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Synthesis, Characterization and Evaluation of Anticancer Activity of Agal Extract Mediated Gold Nanoparticles

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Gold nanopartilces was synthesized by biogenic method using Dyctyota dichotoma aqueous extract under the bright sunlight in 30 min at pH 7.4. Dyctyota dichotoma aqueous extract acts as a capping agent and reduced Au3+ to Au. The synthesized Dd-AuNPs were characterized by UV-vis spectroscopy, HR-TEM, DLS, zeta potential. The SPR peak of Dd-AuNPs was obtained at 526nm. The Dd-AuNPs was found to be spherical shape with average sizs of 30nm. The synthesized Dd-AuNPs was highly hemocompatible in human RBCs, which produced <2.5% hemolysis at 100µg/ml concentration. The Dd-AuNPs has potential anticancer activity and inhibited the growth of A549 lung cancer cells in the concentration manner and IC50 was found to be 80µg/ml. Further the anticancer activity of Dd-AuNPs was confirmed through the apoptosis analysis by using fluorescence staining. Hence, the hemocompatible Dd-AuNPs can be used in biomedical application for cancer therapy, drug delivery and so on.

Keywords: Gold nanoparticles, Biogenic synthesis, Dictyota dichotoma, Sunlight irradiation, Hemocompatibility, Anticancer activity.

1. Introduction

Nanotechnology is a promising field that addresses to the challenges of traditional medicine. Nanoparticles has special attention duet to size, shape, adaptable surface modification,

bioconjugation, optical, electrical and thermal properties[1]. Nanoparticles within the size range of 1-100nm can be utilized in biomedical applications. Among the different metal nanoparticles, gold nanoparticles have great potential due to unique physicochemical feature and it has diverse applications in the biomedical field such as gene delivery, drug delivery, bioimging, and biosensing[2]. Facile synthesis of metal nanoparticles by reducing their metal salt has been interested in the scientific fields. The nanoparticles can be synthesized by physical, chemical and biological methods. Biological synthesis of nanoparticles has gained attention compare to physical and chemical methods. The biogenic nanoparticles synthesis methods are non toxic, ecofriendly, economic and facile. A physical and chemical method of nanoparticles synthesis is required high energy, pressure, sophisticated instruments, and involves toxic chemicals[3]. Previously, several research works have been published on synthesis of metal nanoparticles using medicinal plant and plant components extract[4,5]. Recently, Ipek et al., 2024 reported that the suynthesis of gold nanoparticles using Allium cepa L. peel aqueous extract[6]. Kumar et al., 2024 described green synthesis of gold nanoparticles using Canthium parviflorum extract[7].

In human, cancer is one of the major causes of morbidity and mortality. Thus, the advance methods are necessary in cancer therapy for effective treatment of tumor cells[8]. Lung cancer is the leading cause of cancer death in the United States and around the world. Almost as many Americans die of lung cancer every year than die of prostate, breast, and colon cancer combined. Lung cancer arises from the cells of the respiratory epithelium and can be divided into two broad categories. They are Small cell lung cancer (SCLC) is a highly malignant tumor accounts for 15% of lung cancer cases and Non–small cell lung cancer (NSCLC), which accounts for the remaining 85% of cases[9]. Lung cancer is the second most common cancer diagnosed in both men and women in the United States and the leading cause of cancer death. In 2023, the American Cancer Society estimates that there will be 238,340 new cases of lung cancer, and 127,070 people will die from lung cancer, accounting for approximately 20% of all cancer deaths. The principal cause of lung cancer is cigarette smoking, which accounts for approximately 80% of cases[10]. The treatment of lung cancer relies on chemotherapy, immunotherapy, surgery, and radiation therapy[11]. All these methods have demerits like expensive, time consuming, side effect and so on. Hence, the advance, rapid and cost effective therapy method is necessary for cancer. Researchers have been explained that nanoparticles mediated cancer diagnosis and treatments are new emerging field in the biomedical sciences. Several research works have been published that medicinal plant extract capped nanomaterials are inhibited the growth of a malignant cells. Recently, Yayintas et al., 2024 reported that AuNPs were successfully synthesized from Nasturtiu. officinale extract. The biosynthesized AuNPs exhibited toxicity to and apoptotic effects on A549 lung cancer cells[12]. Palaniyandi et al., 2023 published that the synthesis of AuNPs with the extracts of red algae Halymenia pseudofloresii using the green method and synthesized AuNPs AuNPs showed potential cytotoxic activity against A549 lung cancer[13]. Dictyota dichotoma (Hudson) Lamoroux is brown seaweed and distributed worldwide from temperate to subtropical regions. The species was first described by Hudson (1762) from Walney Island in Lancashire, United Kingdom[14]. The polysaccharide of this seaweed has potential biological activities such anticoagulant, antithrombotic, anti-inflammatory, antitumoral, contraceptive, apoptotic, antioxidants and antiviral[15]. The present research work describes the biogenic synthesis of gold nanoparticles using Dyctyota dichotoma aqueous extract under the sunlight in biological pH. The

synthesized gold nanoparticles were characterized by UV-vis spectroscopy, HR-TEM, DLS, zeta potential. Further, the hemocompatibility of Dd-AuNPs were analyzed using human RBCs by hemolytic assay and the anticancer activity of synthesized Dyctyota dichotoma extract capped gold nanoparticles was evaluated against A549 lung cancer cell line by MTT assay and apoptosis analysis.

2. Materials and Methods

Materials

Chloroauric acid was procured from Loba Chemie Pvt Ltd, India. Nutrient Mixture F-12 Ham Kaighn's Modification media, Trypsin phosphate versene glucose, Fetal bovine serum, Antibiotic-antimycotic solution were purchased from Himedia Laboratories Pvt Ltd, India. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Acridine orange, and Ethidium bromide were purchased from Sigma Aldrich, USA.

Preparation of Dyctyota dichotoma aqueous extract

Dyctyota dichotoma was collected from Mandapam coastal region, Ramanathapuram district, Tamil Nadu, India. The epiphytes and other contaminants were removed from the sample by repeated washing with seawater, fresh water and distilled water. 30% (W/V) of aqueous extract of Dyctyota dichotoma was prepared using sterile distilled water, clean morter and pestle. The extract was centrifuged and filtered by whatman No:1 filter paper and stored at 4^{o}C for nanoparticles synthesis.

Synthesis of Gold Nanoparticles

50ml of Chloroauric acid (1mM) was taken in a clean conical flask, in which 1.25ml of Dyctyota dichotoma aqueous extract was added and pH of the solution was adjusted to 7.4 using sodium hydroxide solution. Then the solution mixture was kept under the bright sun light. The yellow colour choloroauric acid solution was turned to red colour in 30 minutes that confirmed the synthesis of gold nanoparticles.

Characterization of gold nanoparticles

UV-visible Spectrophotometer Analysis

2ml of synthesized gold nanoparticles was taken in a quartz cuvette, the surface plasmon resonance (SPR) peak of Dyctyota dichotoma capped gold nanoparticles (Dd-AuNPs) was analyzed using Shimadzu –UV 1601spectrometer and the SPR peak was recorded.

High Resolution - Transmission Electron Microscopy Analaysis

The size and shape of Dd-AuNPs was analyzed using HR-TEM (FEI TECHNAI G2 MODEL T-30-S-TWIN, USA). 5µl of sample was placed in a carbon coated copper grid and allowed it to dry overnight. Then the grid was examined in HR-TEM.

Dynamic Light Scattering Analysis

In aqueous medium, the hydrodynamic diameter of nanoparticle size was analyzed using DLS method (Malvern Instruments, United Kingdom). Synthesized Dd-AuNPs was diluted and

loaded in the sample holder using cuvette. The instrument was run at 100VA at 25°C and 232 kcps with the duration of 60 seconds.

Zeta Potential Analysis

The net charge of synthesized Dd-AuNPs was estimated using Nano Series (Malvern Instruments, United Kingdom). The Dd-AuNPs solution was taken in an electrode cuvette and placed in the sample holder and the instrument was run at 100VA at 25°C AND 232 kcps with the duration of 60 seconds.

Hemolytic Assay

The hemolytic assay was performed by previously adopted method Ramadurai et al., 2020 with little modification[16]. The anticoagulated blood sample was centrifuged (5000 rpm for 10min) and the RBCs cell suspension (5% v/v) was prepared with sterile saline solution in separate eppendorf tubes. The different concentrations of Dd-AuNPs (20, 40, 60, 80 and 300µg/mL) were added to the RBCs suspension and incubated at 37°C for 1h. Then, all the tubes were centrifuged at 5000rpm for 10min and the supernatant solution was used for hemolysis analysis and the OD value was calculated at 540nm. The hemolysis (%) was calculated by the following formula.

$$\text{Hemolysis (\%)} = [OD_{test} - OD_{blank}] / [OD_{positive\ control} - OD_{blank}] \times 100$$

In-vitro Anticancer Efficacy of Dd-AuNPs

A549 human lung cancer cell line was obtained from National Centre for Cell Science (NCCS), Pune, India. The cells were maintained in Dulbecco's Modified Eagle Medium (DMEM). Anticancer activity of Dd-AuNPs was analyzed using A549 human lung cancer cell line by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay[17.18]. Briefly, 1×10^4 cells/well were cultured in 96 well plates and incubated at 37ºC in a 5% CO_2 incubator for 24 h. The cells were treated with different concentration of Dd-AuNPs (20, 40, 60, 80, 100µg/mL) for 24 h. 10µL of MTT was added to all the wells and the plate was incubated 3h in dark condition. Finally, the formazan crystal was dissolved by adding 100µL of DMSO. Then the purple colour, measured by ELISA reader (BIO-TEK, Power wave-XS) at 570 nm. The cells viability was calculated by the following formula.

$$\text{Cell viability (\%)} = [\text{OD of test sample}] / [\text{OD of control}] \times 100$$

Apoptosis Analysis

The effect of Dd-AuNPs in apoptosis was analyzed using Acridine orange/Ethidium bromide (AO/EtBr) fluorescence staining method[19]. A549 cells were cultured in the 6 well plate with DMEM medium. Then the cells were treated with IC_{50} (80µg/mL) concentration of Dd-AuNPs and incubated for 24h. After that, the untreated and treated cells were stained with AO/EtBr stain mixture. The stained cells were analyzed in the fluorescence microscope (Leica DMi8, 10X).

Statistical Analysis

All the data were evaluated using the statistical software SPSS/16. Hypothesis-testing methods included one-way analysis of variance (ANOVA) followed by least significant difference test. P-values less than 0.05 were considered statistically significant. All the results were expressed

as mean ± standard deviation (n = 3).

3. Results and Discussion

Synthesis of Dd-AuNPs

The present research work describes about the biogenic, Dyctyota dichotoma aqueous extract mediated synthesis of gold naoparticles under the bright sunlight. The light yellow colour gold chloride solution was changed to red colour after adding the Dyctyota dichotoma aqueous extract in 30 minutes at pH:7.4 (Figure 1). Dyctyota dichotoma aqueous extract acts as a capping agent and reduced Au^{3+} to Au. The plant and seed extract of enzymes, proteins, sugars, and phytochemicals like phenolics, flavanoids, terpenoids, cofactors and so on acts as reducing and stabilizing agents[20]. This biogenic and sunlight mediated synthesis of nanoparticles is a cost effective, facile, and non toxic eco-friendly method[21]. Here we have used sunlight as a catalyst for synthesis of AuNPs. The physical and chemical methods are costly, toxic chemical were used for nanoparticles synthesis and need sophisticated instruments for nanoparticles synthesis[22,23].

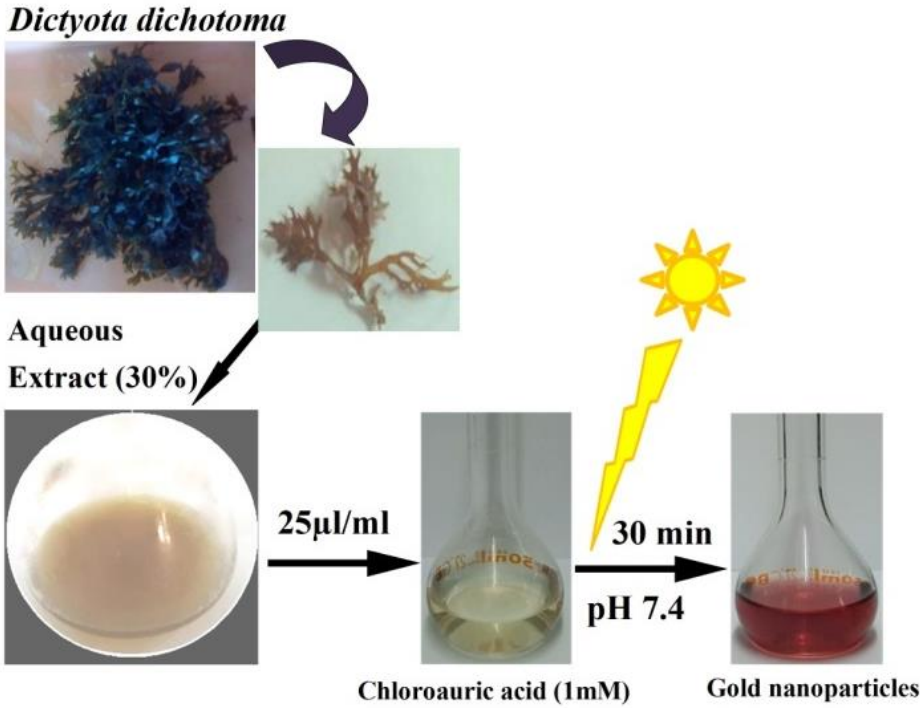

Figure 1: Schematic representation of synthesis of gold nanoparticles using Dyctyota dichotoma aqueous extract under the bright sunlight irradiation.

Characterization of Dd-AuNPs

UV-visible spectroscopy

The UV-visible spectroscopy is a preliminary characterization technique to confirm the optical

nanoparticles synthesis. Generally, The AuNPs are produced SPR peak between 500 to 550nm[24]. The synthesized Dd-AuNPs was produced SPR peak at 526nm that confirmed the AuNPs synthesis (Figure 2). The obtained SPR peak was appeared in sharp, single peak that means the particles is in small size and homogenous. The morphology of the NPs is depending on the extract concentration, composition, pH, temperature and reaction time[25,26.]

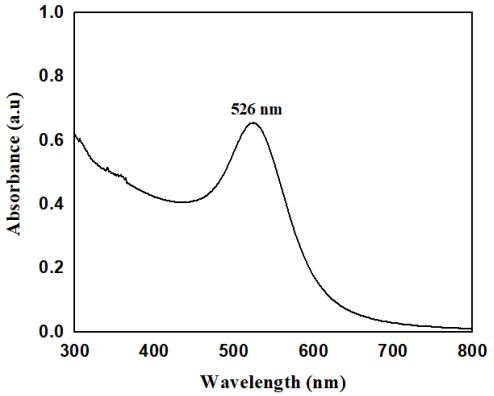

Figure 2: UV-visible spectroscopy analysis of Dd-AuNPs

Dynamic light scattering (DLS) analysis

The size of the nanoparticles in the liquid medium was calculated by DLS method. The average size of the Dd-AuNPs in the water was found to be 47.6nm (Figure 3). Dd-AuNPs size was based on measuring the time dependent fluctuation of scattering of laser light by NPs undergoing Brownian movement[27]. The particles with such smaller size can be widely used for drug delivery applications because they will easily penetrate into the cell membranes and have good suspensibility[28].

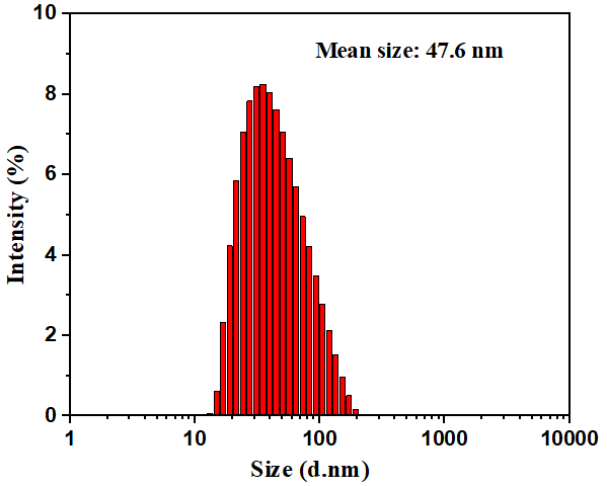

Figure 3: DLS analysis of Dd-AuNPs produced average size of 47.6nm

Zeta potential analysis

The total surface charge of the nanomaterials was calculated by zeta potential and it was found to be -29.5mV (Figure 4). The zeta potential is deduced from the electrophoretic mobility of nanoparticles, it indicates the degree of repulsion between adjacent, similarity charged particles[29]. This strong negative charge of Dd-AuNPs is responsible for prevent the aggregation of particles, exhibiting excellent stability and suggesting less toxicity to normal cells[30].

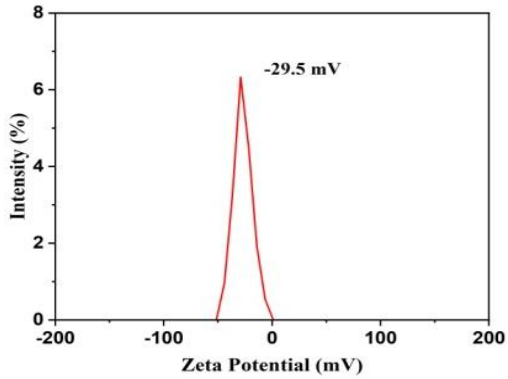

Figure 4: Zeta potential of Dd-AuNPs was found to be -29.5Mv

HR-TEM analysis of Dd-AuNPs

The size and shape of Dd-AuNPs was analyzed through the HR-TEM. The Dd-AuNPs were spherical in shape with monodispersity. The particle size was between 10 to 45nm, and the average particles size was found to be 30nm (Figure 5). The detection of size and shape of nanoparticles is very important in biological applications. A small size nanomaterials is easily cross the cell membrane and it can be used for diverse biomedical application such as drug delivery, gene delivery, biosensing, and so on.

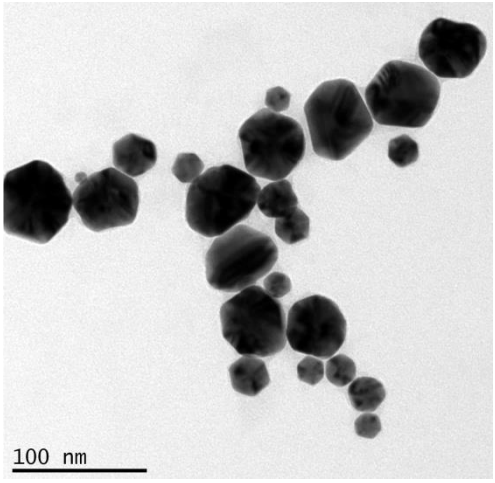

Figure 5: The size and shape of Dd-AuNPs were analyzed by HR-TEM.

Hemolytic Assay

Hemolytic assay is an important and reliable experiment to check the biocompatibility of a material. Hemolysis in-vivo causes harmful effect, so the blood compatibility of nanomaterials is initially evaluated by hemolytic assay using human RBCs[31]. The synthesized Dd-AuNPs induced 0.5% and 2.25% hemolysis for 20µg/mL and 100µg/mL concentration respectively (Figure 6). Previously reported research work has explained that any materials induced >5% of hemolysis at a particular concentration, it will not be suitable for biological applications[32].

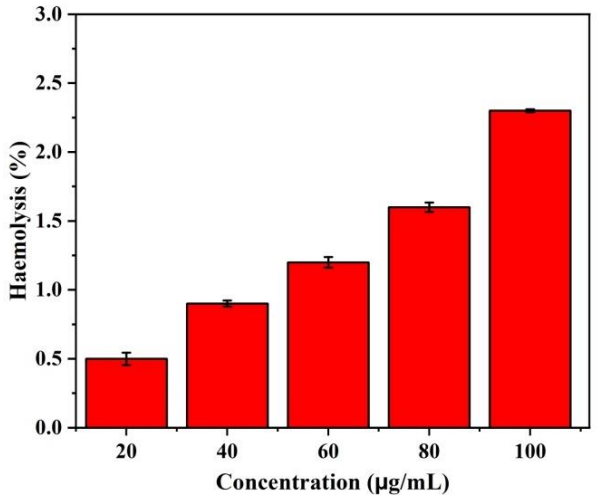

Figure 5: The hemocompatibility of Dd-AuNPs was analyzed by hemolytic assay.

Anticancer activity of Dd-AuNPs

MTT assay is widely used to determine the cytotoxicity of a material. Hence, antiproliferative effect of Dd-AuNPs was analyzed by MTT assay. The viable cells are utilized MTT and converted to formazan crystals by their mitochondrial dehydrogenase enzymes. Further, the formazan crystal is dissolved by DMSO and the cell viability was calculated based on the intensity of OD value[33]. The cell viability of Dd-AuNPs were found to be 90.34%, 78.68%, 64.18%, 50.28%, and 33.96% for 20, 40, 60, 80 and 100µg/ml concentration respectively (Figure 7). Dd-AuNPs inhibited the growth of A549 lung cancer cells in the concentration manner. Cancer cells are highly metabolic and porous in nature, hence internalize the solutes rapidly than the normal cells[34]. The IC_{50} concentration of Dd-AuNPs was found to be 80µg/ml, this concentration was used for the further apoptosis analysis.

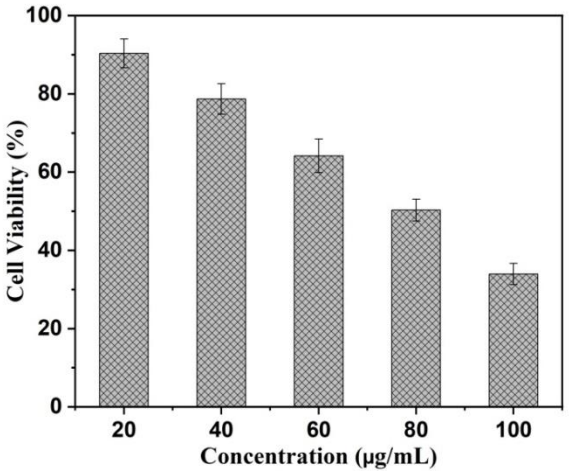

Figure 7: Anticancer activity of Dd-AuNPs analyzed by MTT assay

Apoptosis analysis

Apoptosis is a programmed cell death. The apoptotic effect of Dd-AuNPs was analyzed using acridine orange/ethidium bromide staining. In figure 8, the live and normal cells were appeared in green color in the control, whereas Dd-AuNPs treated cells appeared yellowish green, orange and reddish orange of apoptotic cells[35]. The obtained results confirm that the Dd-AuNPs inhibited the growth of A549 lung cancer cells through the Apoptosis.

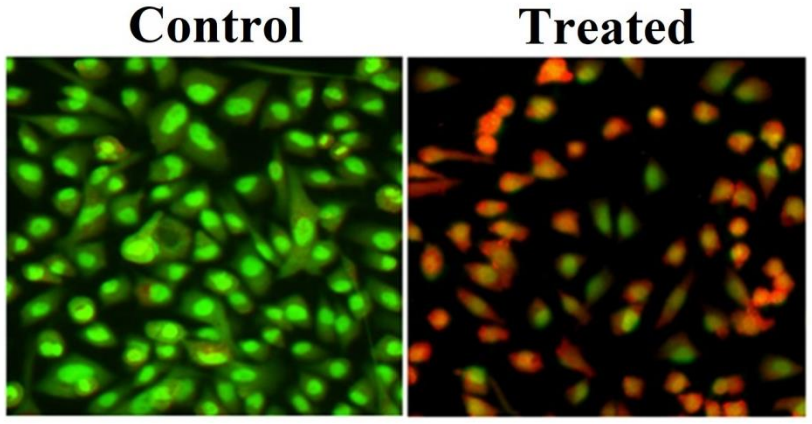

Figure 8: Anticancer activity of Dd-AuNPs analyzed by apoptosis using AO/EtBr fluorescence staining

4. Conclusion

The AuNPs was synthesized in a facile, cost effective, eco-friendly biogenic method using Dyctyota dichotoma aqueous extract. The Synthesized Dd-AuNPs was exhibited in spherical shape with average size of 30nm. Due to the strong negative surface netcharge of Dd-AuNPs

(-29.5mV), it has high stability and the particles will not get aggregation. This stable Dd-AuNPs has highly hemocompatible nature in RBCs and anticancer activity against A549 human non small lung cancer cell line. It inhibited 50% of cancer cell growth at 80µg/ml concentration, which was analyzed by MTT cell viability assay and apoptosis analysis by AO/EtBr fluorescence staining. Hence, this biogenic Dd-AuNPs can be used for various biomedical applications of cancer therapy.

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References

1. Petros, R. A., & DeSimone, J. M. (2010). Strategies in the design of nanoparticles for therapeutic applications. Nature reviews Drug discovery, 9(8), 615-627
2. Rajendran, I., Dhandapani, H., Anantanarayanan, R., & Rajaram, R. (2015). Apigenin mediated gold nanoparticle synthesis and their anti-cancer effect on human epidermoid carcinoma (A431) cells. Rsc Advances, 5(63), 51055-51066.
3. Rastogi, L., & Arunachalam, J. (2011). Sunlight based irradiation strategy for rapid green synthesis of highly stable silver nanoparticles using aqueous garlic (Allium sativum) extract and their antibacterial potential. Materials Chemistry and Physics, 129(1-2), 558-563.
4. Ejaz, A., Mamtaz, Z., Yasmin, I., Shaban, M., Siddique, A. B., Irfan, M. I., ... & Abbas, A. (2024). Cyperus scariosus extract based greenly synthesized gold nanoparticles as colorimetric nanoprobe for Ni2+ detection and as antibacterial and photocatalytic agent. Journal of Molecular Liquids, 393, 123622.
5. Lavanya, G., Anandaraj, K., Selvam, K., Gopu, M., Selvankumar, T., Govarthanan, M., & Kumar, P. (2024). Green synthesis of gold nanoparticles using macroalgae Halimeda macroloba extract and their photocatalytic degradation of methylene blue and methyl orange. Polymers for Advanced Technologies, 35(4), e6383.
6. İpek, P., Baran, M. F., Baran, A., Hatipoğlu, A., Keskin, C., Yildiztekin, M., ... & Cho, W. C. (2024). Green synthesis and evaluation of antipathogenic, antioxidant, and anticholinesterase activities of gold nanoparticles (Au NPs) from Allium cepa L. peel aqueous extract. Biomass Conversion and Biorefinery, 14(9), 10661-10670.
7. Kumar, G. S., Reddy, N. R., Siddiqui, Q. T., Yusuf, K., Pabba, D. P., Kumar, A. S., ... & Joo, S. W. (2024). A facile green synthesis of gold nanoparticles using Canthium parviflorum extract sustainable and energy efficient photocatalytic degradation of organic pollutants for environmental remediation. Environmental Research, 258, 119471.
8. Saravanakumar, K., Chelliah, R., Hu, X., Oh, D. H., Kathiresan, K., & Wang, M. H. (2019). Antioxidant, anti-lung cancer, and anti-bacterial activities of Toxicodendron vernicifluum. Biomolecules, 9(4), 127.
9. Cruz, C. S. D., Tanoue, L. T., & Matthay, R. A. (2011). Lung cancer: epidemiology, etiology, and prevention. Clinics in chest medicine, 32(4), 605-644.
10. Wolf, A. M., Oeffinger, K. C., Shih, T. Y. C., Walter, L. C., Church, T. R., Fontham, E. T., ... & Smith, R. A. (2024). Screening for lung cancer: 2023 guideline update from the American Cancer Society. CA: A Cancer Journal for Clinicians, 74(1), 50-81.
11. Capella, M. P., Pang, S. A., Magalhaes, M. A., & Esfahani, K. (2024). A Review of Immunotherapy in Non-Small-Cell Lung Cancer. Current Oncology, 31(6), 3495-3512.
12. Yayintas, O. T., Demir, N., Canbolat, F., Ayna, T. K., & Pehlivan, M. (2024). Characterization, biological activity, and anticancer effect of green-synthesized gold nanoparticles using

Nasturtium officinale L. BMC Complementary Medicine and Therapies, 24(1), 346.
13. Palaniyandi, T., Viswanathan, S., Prabhakaran, P., Baskar, G., Wahab, M. R. A., Sivaji, A., ... & Kumarasamy, S. (2023). Green synthesis of gold nanoparticles using Halymenia pseudofloresii extracts and their antioxidant, antimicrobial, and anti-cancer activities. Biomass Conversion and Biorefinery, 1-12.
14. Hwang, I. K., Kim, H. S., & Lee, W. J. (2005). Polymorphism in the brown alga Dictyota dichotoma (Dictyotales, Phaeophyceae) from Korea. Marine biology, 147, 999-1015.
15. Rabanal, M., Ponce, N. M., Navarro, D. A., Gómez, R. M., & Stortz, C. A. (2014). The system of fucoidans from the brown seaweed Dictyota dichotoma: Chemical analysis and antiviral activity. Carbohydrate polymers, 101, 804-811.
16. Ramadurai, M., Rajendran, G., Bama, T. S., Prabhu, P., & Kathiravan, K. (2020). Biocompatible thiolate protected copper nanoclusters for an efficient imaging of lung cancer cells. Journal of Photochemistry and Photobiology B: Biology, 205, 111845.
17. Muthukumarasamyvel, T., Rajendran, G., Santhana Panneer, D., Kasthuri, J., Kathiravan, K., & Rajendiran, N. (2017). Role of surface hydrophobicity of dicationic amphiphile-stabilized gold nanoparticles on A549 lung cancer cells. ACS omega, 2(7), 3527-3538.
18. Rajendran, K., Rajendran, G., Kasthuri, J., Kathiravan, K., & Rajendiran, N. (2019). Sweet corn (Zea mays L. var. rugosa) derived fluorescent carbon quantum dots for selective detection of hydrogen sulfide and bioimaging applications. ChemistrySelect, 4(46), 13668-13676.
19. Rajendran, G., Rajamuthuramalingam, T., Jesse, D. M. I., & Kathiravan, K. (2019). Synthesis and characterization of biocompatible acetaminophen stabilized gold nanoparticles. Materials Research Express, 6(9), 095043.
20. Kharissova, O. V., Dias, H. R., Kharisov, B. I., Pérez, B. O., & Pérez, V. M. J. (2013). The greener synthesis of nanoparticles. Trends in biotechnology, 31(4), 240-248.
21. Annadhasan, M., SankarBabu, V. R., Naresh, R., Umamaheswari, K., & Rajendiran, N. (2012). A sunlight-induced rapid synthesis of silver nanoparticles using sodium salt of N-cholyl amino acids and its antimicrobial applications. Colloids and surfaces B: Biointerfaces, 96, 14-21.
22. Dozol, H., Mériguet, G., Ancian, B., Cabuil, V., Xu, H., Wang, D., & Abou-Hassan, A. (2013). On the synthesis of Au nanoparticles using EDTA as a reducing agent. The Journal of Physical Chemistry C, 117(40), 20958-20966.
23. Wan, J., Wang, J. H., Liu, T., Xie, Z., Yu, X. F., & Li, W. (2015). Surface chemistry but not aspect ratio mediates the biological toxicity of gold nanorods in vitro and in vivo. Scientific reports, 5(1), 11398.
24. Bhaumik, J., Thakur, N. S., Aili, P. K., Ghanghoriya, A., Mittal, A. K., & Banerjee, U. C. (2015). Bioinspired nanotheranostic agents: synthesis, surface functionalization, and antioxidant potential. ACS Biomaterials Science & Engineering, 1(6), 382-392.
25. Dwivedi, A. D., & Gopal, K. (2010). Biosynthesis of silver and gold nanoparticles using Chenopodium album leaf extract. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 369(1-3), 27-33.
26. Gericke, M., & Pinches, A. (2006). Biological synthesis of metal nanoparticles. Hydrometallurgy, 83(1-4), 132-140.
27. Prathna, T. C., Raichur, A. M., Chandrasekaran, N., & Mukherjee, A. (2014). Sunlight irradiation induced green synthesis of stable silver nanoparticles using citrus limon extract. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 84, 65-70.
28. Periyathambi, P., Vedakumari, W. S., Bojja, S., Kumar, S. B., & Sastry, T. P. (2014). Green biosynthesis and characterization of fibrin functionalized iron oxide nanoparticles with MRI sensitivity and increased cellular internalization. Materials Chemistry and Physics, 148(3), 1212-1220.
29. Gautam, A., & van Veggel, F. C. (2013). Synthesis of nanoparticles, their biocompatibility, and toxicity behavior for biomedical applications. Journal of Materials Chemistry B, 1(39), 5186-

5200.
30. Paul, P., Chatterjee, S., Pramanik, A., Karmakar, P., Chandra Bhattacharyya, S., & Kumar, G. S. (2018). Thionine conjugated gold nanoparticles trigger apoptotic activity toward HepG2 cancer cell line. ACS Biomaterials Science & Engineering, 4(2), 635-646.
31. Liu, X., Huang, H., Liu, G., Zhou, W., Chen, Y., Jin, Q., & Ji, J. (2013). Multidentate zwitterionic chitosan oligosaccharide modified gold nanoparticles: stability, biocompatibility and cell interactions. Nanoscale, 5(9), 3982-3991.
32. Sarath Chandra, V., Baskar, G., Suganthi, R. V., Elayaraja, K., Ahymah Joshy, M. I., Sofi Beaula, W., & Narayana Kalkura, S. (2012). Blood compatibility of iron-doped nanosize hydroxyapatite and its drug release. ACS applied materials & interfaces, 4(3), 1200-1210.
33. Radha, G., Balakumar, S., Venkatesan, B., & Vellaichamy, E. (2015). Evaluation of hemocompatibility and in vitro immersion on microwave-assisted hydroxyapatite–alumina nanocomposites. Materials science and engineering: C, 50, 143-150.
34. Nune, S. K., Chanda, N., Shukla, R., Katti, K., Kulkarni, R. R., Thilakavathy, S., ... & Katti, K. V. (2009). Green nanotechnology from tea: phytochemicals in tea as building blocks for production of biocompatible gold nanoparticles. Journal of materials chemistry, 19(19), 2912-2920.
35. Krishnaraj, C., Muthukumaran, P., Ramachandran, R., Balakumaran, M. D., & Kalaichelvan, P. T. (2014). Acalypha indica Linn: biogenic synthesis of silver and gold nanoparticles and their cytotoxic effects against MDA-MB-231, human breast cancer cells. Biotechnology Reports, 4, 42-49.