcing the notion that Se-NPs can effectively trigger apoptosis in cancer cells [2].

Morphological changes observed in MCF-7 cells after treatment with Se-NPs at 75 µg/mL, such as cell shrinkage, membrane blebbing, and rounding, are indicative of apoptosis. These findings are consistent with those of Toubhans et al. (2020), who demonstrated that Se-NPs induce significant biomechanical alterations in ovarian cancer cells, a process linked to apoptosis [5]. Furthermore, the EtBr/AO staining method used in this study revealed that early apoptotic cells were prevalent following Se-NP treatment. This observation aligns with the work of Nagalingam et al. (2022)and Mi et al., (2022) which reported similar apoptotic effects of Se-NPs on cancer cells viz., HepG2 hepatocellular carcinoma cells and gastric cancer cells, confirming that Se-NPs effectively induce early apoptosis [16].

The significant reduction in cell viability and the induction of apoptosis observed in our study underscore the potential of Se-NPs as effective therapeutic agents in breast cancer treatment. The dose-dependent response and observable morphological changes provide compelling evidence of their cytotoxic and pro-apoptotic properties. Future studies should explore the molecular mechanisms underlying these effects and evaluate the in vivo efficacy of Se-NPs to better understand their therapeutic potential.

ACKNOWLEDGEMENT

The authors would like to thank the management of Saveetha Institute of Medical and Technical Sciences for providing us the platform for carrying out the research work and their support to conduct the study.

CONFLICT OF INTEREST

The authors declared no conflict of interest pertaining to the study.

References

1. Fernandes AP, Gandin V. Selenium compounds as therapeutic agents in cancer. Biochimica et Biophysica Acta (BBA)-General Subjects. 2015 Aug 1;1850(8):1642-60.
2. Spyridopoulou K, Aindelis G, Pappa A, Chlichlia K. Anticancer Activity of Biogenic Selenium Nanoparticles: Apoptotic and Immunogenic Cell Death Markers in Colon Cancer Cells. Cancers (Basel). 2021 Oct 24;13(21):5335. doi: 10.3390/cancers13215335.
3. Combs Jr GF. Biomarkers of selenium status. Nutrients. 2015 Mar 31;7(4):2209-36.
4. Sanmartín C, Plano D, Sharma AK, Palop JA. Selenium compounds, apoptosis and other types of cell death: an overview for cancer therapy. Int J Mol Sci. 2012;13(8):9649-9672. doi: 10.3390/ijms13089649. Epub 2012 Aug 2. PMID: 22949823; PMCID: PMC3431821.
5. Toubhans B, Gazze SA, Bissardon C, Bohic S, Gourlan AT, Gonzalez D, Charlet L, Conlan RS, Francis LW. Selenium nanoparticles trigger alterations in ovarian cancer cell biomechanics. Nanomedicine: Nanotechnology, Biology and Medicine. 2020 Oct 1;29:102258.
6. Geoffrion LD, Hesabizadeh T, Medina-Cruz D, Kusper M, Taylor P, Vernet-Crua A, Chen J, Ajo A, Webster TJ, Guisbiers G. Naked selenium nanoparticles for antibacterial and anticancer treatments. ACS omega.

2020 Feb 5;5(6):2660-9.
7. Kumar, TM Sathish. "Low-Power Design Techniques for Internet of Things (IoT) Devices: Current Trends and Future Directions." *Progress in Electronics and Communication Engineering* 1.1 (2024): 19-25.
8. Song X, Chen Y, Zhao G, Sun H, Che H, Leng X. Effect of molecular weight of chitosan and its oligosaccharides on antitumor activities of chitosan-selenium nanoparticles. Carbohydrate polymers. 2020 Mar 1; 231:115689.
9. Johnson J, Shanmugam R, Lakshmi T. A review on plant-mediated selenium nanoparticles and its applications. Journal of Population Therapeutics and Clinical Pharmacology. 2022 Jan 6;28(2):e29-40.
10. Shahverdi AR, Shahverdi F, Faghfuri E, Mavandadnejad F, Yazdi MH, Amini M. Characterization of folic acid surface-coated selenium nanoparticles and corresponding in vitro and in vivo effects against breast cancer. Archives of medical research. 2018 Jan 1;49(1):10-7.
11. Muralidharan, J. "Machine Learning Techniques for Anomaly Detection in Smart IoT Sensor Networks." *Journal of Wireless Sensor Networks and IoT* 1.1 (2024): 10-14.
12. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2021 May;71(3):209-49.
13. Li S, Silvestri V, Leslie G, Rebbeck TR, Neuhausen SL, Hopper JL, Nielsen HR, Lee A, Yang X, McGuffog L, Parsons MT. Cancer risks associated with BRCA1 and BRCA2 pathogenic variants. Journal of Clinical Oncology. 2022 May 10;40(14):1529-41.
14. Narwade M, Shaikh A, Gajbhiye KR, Kesharwani P, Gajbhiye V. Advanced cancer targeting using aptamer functionalized nanocarriers for site-specific cargo delivery. Biomaterials Research. 2023 May 6;27(1):42.
15. Sadulla, Shaik. "Next-Generation Semiconductor Devices: Breakthroughs in Materials and Applications." Progress in Electronics and Communication Engineering 1.1 (2024): 13-18.
16. Hussain A, Lakhan MN, Hanan A, Soomro IA, Ahmed M, Bibi F, Zehra I. Recent progress on green synthesis of selenium nanoparticles–a review. Materials Today Sustainability. 2023 Sep 1;23:100420.
17. Surendar, A. "Emerging Trends in Renewable Energy Technologies: An In-Depth Analysis." *Innovative Reviews in Engineering and Science* 1.1 (2024): 6-10.
18. Cittrarasu V, Kaliannan D, Dharman K, Maluventhen V, Easwaran M, Liu WC, Balasubramanian B, Arumugam M. Green synthesis of selenium nanoparticles mediated from Ceropegia bulbosa Roxb extract and its cytotoxicity, antimicrobial, mosquitocidal and photocatalytic activities. Scientific reports. 2021 Jan 13;11(1):1032.
19. Nagalingam M, Rajeshkumar S, Balu SK, Tharani M, Arunachalam K. Anticancer and antioxidant activity of MorindaCitrifolia leaf mediated selenium nanoparticles. Journal of Nanomaterials. 2022;2022(1):2155772.
20. Sadulla, Shaik. "A Comparative Study of Antenna Design Strategies for Millimeter-Wave Wireless Communication." *SCCTS Journal of Embedded Systems Design and Applications* 1.1 (2024): 11-15.
21. Mi XJ, Choi HS, Perumalsamy H, Shanmugam R, Thangavelu L, Balusamy SR, Kim YJ. Biosynthesis and cytotoxic effect of silymarin-functionalized selenium nanoparticles induced autophagy mediated cellular apoptosis via downregulation of PI3K/Akt/mTOR pathway in gastric cancer. Phytomedicine. 2022 May 1;99:154014.