

RESEARCH ARTICLE

Green Synthesis of Gold Nanoparticles Using Pomegranate Peel Extract and Its Antioxidant and Anticancer Activity against Liver Cancer Cell Line

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ABSTRACT

Decrease of gold metal particles in contact with the watery strip concentrate of pomegranate organic product finishes the bio-union of gold nanoparticles. At the frequency scope of 350-700nm, an assimilation pinnacle of the biosynthesized gold nanoparticles is noticed. At 530nm, the UV-Visible range of gold nanoparticles orchestrated by pomegranate strip remove shows most extreme pinnacle assimilation. SEM has contemplated the morphological qualities of gold nanoparticles, affirming the round type of gold nanoparticles and their size of around 16nm. The essential investigation of gold nanoparticles blended by pomegranate strip remove has a gold level of 59.90 percent by weight. The gold nanoparticles combined by pomegranate strip extricate showed high cell reinforcement action. The examination shows that orchestrated gold nanoparticles exhibit conceivable anticancer action to build the grouping of gold nanoparticles against the HepG-2 liver malignant growth cell line.

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Background

Nanotechnology is an innovation that manages an assortment of nanoscale materials and their applications, going from 1 to 100 nm[1]. Metal nanoparticles have increased noteworthy thought among various types of nanomaterials because of their exceptional reactant, electronic and optical properties[2]. Gold is utilized as medication in the plan of nano-level SwarnaBhasma for the treatment of tuberculosis, iron deficiency, and hack in the outdated Indian ayurvedic framework and is additionally trusted as an enemy of maturing agent[3]. Because of their uniqueness, especially in bio-prescriptions, gold nanoparticles (Au-NPs) have commonly been investigated[4]. When gone up against with oxygen or in destructive conditions, gold and gold nanoparticles are valuable, dormant and not effectively oxidized.

Contingent upon the scale, shape and measure of the mixture, gold nanoparticles show different tones, for example, red, blue [5,6].

These noticeable tones speak to the movements at appropriate frequencies of conduction band electrons[7]. Gold nanoparticles are profoundly consistent, delicate and have higher consistency norms. They are colossally esteemed and utilized for biomedical applications due to these properties, for example, drug conveyance, imaging, photothermal therapy and immunochromatographic microbe distinguishing proof in food and clinical examples[8].

Different strategies, for example, physical, synthetic and natural procedures, are utilized for the amalgamation of gold nanoparticles. Among these strategies, the green amalgamation method is an eco-circumspect, safer, clean, and less repetitive strategy utilizing natural systems[7]. Punicagranatum (Punicaceae) organic product strips (Pomegranate) are rich in the polyphenolic class of enemies

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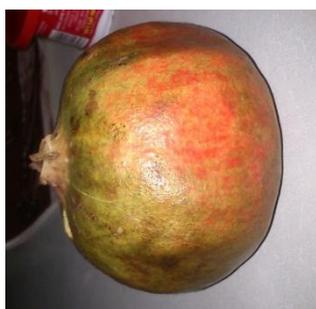
of oxidants containing tannins and flavonoids[9]. Pomegranate strips have been accounted for to show conceivable enemy of oxidant and hostile to malignant growth exercises since they contain polyphenols, for example, tannins, gallic corrosive, and ellagicacid[10, 11]. The strips contain a higher phenolic content than the pomegranate natural product, as detailed in[12]. A straightforward, ease, green blend procedure for integrating gold nanoparticles utilizing pomegranate strip separate was examined in the current investigation. The objective of the examination is to uncover the counter oxidant and hostile to malignant growth capability of pomegranate strip remove combined gold nanoparticles.

Material and Methods

Sigma Aldrich has purchased chemicals such as chloroauric acid (HAuCl_4), 3-(4,5-di-methyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT), Dimethyl sulphoxide (DMSO), DPPH, and Ascorbic acid.

Preparation of Pomegranate Peel Extract

Pomegranate organic products were bought at the nearby organic product market in Poonamallee. The natural product strips were assembled and dried in the shade for 4-5 days. The dried strips were then ground into the best powder. 1g of grounded strip powder was gauged and disintegrated in 100ml of refined water to set up the pomegranate strip remove. The combination was cooked for 10 minutes by warming the mantle at 70°C . The bubbled concentrate of the pomegranate strip was sifted utilizing Whatmann No.1 channel paper. The sifted extricate was prepared for additional utilization in a virus state.



(A) Pomegranate fruit



(B) Pomegranate fruit peels



(C) Fruit peel extract

Fig. 1.

Synthesis of Gold Nanoparticles

10mL of pomegranate strip separate was applied to 90mL of fluid 1mM gold chloride answer for integrate gold nanoparticles. The response blend was prepared for 72 hours in an attractive stirrer. The arrangement of gold nanoparticles was intermittently observed by gold chloride arrangement estimations of the UV-Vis spectra (350-700nm). The shading movement of the gold chloride arrangement, which for starters shows the bio-decrease and development of gold nanoparticles was noticed and noted. Biosynthesized gold nanoparticles were gathered for 10 minutes by centrifugation at 8000rpm. The gold nanoparticle pellet acquired was washed 3-4 times with twofold refined water and afterward washed for 2 hours in a hot air stove at 70°C . The powdered gold nanoparticles were put away for additional investigations in water/air proof vials.

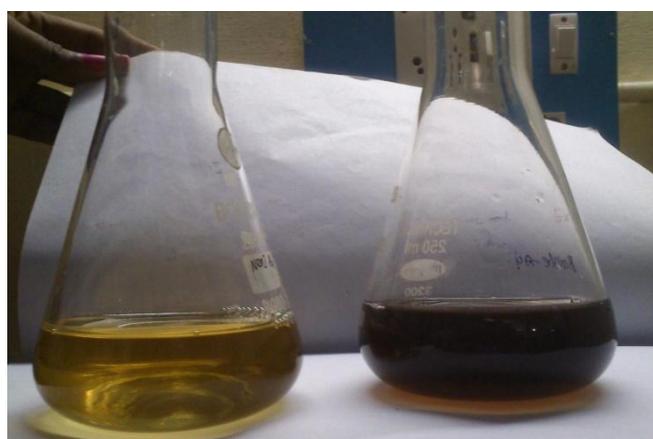


Fig. 2. Gold solution and Gold solution with peel extract at initial time period.

Characterization

Using the UV-Vis spectrophotometer (uv-2450, Shimadzu), the optical properties of blended Pomegranate strip extricate interceded gold nanoparticles were estimated in the frequency scope of 350-700nm. Checking electron magnifying lens (SEM) examination was utilized to analyze the morphological qualities of biosynthesized gold nanoparticles, for example, scale, shape. Furthermore, the EDX finder has affirmed the presence of essential gold.

Cancer Prevention Agent Action

The framework DPPH (1,1-diphenyl-2-picryl-hydrazil) was utilized to break down the cell reinforcement action of gold nanoparticles interceded by Pomegranate strip remove and the strategy was archived (Rajeshkumar,2017). Various focuses (2-10 $\mu\text{g/ml}$) of mediated gold nanoparticle pomegranate strip were joined with 1mL of 0.1 mM DPPH in methanol and 450 μl of DPPH in methanol Incubated for 30 minutes with 50 mMTrisHCl cradle (pH 7.4). The abatement in the measure of DPPH free extremists was estimated based on absorbance at 517 nm after hatching. As force, BHT was used. From the going with condition, the rate limitation was determined, Incubated for 30 minutes with 50 mMTrisHCl cradle (pH 7.4). The reduction in the measure of DPPH free extremists was estimated based on absorbance at 517 nm

after brooding. As force, BHT was used. From the going with condition, the rate limitation was determined,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Absorbance of Control

Anticancer Action

The cytotoxicity of gold nanoparticles intervened by pomegranate strip extricate was inspected utilizing the MTT test as per the technique expressed in (Rajeshkumar, 2016). In 96 well plates at a convergence of 1×10^4 cells/well, the HepG-2 cells were plated independently. Cells were washed twice with 100 μ L of serum medium following 24 hours and starved at 37 °C for 60 minutes. The cells were treated with various groupings of biosynthesized gold nanoparticles (1, 10, 25, 50, 100 μ g/ml) for 24 hours after starvation. The medium was sucked to the furthest limit of the treatment time-frame and serum free medium containing MTT (0.05 mg/ml) was incorporated and brooded in a CO2 hatcher for 4 h at 37 °C. For normal untreated cell lines, the inhibitory focus esteem (IC) of the biosynthesized gold nanoparticles was set up. The medium containing MTT was then discarded and the cells were washed (200 μ L) with PBS. By including 100 μ L of DMSO, the precious stones were then broken up and this was mixed effectively. In a microplateperuser, the assimilation of the purple-blue formazan shading was estimated at 570nm (Biorad 680). Utilizing Graph cushion crystal 5 program, cytotoxicity was survived. The cycle depends on the decrease by metabolically powerful cells of dissolvable yellow tetrazolium salt to insoluble purple formazan gems. Tetrazolium salt must be devoured by live cells. The impetus (succinate dehydrogenase) present in live cell mitochondria will move from tetrazolium salt to formazan precious stone to formazan gem.

Result and Discussion

Visual Perception

At the point when the pomegranate strip separate was presented to the gold particle arrangement, the bio-decrease of gold particles into gold nanoparticles was outwardly distinguished by a shading change from yellow to dim pink. In refined water, gold particles normally show a yellow tint. The pomegranate strip extricate fills in as a decrease specialist to change over gold particles into gold nanoparticles that are outwardly perceived by the progress in shading from yellow to pink. . Variety in shading shift was noticed and it was basically reliant on the time interval and the concentrate of pomegranate strip phytoconstituents. Because of the excitation of surface plasmon vibration with gold nanoparticles [13,14], shading changes happen.

Following one hour of blending the response combination into the attractive stirrer, the dull pink tone is framed. Following 24 hours of hatching in the attractive fomenter, the decrease cycle was finished and the precipitation of gold nanoparticles at the lower part of the cone shaped carafe was identified[15].



Fig. 3. Bioreduction of gold ion into gold nanoparticle (yellow to dark pink)

Characterization

UV-visible Spectroscopy

The surface plasmon reverberation of gold nanoparticles brings about an expansive absorbance band in the frequency scope of 350-700nm that can be estimated by UV-Visible spectroscopy[16,17]. Gold nanoparticles display a particular optical trademark commonly known as surface plasmon reverberation (SPR). The UV-Visible range of gold nanoparticles incorporated by pomegranate strip remove shows its greatest retention top at 530 nm in Fig 4. The greatest retention top at 530 nm demonstrates that gold nanoparticles are framed and the capacity of pomegranate strip remove is additionally diminished.

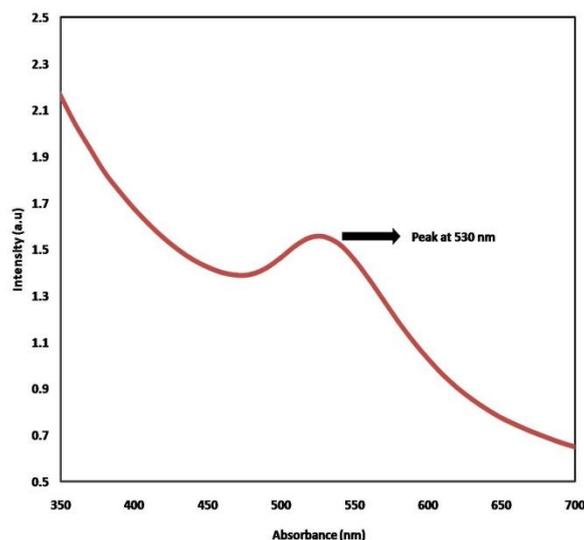


Fig. 4. UV-Visible spectra of bio-synthesized gold nanoparticles

SEM-EDX Analysis

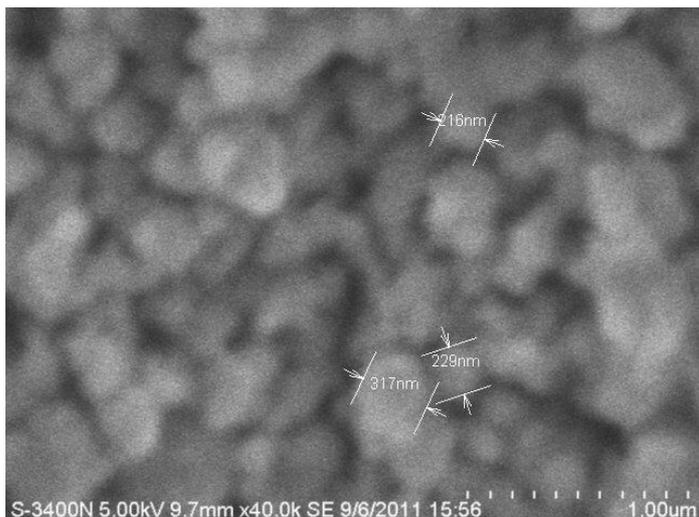


Fig. 5. SEM image of gold nanoparticles synthesized using pomegranate peel extract.

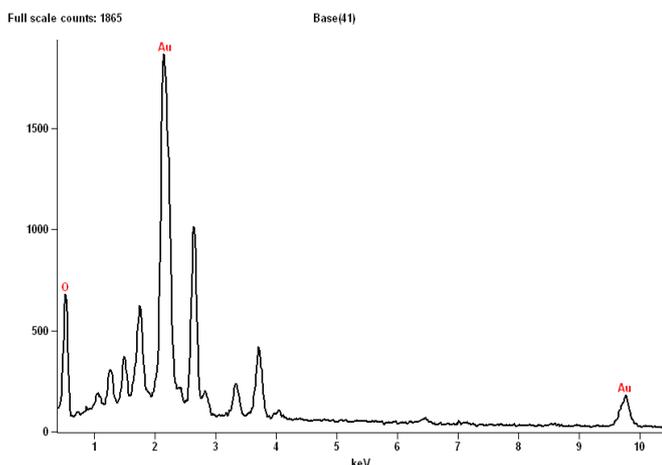


Fig. 6. EDX spectra of the biosynthesized gold nanoparticles.

Fig 5 shows the SEM outline of bio-incorporated gold nanoparticles. Utilizing an examining electron magnifying instrument, the morphological attributes of gold nanoparticles were broke down. The SEM picture plainly unveils the improvement of round gold nanoparticles and more modest nanoparticles were found at a normal size of 16 nm. The decrease and covering limit of phyto exacerbates present in pomegranate strip extricate is likewise helped. Fig. Fig. 6 mirrors the range of energy dispersive X-beam investigation of gold nanoparticles combined as a diminishing specialist utilizing pomegranate strip extricate. In the gold field, the biosynthesized gold nanoparticles unveil the most grounded flag and affirm the arrangement of gold nanoparticles. The EDX spectra shows that the gold nanoparticles combined by the concentrate of the pomegranate strip have a gold weight level of 59.90 percent and pollutants, for example, silicon (Si) alongside them.

Anticancer Action

The anticancer impact of gold nanoparticles interceded by pomegranate strip extricate was assessed against the HepG-2 liver malignancy cell line. To survey the anticancer movement, various convergences of biosynthesized gold nanoparticles, for example, 1µg, 10 µg, 25 µg, 50 µg and 100 µg were utilized. As the gold nanoparticle fixation expands, the anticancer impact of gold nanoparticles against the HepG-2 liver malignant growth cell line shows higher movement. The level of cell practicality was appeared in the HepG-2 line of liver malignancy cells (Fig 6). To analyze the anticancer impact of gold nanoparticles, cyclophosphamide is utilized as the standard. When contrasted with ordinary cyclophosphamide, gold nanoparticles show great execution. The counter malignancy impact of gold nanoparticles is the result of communications between gold molecules and the practical gatherings of DNA intracellular proteins, nitrogen bases, and phosphate groups[18]. In this review.

Table 1. Represents anticancer effect of bio-synthesized gold nanoparticles

HepG2	1 µg	10 µg	25 µg	50 µg	100 µg
Treatment	% of cell viability				
Gold NPs	84.81	75.87	33.38	22.81	16.9
Cyclophosphamide	87.81	73.4	23.21	1.93	1.48

Antioxidant Activity

The free revolutionary rummaging action of pomegranate strip remove intervened gold nanoparticles was dictated by utilizing DPPH test. DPPH is a steady free radicle. Any atom which contributes an electron or hydrogen to DPPH, it reacts and brings about difference in shading [19]. The level of staining uncovers the searching capacity of the cell reinforcement compound [20]. As convergence of bio-orchestrated gold nanoparticles builds there is a slow decrease in the absorbance esteems at 517 nm. Subsequently this outcome affirms the planned cell reinforcement impact of the pomegranate strip remove intervened gold nanoparticles.

Conclusion

This exploration utilizes pomegranate strip concentrate to apply an eco-accommodating way to deal with the combination of gold nanoparticles. The concentrate uncovers the properties of the decrease and balancing out specialist because of the presence of different mixes in the pomegranate natural product strips. Orchestrated gold nanoparticles were at first perceived by a dull pink shading arrangement and the surface plasmon reverberation band at 530 nm was seen by UV-Visible spectrophotometer investigation. The SEM picture shows the nanoparticles' scale, shape and appropriation. The anticancer action of bio-integrated gold nanoparticles was performed by the Hep-G2 liver malignancy cell line test of MTT. The intensified anticancer and cancer prevention agent movement in this examination was discovered to be at a raised centralization of gold nanoparticles. Results are brought about by the chemotherapy specialists utilized in

malignant growth care, while biosynthesized gold nanoparticles fill in as a danger free elective medicine. Along these lines, bio-incorporated nanoparticles have been able for their high enemy of malignant growth viability in biomedical applications in disease therapy.

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