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Anticancer Effect of Green Synthesized Gold Nanoparticles Using Orchid Extract and their Characterizations on Breast Cancer AMJ-13 Cell Line.

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ABSTRACT
Gold nanoparticles (AuNPs) have shown numerous unique characteristics, such as biocompatibility, and are an excellent factor for biological applications. In this work,Gold nanoparticle green synthesis was tested using orchid plant extract, and their efficacy against EMJ cells was evaluated in vitro breast cancer.The synthesized AuNPs were described by UV-Vis (UV-Vis spectrophotometer), FTIR (Fourier Transform Infrared Spectroscopy), and AFM(atomic power microscopy) TEM (transmission electron microscopy). The absorbent gold nanoparticles in the UV spectrum were 528 nm. Sphere morphology for synthesized AuNPs ranging in size from 14 to 50 nm was shown for the AFM and TEM analyses. The cytotoxicity of AuNPs was measured with MTT testing on breast cancer cell lines. Selected gold nanoparticles have different concentrations (6.25.12.5,25,50,100) µg / ml. The inhibition rate of cancer cells depends on the AuNPs concentration, as the concentration increase the percent of inhibition increase. The results confirmed the strong anti-cancer agent of green synthesized AuNPs, which is promising for biomedical applications.

Keywords: green synthesis, gold nanoparticles, orchid plant, anti-cancer.

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Introduction

Their new and different characteristics attract the synthesis of noble metal nanoparticles compared to macroscopic ones who make it possible to apply in various fields, such as medicine, biotechnology, antimicrobials, catalysis, optics, etc.[1].Nowadays, the use of therapeutic nanomaterials in medicine is increasing continuously; also, The materials used for nanomaterial synthesis should be taken into account. Physically and chemically synthesized nanoparticles are less active or cause toxic side effects. Hence, using biological systems is an eco-friendly, less toxic, safe, and less time-consuming process called green chemistry compared to other synthesis methods [2]. Therefore, the green synthesis of nanoparticles is more biologically significant [3]. The synthesis of nanoparticle using beneficial plant materials is an interesting method as compared withthe common method of synthesis. As plants are available, Widespread distribution, easily and at the same time safe in use, therefore, this method of synthesis will be developing and implementing [4,5]. The characteristic features of nanoparticles, such as shape, size, surface area distribution, and aggregation, need to be estimated before studying toxicity or biocompatibility [6]. Since nanoparticles have the attraction towards acidic environments that represented in cancer cells, it was thought that selective targeting planning with nanoparticles encourages the effectiveness of cancer detection and therapy with reduced side effects that affect the normal cells [7,8]. The interfaces with biological molecules or structures provide all nanomaterial functionalities due to the similar size of nanomaterials and most biological molecules. So, for both in vivo and in vitro biomedical experiments and applications, Nanoparticles may be useful [9]. Green synthesized gold nanoparticles (AuNPs) are more efficient between all nanoparticles due to their noticeable Plasmon resonance and bio-conjugation property withbimolecular mediums [10-12].AuNPs have perfect features such as biocompatibility, controlled size, and stability, which make them the best drug nano-carriers and prospect agent in diagnosis and therapy of cancer [13]. Cancer is one of the major factors that threatening the health of the human population,Which classifies the world's second cause of death [12].So, it is necessary to find a herbal plant medicine in order to synthesize gold nanoparticles, which is an efficient anti-cancer with few side effects as well as cost-effective. Gold nanoparticles used extensively for medical and biological applications such as antioxidant, antibacterial, diagnostics, treatment, hygiene, and prevention [14-17]. The synthesis of AuNPs using plant extracts like pineapple [18], acacia gum [19], and other plant materials [20,21] have been reported. In the current study, gold nanoparticles were synthesized by using orchid plant extract and determined its anti-cancer activity.

Materials and methods

Materials

Chemicalmaterials were supplied by HIMEDIA company-India and MERCK Company-Germany. Chloroauric acid (HAuCl4.3H2O) from MERCK Company-Germany. Acacia gum.

Methods

Gold nanoparticles synthesis using orchid plant extract:

One gram of HAuCl4.3H2O was dissolved in 200 mL of distilled water by a volumetric flask (500 mL) and then completed to 250 mL to make a ten mM. This stock solution of gold (III) salt. Then 10ml of HAuCl4.3H2O solution was added to 240ml distilled water in the beaker or Erlenmeyer flask on a stirring hotplate with a magnetic stir bar. The solution was heated to 60-70°C. Then, quickly 0.2g from orchid was added with small pieces of Arabic gum (as a stabilizing agent) to the rapidly stirred solution. The solution was picked away from the heater when the solution turned ruby red, which indicates the gold nanoparticles formation and reduce Au^{+3} to Au^0.

Gold nanoparticles characterization

UV-Visible spectrometer: was used to characterize the synthesized AuNPs for their absorption peak; the absorbance spectra were obtained using UV-Vis spectroscopy (Shimadzu, Japan).

Atomic force microscope (AFM): Provides 3D visualization capability. AFM - (SPM AA 3000, USA) was used to describe quantitative and qualitative information on numerous physical characteristics such as morphology, size, surface texture, and roughness.

Transmission electron microscope (TEM): The morphological characteristic, including distribution, size, and shape, was analyzed by using TEM (Philips CM 100, Holland).

Fourier transform infrared spectroscopy (FTIR): The FTIR was used to determine the active biomolecules associated with gold nanoparticles. FTIR technique used to achieve an infrared spectrum of nanoparticles absorption and emission. The measurement of the absorption spectrum of the FTIR was done at room temperature between 4000–400 cm_1 for the extraction of the aqueous plant by using FT-IR (Shimadzu-8400) spectrometer.

MTT cell viability assay

The synthesized gold nanoparticular cytotoxic effects were performed with the AMG-13 cell line breast cancer by using MTT assay. The breast cancer cells were distributed into 96-well plateswith a density of1 × 10^4cells/well for24 h. Subsequently, AMJ-13cells were treated with AuNPs at different concentrations (6.25,12.5,25,50,100 µg/ml). After 72 hours of therapy, cell viability is assessed with medium removal, 28 µL 2 mg / mL MTT solution, and cell incubation at 37 ° C for 2.5 hours. After removing the MTT solution, 130 µL DMSO (Dimethyl sulfoxide) was added to the remaining crystals in the wells to solubilized it then followed by incubation at 37 ° C with shaking for about 15 minutes [22]. The absorbency was estimated at 492 nm (test wavelength) on a microplate reader; the test was performed in triplicate. The cell growth inhibition rate (cytotoxicity percentage) was calculated according to the following equation: -

Cytotoxicity = A-B/A *100, where Aand B are the control optical density (untreated cells) and the optical test density (treated cells with AuNPs).

Statistical analysis

All data obtained is evaluated statistically using unpaired t-test with Graph Pad Prism 6. The values were reported as the mean ± SEM of three independent observations [23].

Results and discussion

Synthesis and development of nanoparticles with optoelectronic and exceptional Physico-chemical properties is the basis in nanoscience. Current research reveals the gold ions (Au^{+3}) are reduced into gold nanoparticles by green synthesis method using natural material reduces aqueous $HAuCl_4.3H_2O$ and pharmacologically important orchid flower extract.

Characterizations of gold nanoparticles

The AuNPs formation was monitored when the color of the solution was changed from yellow to ruby-red, and the typical Plasmon resonance peak was present around 520–540 nm [12].UV–visible absorption spectrum of green synthesized AuNPs with orchid as reducing agent showed surface Plasmon resonance absorption peak centered around 533 nm (Fig.1). The summit is distinctive in spherical AuNPs of 30 – 50 nm in diameter. [24,25].

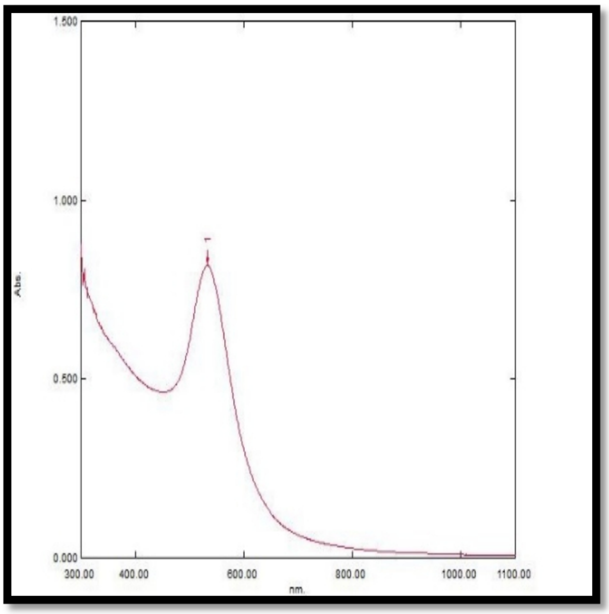


Figure. 1. Spectrum absorption of AuNPs using an orchid plant as an agent for reducing with maximum peak at 533nm.

AFM analysis assessed the presence and size of synthesized AuNPs. Furthermore, it clarifies quantitative and qualitative information on many physical characteristics, including morphology, size, roughness, and surface texture. Two and three-dimensional images were created from AFM analysis, AuNPs images were confirmed to have constant distribution, spherical in shape and the nanosized particles of approximately 40nm (Fig.2).

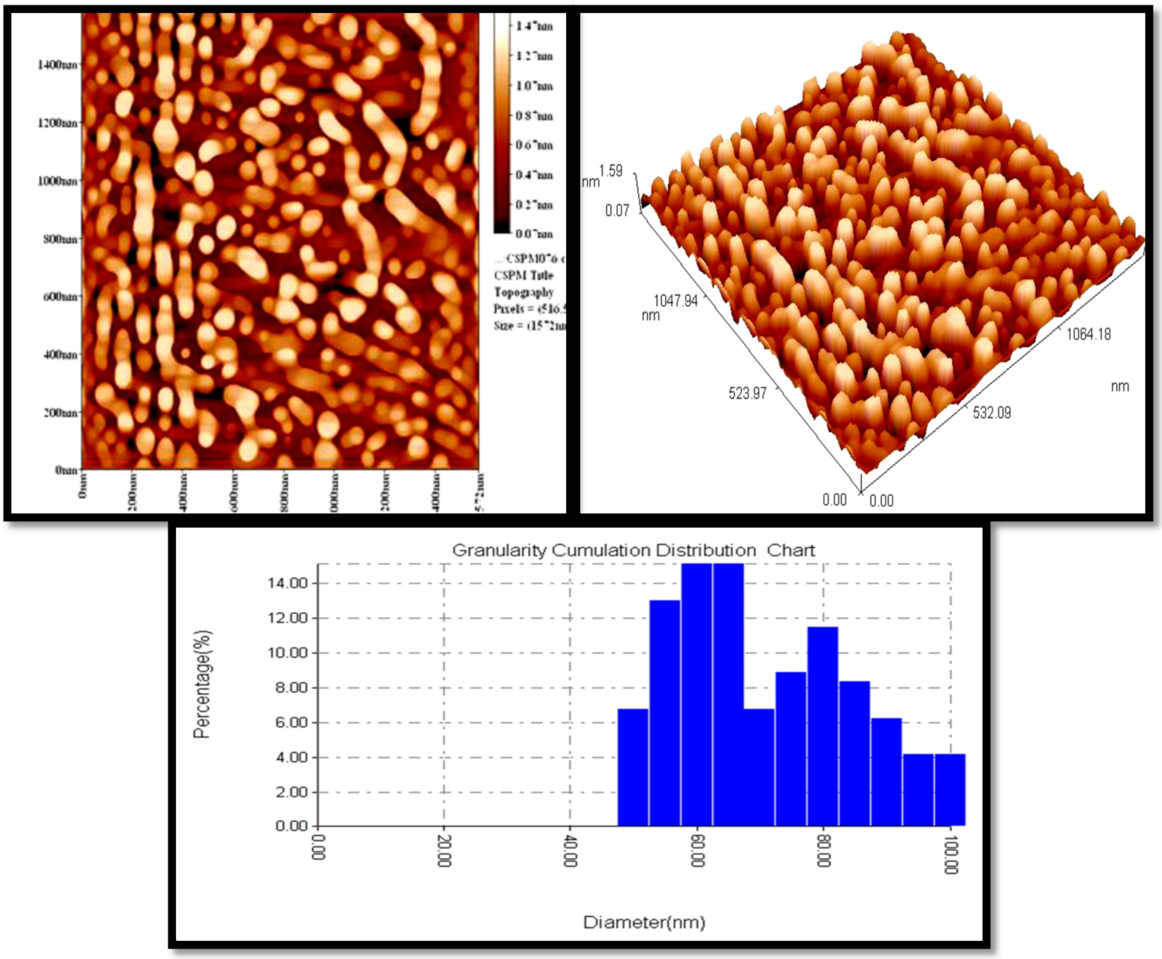
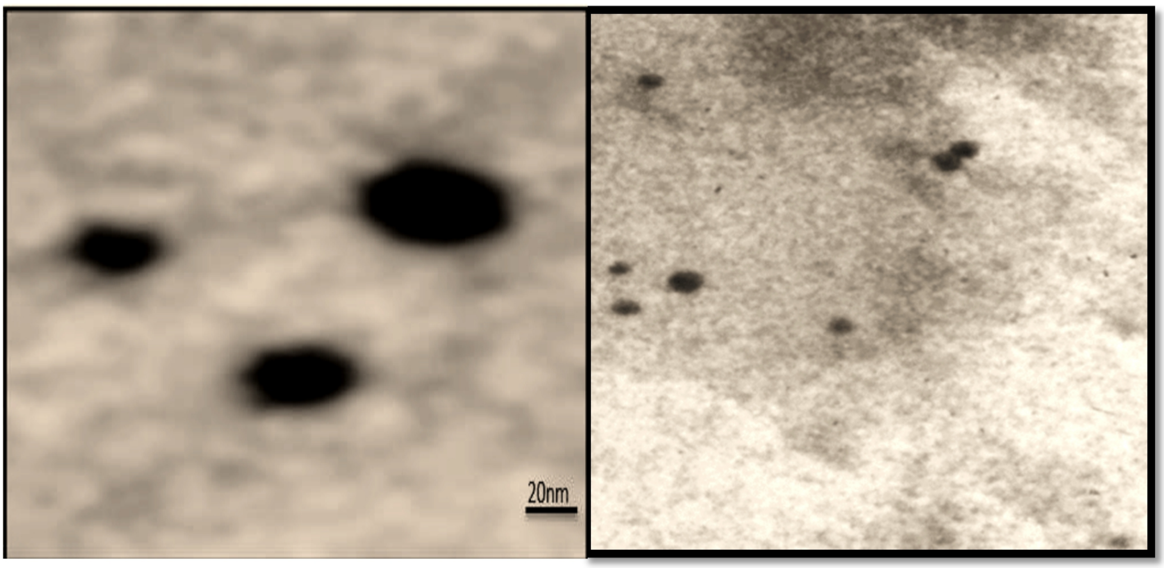


Figure. 2. AFM image (2D and 3D) and distribution chart of synthesis AuNPs using orchid as a reducing agent.

TEM analysis: was employed to confirm the formation of AuNPs and properties determined the crystal structure, morphology, and AuNPs particle size. Fig.3 shows the TEM image of the nanosize distribution of the AuNPs, which possess spherical morphology 14-50 nmin diameter. The present result was consistent with UV–Vis and analysis of AFM. Where the green synthesizes of AuNPs by using orchid extract as a reducing agent are spherical in shape and the hexagonal shape (Figure 3),

Figure 3. AuNP's TEM image with orchid as a reduction agent resulting in 14-50 nm particle size **(high and low magnification).**



FTIR analysis was used in order to identify the potential bio-molecules which are responsible for the gold ions reduction to bio-reduced AuNPs.Figure 4 revealed the FTIR spectrum of synthesized AuNPs.AuNPs' maximum peak range between

1000-3500cm 1.The 1355 cm-1 spectrum of AuNPs equates to C-O extending phenyl vibration. The 1623 cm-1 band combines polysaccharides and pectin. The 2207 cm-1 band corresponds to the water molecule. The band that equal to 1009 cm-1 was related to ribose extract. The C=O amide band is equal to 1639 cm-1, The 2042 cm-1 band is equal to fatty acid and lipids, and the band is equal to OH bond at 3442 cm-1, And the NH amide is at 3249cm-1.

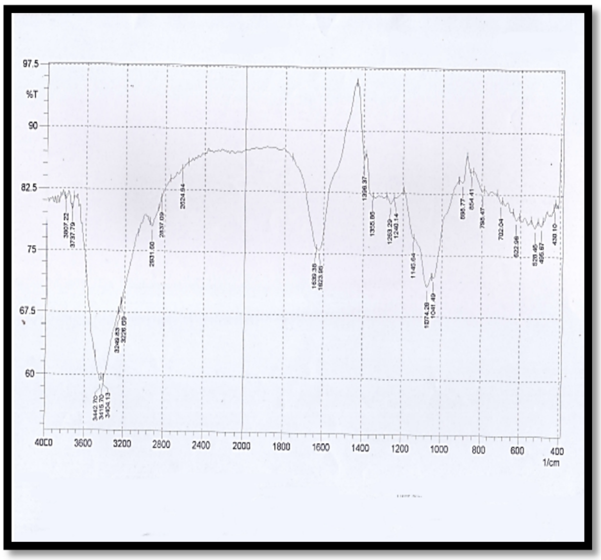


Figure 4. FTIR spectra of synthesized AuNPs using orchid as a reducing agent.

Cytotoxicity effect of AuNPs:

Breast cancer is a major health problemandthe most common cancer in women worldwide. Therefore, Nanotechnology offers promising possibilities for the diagnosis and treatment of human cancer. In this study, the cell lines of AMJ13 breast cancer were used to estimate the in vitro cytotoxic activity of gold nanoparticles. The treatment of cell lines was done with different concentrations of AuNPs (6.25, 12.5, 25, 50, 100 µg/ml) for 72 hours. Table (1) shows the results which varied according to the concentration and the inhibition percentage of cancer cells, which was found to increase with AuNPs concentration. In Fig. 5, the cytotoxic effect of green Synthesized AuNPs have been shown to decrease breast cancer cell viability in the manner of dose-dependent. MTT tests were conducted to evaluate AuNPs with 50 % inhibition of cancer cell line, IC50 achieved with a value of 14.56 µg / ml in the AMJ13.

Table 1. Cytotoxic activity of AuNPs in AMJ13 breast cancer cells after 72 hours.

AuNPsconcentration(µg/ml)	Cytotoxicity %
6.25	22.00±2.517
12.50	43.33±3.480
25.00	57.67±3.520
50.00	75.68±2.963
100.00	83.00±2.880

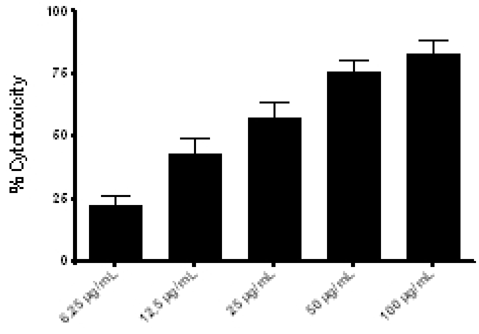


Figure 5. Cytotoxic effect of AuNPs nanoparticles in AMJ-13 cells. IC50=14.56 µg/ml

The MTT (cytotoxic) test exposed the activity of AuNPs as a strong anti-cancer against AMJ-13 cells depending on nanoparticles concentration. The significantincrease in the concentration of green synthesized AuNPs leads to increased cell death ofcancer.Generally, AuNPs in normal cells is considered to be nontoxic and biocompatibleamong all the metalnanoparticles [5].The adsorbed effective molecules contained in the orchid plant extracts can recognize the anti-cancer activity of AuNP.AuNPs were analyzed for a cytotoxic effect for 72 h on the viability of cancer cell lines and shown in Fig.6. The proliferation of cellswas reduced significantly in comparisonto the untreated control cells. The control cells maintain the original morphology and have been mostly fixed to the plate. Contrary, after 72hr, the treated cells had high antiproliferation activity and cell morphological changes. A typical characteristic of apoptotic cells, such as isolation from adjacent cells membrane blebbing, was observedand a decrease in the number of cells. This indicates that the nanoparticles can be used in vitro. This study has been achieved the biocompatible gold nanoparticles from plant extract compounds and theirtherapeutic activity against cancer cells.

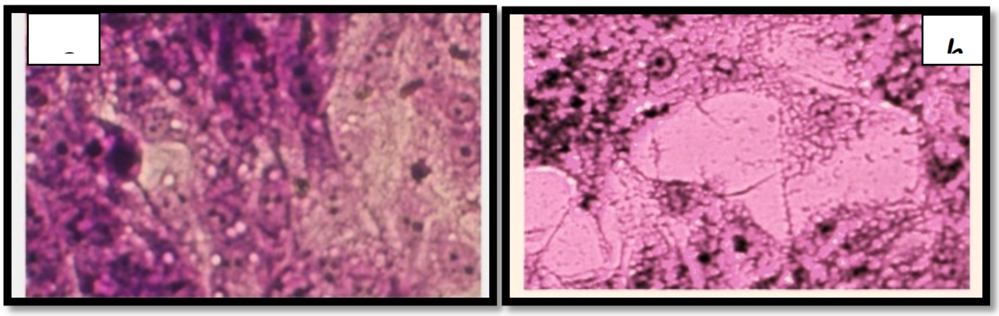


Fig. 6: AuNPs' cytotoxic effect on cancer cell lines viability. a: control cells, b: gold nanoparticles treated cells.

From the previous results, it became clear that the green synthesis of gold nanoparticles is a very important, fast, easy, and effective method. UV-Vis spectroscopy, AFM, TEM, FTIR, Zeta Potential Analysis showed that gold particles were present, confirming nanoparticles of gold formation. At 533 nm, the absorbance of AuNPS, according to the gold surface

Plasmon resonance standard, was observed.These findings correspond to previous literature, whichshows the strong absorption peak of gold nanoparticles around 520-540 nm [12].TEM analysis was carried out to determine the synthesized AuNPs shape, which clearly represents the spherical appearance of gold nanoparticles with size between 14-50 nm and also confirmed with the atomic force microscopic analysis.The spherical gold nanoparticles with nanosize have unique optical and electronic properties [26]. The green synthesized AuNPswere subjected to cytotoxicity analysis with AMJ-13 cancer cell lines in order to assess their anti-cancer activity. As a result, the synthesized AuNPs from the plant extract have effectively inhibited the cancer cells with a dose-dependent manner, which is compatible with previous studies that are used gold nanoparticles as anti-cancer materials with different cancer cells line [27].

Conclusion

In this work, the green synthesis of gold nanoparticles was achieved using the orchid plant as a reducing agent and was confirmed by several characterization analyses such as UV absorbance, AFM, TEM, and FTIR.

The surface plasm resonance peak was initially defined by the ruby-red formation and UV-vis analysis at 533 nm. Also, fine distribution,the nanosize, spherical shape of gold nanoparticles were determined by usingAFM and TEM analysis.The activity of synthesized gold nanoparticles as an anti-cancer agent was achieved by the MTT test in breast cancer AMJ-13 cell line.The cell proliferation was inhibited by AuNPs and induce cytotoxic effect as a dose-dependent concentrations manner in cancer cells.The activity of anti-cancer was increased with increased AuNP concentration.Thus green synthesized gold nanoparticles were more effective to use in nanomedicine applications in cancer treatment due to high anti-cancer activity.

References

1. Suganthy N., Sri Ramkumar V., Pugazhendhi A., Benelli G., Archunan G. **2017**. Biogenic synthesis of gold nanoparticles from Terminalia arjuna bark extract: Assessment of safety aspects and neuroprotective potential via antioxidant, anticholinesterase, and antiamyloidogenic effects. Environ. Sci. Pollut. Res.
2. Bhau B. S., Ghosh S., Puri S., Borah B., Sarmah D. K., Khan R. **2015**. Green synthesis of gold nanoparticles from the leaf extract of Nepenthes Khasiana and antimicrobial assay. Adv. Mater. Lett**.** 6(1): 55-58.
3. Shankar S.S., Rai A., Ahmad A., et al. **2004**. Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (Azadirachtaindica) leaf broth. J Colloid Interface Sci. 275: 496–502.
4. Abid Ali Khan M.M., Jain D.C., Bhakuni R.S., et al. **1991**. Occurrence of some antiviral sterols in Artemisia annua. Plant Sci.75: 161–165.
5. Tao Wu, Xi Duan, Chunyan Hu, Changqiang Wu, Xiaobin Chen, Jing Huang, Junbo Liu & Shu Cui **2019**. Synthesis and characterization of gold nanoparticles from Abiesspectabilisextract and its anti-cancer activity on bladder cancer T24 cells, Artificial Cells, Nanomedicine, and Biotechnology. 47(1), 512-523.
6. Murdock R.C., Braydich-Stolle L., Schrand A.M., Schlager J.J., Hussain S.M. **2008**. Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique. Toxicol Sci.101:239-53.
7. Yu MK., Park J., Jon S. **2012** Targeting strategies for multifunctional nanoparticles in cancer imaging and therapy. Theranostics. 2:3-44
8. Nawfal N. R. ALrawi, Mohammed Q. AL-Ani, Nahi Y. Yaseen. **2018**. Cytotoxic effect of synthesized silver nanoparticles by pulse laser ablation on breast cancer cell line (amj13) from iraqi patient and normal human lymphocytes. Asian J Pharm Clin Res. 11(4):375-382.
9. Asia H. Al-Mashhadani and Rana M. Yas. **2017**. Study of in vitro and in vivo free radical scavenging activity for radioprotection of cerium oxide nanoparticles. Iraqi Journal of Physics. 15(35): 40-47.
10. Bhattacharya R., Murkherjee P. **2008**. Biological properties of “naked” metal nanoparticles. Adv Drug Deliv Rev. 2008.60:1289–1306.
11. Bhumkar DR, Joshi HM, Sastry M, Pokharkar VB. **2007**. Chitosan reduced gold nanoparticles as novel carriers for transmucosal delivery of insulin. Pharm Res. 24:1415–1426.
12. Rui Liu, Qiang Pei, Tao Shou, Wenjing Zhang, Jing Hu, Wei Li. **2019**.Apoptotic effect of green synthesized gold nanoparticles from Curcuma wenyujin extract against human renal cell carcinoma A498 cells, International Journal of Nanomedicine. 14: 4091–4103.
13. Butong Sun., Nanjun Hu., Leng Han, Yanan Pi, Yu Gao and Kang Chen. **2019**. Anti-cancer activity of green synthesized gold nanoparticles from Marsdeniatenacissima inhibits A549 cell proliferation through the apoptotic pathway, ARTIFICIAL CELLS, NANOMEDICINE, AND BIOTECHNOLOGY. 47(1): 4012–4019.
14. HammadR.Humud, Rasha Waleed AbdulHadi, Asia H. Al-Mashhadani and RanaM.Yas. **2018**. Free radical scavenging activity of gold nanoparticles due to ionizing radiation effect. Indian Journal of Natural Sciences. 8 (46): 13052-13059.
15. Vijayan R., Joseph S., Mathew B. 2018. Eco-friendly synthesis of silver and gold nanoparticles with enhanced antimicrobial, antioxidant, and catalytic activities. IET Nanobiotechnol. 12:850–856.
16. Rana M. Yas and Asia H. Al-Mashhadani. **2016.** In vivo study of nanoparticles as free radical scavengers for radiation protection. Der Pharma Chemica. 8(5):194-200.
17. Dykman L.A., Khlebtsov N.G. **2011**. Gold nanoparticles in biology and medicine: recent advances and prospects. ActaNaturae. 3: 34–55.
18. Dhelal Abdul Ghafoor, Wahran M. Saod, Nazhan Mohammed. **2020**. Green synthesis of goldnanoparticles using Pineapple extract and study their analytical characterization and antibacterial activity**.** Sys Rev Pharm. 11(2): 462 465.
19. **19.**Dhelal Abdul Ghafoor1, Rana M. Yas, Muna Ahmed Saeed. **2019**. The properties of nano-gold particles synthesized by ascorbic acid with acacia gum and sodium hydroxide as stabilizers, Iraqi Journal of Science. 60(10): 2149-2155.
20. Elia P., Zach P., Hazan S., et al. **2014**. Green synthesis of gold nanoparticles using plant extracts as reducing agents. Int J Nanomedicine. 9:4007–4021.
21. Dzimitrowicz A., Jamroz P., di CenzoGC., et al. **2016**. Preparation and characterization of gold nanoparticles prepared with aqueous extracts of Lamiaceae plants and the effect of follow-up treatment with atmospheric pressure glow microdischarge. Arab J Chem.
22. Al-Shammari AM, Salman MI, Saihood YD, Yaseen NY,

Raed K, Shaker HK, Ahmed A, Khalid A, Duiach A. **2016**. In vitro synergistic enhancement of newcastle disease virus to 5-fluorouracil cytotoxicity against tumor cells. Biomedicines. Jan 29;4(1):3.

23. Jabir M.S., Sulaiman G.M., Taqi Z.J., D. Li. **2018**. Iraqi propolis increases degradation of IL-1β and NLRC4 by autophagy following Pseudomonas aeruginosa infection, Microbes and infection.20.89-100.
24. Barnes W.L., Dereux, A., Ebbesen, T.W. **2003.** Surface plasmon sub wavelength optics. Nature, 424, 824–830.
25. Zuber, A., Purdey, M., Schartner, E., Forbes, C. van der Hoek, B., Giles, D., Abell, A., Monro, T., Ebendorff-Heidepriem, H. **2016.** Detection of gold nanoparticles with different sizes using absorption and fluorescence-based method. Sens. Actuators B, 227, 117–127.
26. Chandran SP., Chaudhary M., Pasricha R., Ahmad A., Sastry M. **2006**.Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. BiotechnolProg. 22(2):577–583.
27. Dhelal A. Shabeeb, Adawiya J. haider and Abdulalah T. Mohammed.**2016**. Synthesis of gold nano particles using poly acrylic acid as reducing agent - characterization and in vitro study of anti-cancer cervix (hela) activity. International Journal of Humanities, Arts, Medicine and Sciences. 4(9): 101-108.