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Thrombolytic, Anticoagulant Activity and Cytotoxic Effect of Carbon Nanoparticles from Vitis Vinifera Seeds and its Polymer Nanocomposite

Lokesh Sankar[1], Akshay J Kumar[2], Rajeshkumar Shanmugam[1*], Pradeep Manigandan[1]

[1]Nanobiomedicine Lab, Centre for Global Health Research, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, India
[2]Department of Orthopaedics,Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, India
Email: rajeshkumars.smc@saveetha.com

Nanotechnology is anemerging field of research that involves using nanoparticles to benefit humankind. Carbon nanoparticles have various properties that can be used in medicine. Their anticoagulant and thrombolytic properties can be used to make drugs that prevent vascular diseases like myocardial infarction, pulmonary thromboembolism, stroke, etc. Exposure to these carbon nanoparticles can be hazardous to humans and the environment, so their cytotoxicity should also be investigated.Green synthesis of these nanoparticles is preferred because they involve fewer toxic chemicals and cheap materials for their synthesis. Grapes(Vitis vinifera) are used for the synthesis of CNPs. The objective of the study is to assess the anticoagulant, thrombolytic, and cytotoxic properties of carbon nanoparticles from Vitis vinifera seeds and their nanocomposite.Their anticoagulant and thrombolytic properties are checked by observing their effect on human blood samples. The cytotoxic effect of the carbon nanoparticles is assessed using a brine shrimp lethality assay.Results from anticoagulant assay showed dose-dependent effects of carbon nanoparticles. Thrombolytic assay showed carbon nanoparticles showed a similar effect to the control. A brine shrimp lethality assay is performed to see their cytotoxicity. Results of these assays showed lethality of brine shrimps increased with an increase in their concentrations. Carbon nanoparticles showed less toxicity at the concentration of 40 and 80 µg/ml.From this study,we could conclude that carbon nanoparticles have potent anticoagulant and thrombolytic properties with less toxicity.

Keywords: Anticoagulant, brine shrimp, cytotoxic effect, carbon nanoparticles, thrombolytic, Vitis vinifera.

1. Introduction

Nanotechnology is a newly emerging and growing field of research thatoffers new technologies. Nanotechnology refers to using materials that are in nano size ranging from 1 to 100 nm. Nanoparticles exhibit various properties due to their small size. Nanoparticles fall into three categories: natural, engineered, and incidental(Fiorito et al., 2006). Their nano size, high surface area, and surface properties are giving them their biological applications. Their safety should also be evaluated (Zakrzewska et al., 2015). The application of nanoparticles in medicine is termed nanomedicine. Studies have shown that nanoparticles have potential effects in reducing cardiovascular diseases. These nanoparticles have also been reported to have cell toxicity(LeCroy et al., 2016).

Carbon nanoparticles (CNPs) are one of the most important nanoparticles due to their useful properties. These particles can be obtained from natural sources. They have been utilized in bioimaging, biosensing,drug delivery, etc., due to their biocompatibility (Lee et al., 2020). CNPs have biomedical properties as a drug carrier through intravenous injection(Tang et al., 2012). CNPs have attracted many researchers in recent decades. These nanoparticles can be obtained from natural sources(Varghese et al., 2013). Green synthesis of these particles reduces the use of solvents,chemicals, and other raw materials.Green synthesis is a stable, reliable, and environmentally friendly method for the synthesis of these nanoparticles. So, green synthesis is better and safer for the synthesis of nanoparticles(Barabadi et al., 2023). Due to various applications of nanoparticles, the exposure to nanoparticles is increased. The studies on the toxicity of these substances are also very few. So,a study on their toxicity is also needed(Jia et al., 2005).

Thrombosis and thromboembolism are the common causes of mortality and morbidity in developed and developing countries. It creates a burden on the health and economical status of patients. This condition requires early diagnosis. The treatment is also very limited. Traditional thrombolytics have a short half-life and they easily get degraded.Thrombosis is related to cardiovascular diseases and lifestyle (Russell et al., 2022). Antiplatelet and anticoagulant drugs are playing a major role in medicine (Bijak et al., 2019). These drugs are efficient but their life-threatening side effects are also documented. So, many researchers are going on to develop natural alternatives. Plants can be used for isolating these natural compounds with least to no side effects. Many studies showed that a polyphenol-rich diet improved vascular health and reduced the risk of vascular diseases. One such polyphenol-rich plant is grapes (Vitis vinifera). It has been eaten as food for centuries and is known to possess medicinal effects (Hassan et al., 2020). Grapes were used in ancient times for curing cardiovascular diseases, controlling cholesterol levels, and controlling high blood pressure. Grape seed extracts are also said to have anticoagulant effects due to being rich in polyphenols. Polyphenols do it by increasing the prothrombin time (Hussein et al., 2021). Grape seeds are a good potent reducing agent. The chemicals present in grapes are also safe for the environment (Vinayagam et al., 2017; Kanniah et al., 2020).

The objective of thestudy is to assess the anti-coagulant and thrombolytic properties of CNPs from Vitis vinifera and itsnanocomposite to check its cytotoxicity. The anticoagulant effect is assessed by seeing the effect of CNPs suspension on human blood. Thrombolytic activity is assessed by seeing its effect on clotted blood. The cytotoxicity is assessed by brine shrimp lethality assay. This study helps to develop an efficient and less toxic drug for vascular diseases like myocardial infarction and stroke.

2. Materials and Methods:

Preparation of Carbon Nanoparticles:

Vitis vinifera seeds were washed using distilled water then dried and then powdered. Then 5g of Vitis vinifera seeds powder is mixed in distilled water of 100 ml and then boiled with the heating mantle. At 700 volts the microwave oven is utilized to synthesize carbon nanoparticles utilizing a crucible. The effluent that is settled in the crucible was pounded well into a fine powder. The powder was then combined with distilled water and placed in a sonicator for 10 minutes. Carbon nanoparticles are kept in a hot air oven for drying and used for further studies. Chitosan of 500g was added along with 1 mL glacial acetic acid to distilled water of 49 mL. The solution was placed in a magnetic stirrer till the solution became a clear solution. 0.2g in 3 ml of chitosan were combined and placed in the sonicator for 15 minutes.

Anticoagulant Activity:

The anticoagulant ability of carbon nanoparticles is evaluated by performing assays on freshly taken human blood samples at normal room temperature for performing this assay carbon nanoparticles (concentration 50 µg/mL) of 1 mL as the suspension is mixed with fresh human blood of 1 mL (vial B) and another blood of 10mLwithout adding anything was taken as control (vial A).These two blood samples were kept for observation for one hour at room temperature for any notable changes((Aina et al., 2019; Dinesh et al., 2021).

Thrombolytic Assay:

A drop of blood was collected in the sterile glass slide and incubated at room temperature for 45 minutes.After clotting blood, the carbon nanocomposite solutions were added in different concentrations(10, 20, 30, 40, 50 µg/ml). The control group only has blood. The glass slide is then kept in incubation at room temperature for 90 minutes, the time was noted for incubation hours to observe the clot lysis(Tabassum et al., 2017; Rifaath et al., 2023; Abitha et al., 2019).

Cytotoxic Effect:

Brine Shrimp Lethality Assay:

2 grams of non-iodized salt was made to dissolve in 200 ml of distilled water and thensaline water of 10 to 12 ml was added onto ELISA plates with 6 wells. Then 10 nauplii were added gently to each well (5, 10, 20, 40, and 80 µg/mL). Then carbon nanoparticles were added according to concentration level and left for incubation for 24 hours.After incubating for 24 hours number of nauplii present in ELISA plates was counted and calculated by using the equation,

Number of dead nauplii / number of dead nauplii + number of live nauplii×100

3. Results and Discussion:

Figure 1: Visual representation of step-by-step processes for synthesizing carbon nanoparticles from Vitis vinifera and its nanocomposite.

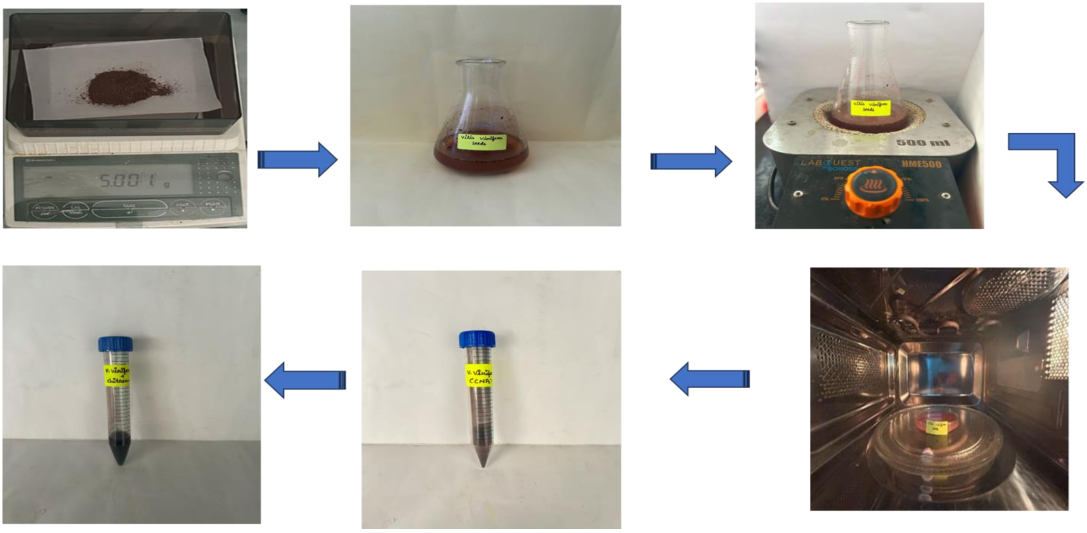
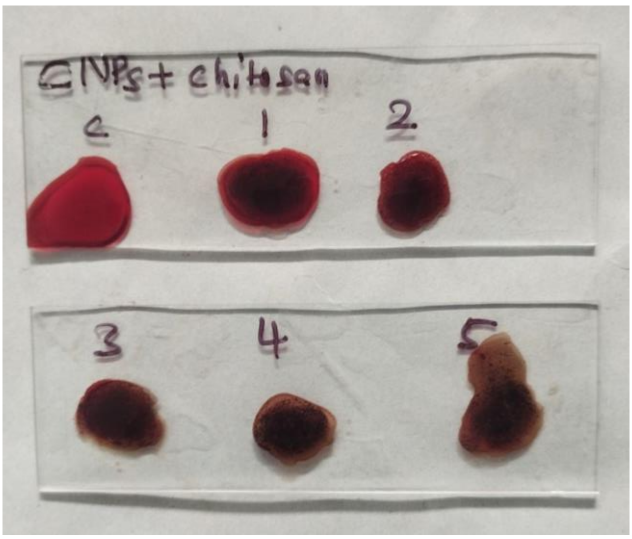


Figure 2: Thrombolytic activity of chitosan incorporated carbon nanocomposite in different concentrations.



The overall synthesis of the CNPs is represented in Figure 1. Thrombolytic activity of carbon nanoparticles on human blood samples. We could visually see control being coagulated and the blood sample with carbon nanoparticles is not coagulated and we can observe the anticoagulant activity of carbon nanocomposite is concentration dependent as shown in Figure 2 and graphical representation in Figure 3.

Figure 3: Graphical representation of the thrombolytic activity of carbon nanocomposites.

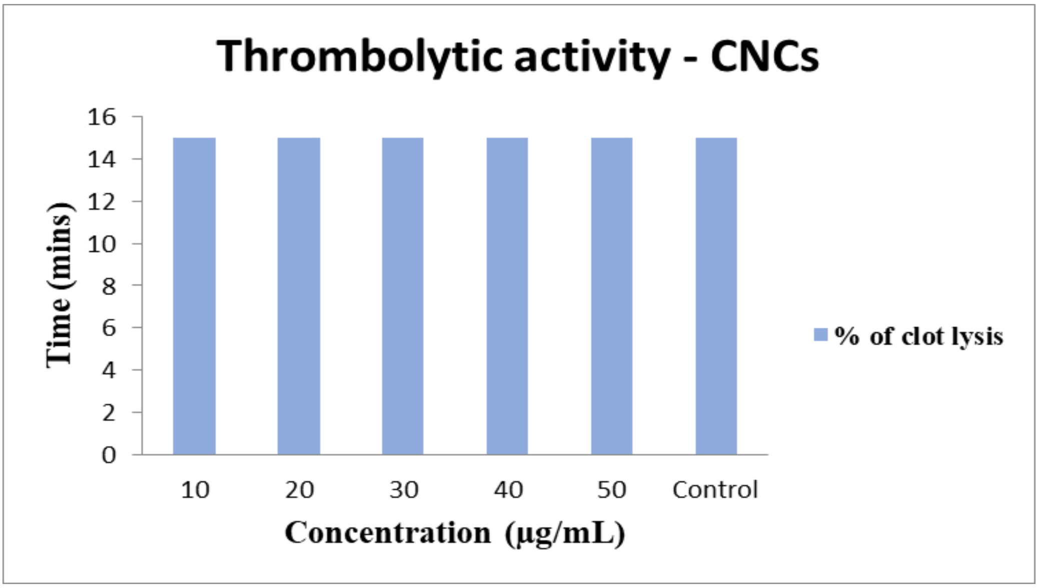
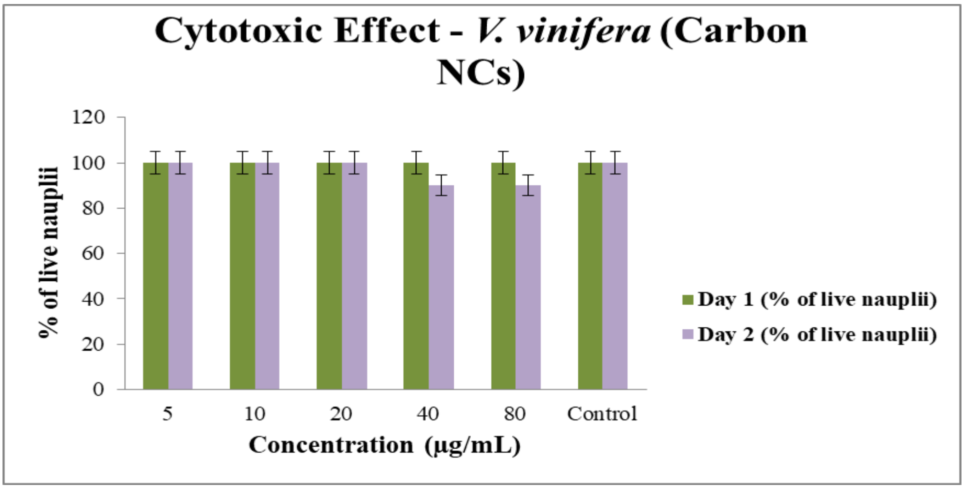


Figure 4: Anticoagulant activity of Vitis vinifera mediated carbon nanocomposites.

The thrombolytic activity of carbon nanoparticles is assessed by seeing its effect on human blood samples. Carbon nanocomposites with various concentrations (10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml) exhibited same thrombolytic activity as control. It is observed that CNPs have great potential in the lysis of blood samples which can be further developed into therapeutic drugs to prevent clot lysis. The anticoagulant activity of carbon nanoparticles is assessed by testing them on human blood samples and comparing them with control. In Figure 4 we were able to visually appreciate clot lysis by Vitis vinifera carbon nanoparticles and its chitosan nanocomposite compared to control.

Figure 5: Cytotoxic effect of chitosan incorporated carbon nanocomposites at different concentrations using brine shrimp lethality assay.



The cytotoxicity of carbon nanoparticles is assessed by performing a brine shrimp lethality assay. Some nauplii alive and dead are counted and the percentage of live nauplii is calculated. In control 100 percent of nauplii are alive. At 5 µg/mL, 100 percent of nauplii are alive on both day one and day two. At 10 µg/mL, 100 percent of nauplii are alive on both day one and day two. At 20 µg/mL, 100 percent of nauplii are alive on both day one and day two. At 40 µg/mL, 100 percent of nauplii are alive on day one, and 90 percent of nauplii are alive on day 2. At 80 µg/mL 100 percent of nauplii are alive on day one and 90 percent of nauplii are alive on day 2 as represented graphically in Figure 5. This demonstrates the potential cytotoxicity of carbon nanoparticles at higher concentrations.

4. Discussion:

The synthesis of carbon nanoparticles from Vitis vinifera seeds was successful. The thrombolytic and anticoagulant activity of carbon nanoparticles was evident through the assays performed in freshly collected human blood samples. It exhibited concentration-dependent thrombolyticactivity. It also exhibited cytotoxicity at higher concentrations. It was evident through the brine shrimp lethality assay.In a previous study conducted by Ju Yi Mao et al., the anticoagulant property of alginate-based carbon nanoparticles was studied. They exhibited potent anticoagulant activity by inhibiting thrombin(Abitha et al., 2019).In a previous study done by Farzaneh et al., the toxicity of single-walled and multi-walled carbon nanotubes were tested against brine shrimps. They showed potent cytotoxicity. Their effect is concentration-dependent (Mao et al., 2019).In comparison, in a study conducted by Jessica et al., the cytotoxic properties of multiwalled carbon nanotubes on the Danio rerio and Artemia salina were studied. The multi-walled carbon nanotubes exhibited potential cytotoxicity (De Souza et al., 2024).In a study conducted by (Cheng et al.,2009)the carbon nanoparticle cytotoxic effect is evaluated by performing cell assays. They noted the cytotoxicity of carbon nanoparticles is understood by high-resolution imaging. Plants Allium sativum, Cucurma longa, Ananascomosus, and Lycopersicumesculentum were tested in vitro, using Heparin and water as controls. Plants exhibited clot lysis percentages of 18.30%, 21.77%, 21.85%, 35.91%, 15.67%, and 24.52%. Only C. longa peel extracts and L.esculentum demonstrated clot lysis, surpassing the negative control. C. longa peels shown a greater proportion of clot lysis as compared to Heparin. The one-way ANOVA analysis revealed a p-value of 0.674574 for the clot lysis activity of all plant extracts. The cytotoxicity of herbal plants was assessed using the brine shrimp lethality test (BSLA), which has less toxicity (Somasundaram et al., 2023).

An in vitro thrombolytic model of water, methanol, and chloroform extracts showed 51.76±2.5%, 58±2.32, and 18±1.84 clot lysis, respectively. The positive control Streptokinase showed 79.6±1.10 while the negative control was minimal (2.44±0.62). The aqueous, methanol, and chloroform extracts killed brine shrimp with LC50 values of 40µg/ml, 30µg, and 104 µg/ml, respectively. T angustifolia extracts, both aqueous and methanolic, have shown promise for thrombolytic and cytotoxic effects as per the previous literature (Umesh et al.2014).In the cytotoxicity test, the sample's LC50 values were (106.41±0.78) µg/mL, while standard vincristinsulfate had an LC50 value of (08.50±0.24) µg/mL as a positive control. The extract demonstrated (12.86±1.02%) clot lytic activity in the thrombolytic assay, while standard streptokinase showed (30.86±0.44%) clot lytic activity. The assessment of phytochemicals reveals the existence of several chemical components such as alkaloids, flavonoids, glycosides, saponins, and carbohydrates (Mohammad et al., 2013).In this previous study, the purpose of this work was to examine the thrombolytic, anticoagulant, antioxidant, and antitumor properties of ascorbic acid-mediated silver nanoparticles (A-SeNPs). Various analytical methods were employed to look at how A-SeNPs formed. Xanthine oxidase inhibition (XOI), uric acid (UA) degradation assay and MSU crystal dissolution were used to test the nanoparticles' anti-tuberculate capability. Excellent antihyperglycemic action was demonstrated by A-SeNPs in a concentration-dependent manner (Muhammad Aamir et al.,2024)

5. Conclusion:

In conclusion, we could conclude that carbon nanoparticles from Vitis vinifera have exhibited good anticoagulant and thrombolytic activity. The tests were conducted on freshly collected human blood samples. They exhibited dose-dependent effects. Their cytotoxicity was also assessed in the nauplii of brine shrimp. The result showed dose-dependent cytotoxicity of carbon nanoparticles. From the study, we can say that carbon nanoparticles synthesized from Vitis vinifera can be used to develop a novel drug for vascular diseases like myocardial infarction, pulmonary thromboembolism, and stroke. Their dose-dependent cytotoxicity should also be considered. These can be confirmed by further studies.

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References

1. Abitha, S. T., Rajeshkumar, S., Lakshmi, T., & Roy, A. (2019, September 1). Cytotoxic effects of silver nanoparticles synthesized using amla fruit seed. | Drug Invention Today

EBSCOhost.https://openurl.ebsco.com/EPDB%3Agcd%3A2%3A28249746/detailv2?sid=ebsco%3Aplink%3Ascholar&id=ebsco%3Agcd%3A139165824&crl=c

2. Aina, D. A., Owolo, O., Lateef, A., Aina, F. O., Hakeem, A. S., Adeoye-Isijola, M., Okon, V., Asafa, T. B., Elegbede, J. A., Olukanni, O. D., &Adediji, I. (2019). Biomedical Applications of Chasmantheradependens stem extract mediated silver nanoparticles as Antimicrobial, Antioxidant, Anticoagulant, thrombolytic, and Larvicidal agents. Karbala International Journal of Modern Science, 5(2). https://doi.org/10.33640/2405-609x.1018
3. Kanniah, P., Radhamani, J., Chelliah, P., Muthusamy, N., Balasingh, E. J. J. S., Thangapandi, J. R., Balakrishnan, S., & Shanmugam, R. (2020). Green Synthesis of Multifaceted Silver Nanoparticles Using the Flower Extract of Aervalanata and Evaluation of Its Biological and Environmental Applications. ChemistrySelect, 5(7), 2322–2331. https://doi.org/10.1002/slct.201903228
4. Barabadi, H., Noqani, H., Ashouri, F., Prasad, A., Jounaki, K., Mobaraki, K., Mohanta, Y. K., & Mostafavi, E. (2023). Nanobiotechnological approaches in anticoagulant therapy: The role of bioengineered silver and gold nanomaterials. Talanta, 256, 124279. https://doi.org/10.1016/j.talanta.2023.124279
5. Rajeshkumar, S., Malarkodi, C., Paulkumar, K., Vanaja, M., Gnanajobitha, G., & Annadurai, G. (2014). Algae Mediated Green Fabrication of Silver Nanoparticles and Examination of Its Antifungal Activity against Clinical Pathogens. International Journal of Metals, 2014, 1–8. https://doi.org/10.1155/2014/692643
6. Cheng, C., Porter, A. E., Muller, K., Koziol, K., Skepper, J. N., Midgley, P., &Welland, M. (2009). Imaging carbon nanoparticles and related cytotoxicity. Journal of Physics Conference Series, 151, 012030. https://doi.org/10.1088/1742-6596/151/1/012030
7. De Souza, J. P. A., Silva, I. F., Carneiro, P. G., Schnitzler, M. C., Thomé, R. G., & Santos, H. B. D. (2024). Toxic Effects of Multiwalled Carbon Nanotubes on the Zebrafish (Danio rerio) and the Brine Shrimp (Artemia salina): a Morphological, Histological, and Immunohistochemical Study. Brazilian Archives of Biology and Technology, 67. https://doi.org/10.1590/1678-4324-2024230143
8. Somasundaram, S., Balusamy, S. R., Perumalsamy, H., Ranjan, A., Abbas, Q., Irfan, N., Shanmugam, R., & Park, S. (2023). Structural modifications and biomedical applications of π-extended, π-fused, and non-fused tetra-substituted imidazole derivatives. Arabian Journal of Chemistry, 16(9), 105030. https://doi.org/10.1016/j.arabjc.2023.105030
9. Evangelista, J., De Vera, M., Garcia, J., Joven, M., &Nerosa, M. (2012). Preliminary assessment of in vitro anticoagulant activity vs. heparin 1,000 IU and cytotoxicity of selected Philippine medicinal plants. International Journal of Chemical and Environmental Engineering, 3(6).
10. Fiorito, S., Serafino, A., Andreola, F., Togna, A., &Togna, G. (2006). Toxicity and Biocompatibility of Carbon Nanoparticles. Journal of Nanoscience and Nanotechnology, 6(3), 591–599. https://doi.org/10.1166/jnn.2006.125
11. Hassan, S. A., Abdelaziz, Z. S., Elbasheir, M. M., Elradi, W. E. O., Allah, E. I. A., &Elmahi, A. Y. (2020). In vitro Anticoagulant Effect of Grape Seed Extract (Vitis vinifera) on Human Normal Blood Sample. European Journal of Medicinal Plants, 40–45. https://doi.org/10.9734/ejmp/2020/v31i1630329
12. Hussein, B. Y., & Mohammed, A. M. (2021). Green synthesis of ZnO nanoparticles in grape extract: Their application as anti-cancer and anti-bacterial. Materials Today Proceedings, 42, A18–A26. https://doi.org/10.1016/j.matpr.2021.03.729
13. Jia, G., Wang, H., Yan, L., Wang, X., Pei, R., Yan, T., Zhao, Y., & Guo, X. (2005). Cytotoxicity of Carbon Nanomaterials: Single-Wall Nanotube, Multi-Wall Nanotube, and Fullerene. Environmental Science & Technology, 39(5), 1378–1383. https://doi.org/10.1021/es048729l
14. Cide, Felip, José Urebe, and Andrés Revera."Exploring Monopulse Feed Antennas for Low Earth Orbit Satellite Communication: Design, Advantages, and Applications." National Journal

of Antennas and Propagation 4.2 (2022): 20-27.

15. LeCroy, G. E., Yang, S. T., Yang, F., Liu, Y., Fernando, K. a. S., Bunker, C. E., Hu, Y.,Luo, P. G., & Sun, Y. P. (2016). Functionalized carbon nanoparticles: Syntheses and applications in optical bioimaging and energy conversion. Coordination Chemistry Reviews, 320–321, 66–81. https://doi.org/10.1016/j.ccr.2016.02.017
16. Lee, T. Y., Jayakumar, T., Thanasekaran, P., Lin, K. C., Chen, H. M., Veerakumar, P., & Sheu, J. R. (2020). Carbon Dot Nanoparticles Exert Inhibitory Effects on Human Platelets and Reduce Mortality in Mice with Acute Pulmonary Thromboembolism. Nanomaterials, 10(7), 1254. https://doi.org/10.3390/nano10071254
17. Mao, J. Y., Lin, F. Y., Chu, H. W., Harroun, S. G., Lai, J. Y., Lin, H. J., & Huang, C. C. (2019). In situ synthesis of core-shell carbon nanowires as a potent targeted anticoagulant. Journal of Colloid and Interface Science, 552, 583–596. https://doi.org/10.1016/j.jcis.2019.05.086
18. Miyawaki, J., Yudasaka, M., Azami, T., Kubo, Y., & Iijima, S. (2008). Toxicity of Single-Walled Carbon Nanohorns. ACS Nano, 2(2), 213–226. https://doi.org/10.1021/nn700185t
19. Rifaath, M., Rajeshkumar, S., Anandan, J., Munuswamy, T., &Govindharaj, S. (2023). Preparation of Herbal Nano-Formulation-Assisted Mouth Paint Using Titanium Dioxide Nanoparticles and Its Biomedical Applications. Curēus. https://doi.org/10.7759/cureus.48332
20. Russell, P., Hagemeyer, C. E., Esser, L., & Voelcker, N. H. (2022). Theranostic nanoparticles for the management of thrombosis. Theranostics, 12(6), 2773–2800. https://doi.org/10.7150/thno.70001
21. Siddique, M. a. R., Khan, M. A., Bokhari, S. a. I., Ismail, M., Ahmad, K., Haseeb, H. A., Kayani, M. M., Khan, S., Zahid, N., & Khan, S. B. (2024). Ascorbic acid-mediated selenium nanoparticles as potential antihyperuricemic, antioxidant, anticoagulant, and thrombolytic agents. Green Processing and Synthesis, 13(1). https://doi.org/10.1515/gps-2023-0158
22. Tabassum, F., Chadni, S. H., Mou, K. N., Hasif, K. I., Ahmed, T., & Akter, M. (2017). In-vitro thrombolytic activity and phytochemical evaluation of leaf extracts of four medicinal plants of Asteraceae family. www.phytojournal.com. https://www.phytojournal.com/archives?year=2017&vol=6&issue=4&ArticleId=1486
23. Tang, A. C. L., Chang, M. Y., Tang, Z. C. W., Li, H. J., Hwang, G. L., & Hsieh, P. C. H. (2012). Treatment of Acute Thromboembolism in Mice Using Heparin-Conjugated Carbon Nanocapsules. ACS Nano, 6(7), 6099–6107. https://doi.org/10.1021/nn301198r
24. Umesh, M. K., Sanjeevkumar, C. B., Hanumantappa, B. N. R. L., & Ramesh, L. (2014). Evaluation of in vitro anti-thrombolytic activity and cytotoxicity potential of Typha angustifolia L leaves extracts. Int J Pharm PharmSci, 6(5), 81-85.
25. Varghese, S., Kuriakose, S., & Jose, S. (2013). Antimicrobial Activity of Carbon Nanoparticles Isolated from Natural Sources against Pathogenic Gram-Negative and Gram-Positive Bacteria. Journal of Nanoscience, 2013, 1–5. https://doi.org/10.1155/2013/457865
26. JONNERBY, JAKOB, A. BREZGER, and H. WANG. "Machine learning based novel architecture implementation for image processing mechanism." International Journal of communication and computer Technologies 11.1 (2023): 1-9.
27. Vinayagam, R., Varadavenkatesan, T., & Selvaraj, R. (2017). Evaluation of the Anticoagulant and Catalytic Activities of the Brideliaretusa Fruit Extract-Functionalized Silver Nanoparticles. Journal of Cluster Science, 28(5), 2919–2932. https://doi.org/10.1007/s10876-017-1270-5
28. Zakrzewska, K. E., Samluk, A., Wierzbicki, M., Jaworski, S., Kutwin, M., Sawosz, E., Chwalibog, A., Pijanowska, D. G., & Pluta, K. D. (2015). Analysis of the Cytotoxicity of Carbon-Based Nanoparticles, Diamond and Graphite, in Human Glioblastoma and Hepatoma Cell Lines. PLoS ONE, 10(3), e0122579. https://doi.org/10.1371/journal.pone.0122579