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Research Article

The Potential of 3,6 Dihydroxyflavone Embedded Gold Nanoparticles for Inhibition of Tumor Growth in Presence of Dietary Supplements

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Abstract

Objective: 3,6 dihydroxyflavone reported to be an effective bioreducing agent in the production of metal nanoparticles and also as a chemopreventive agent. The antioxidative and antibacterial effect of 3,6 dihydroxyflavone along with selective dietary compounds both single and in combination has been studied and was reported to be associated with lowering the effect of diseases like cancer and certain other types of degenerative oxidative stress.

Method: Biogenesis of stabilized gold nanoparticles was carried out successfully using a hydrated auric chloride solution reduced with a targeted flavonoid 3,6 dihydroxyflavone following the conditions as reported. The antioxidative and antibacterial analysis of selective compounds 3,6 dihydroxy flavone along with lycopene (antioxidant) and selenium methyl selenocysteine (sensitizer) was estimated using different free radical scavenging assays like -DPPH, -H_2O_2, -OH, etc.

Result: It has been reported from the observed study that all selective dietary phytochemicals showed significant free radical scavenging activity and the triple combination of all the three in equimolar concentration (1:1:1) showed remarkable enhancement in the antioxidative as well as antibacterial activity. The cytotoxic studies relating to the biocompatibility of 3,6 dihydroxyflavone embedded gold nanoparticle also shows excellent cell viability results even up to 100 µl concentration of (AuNp-DHF) solution.

Conclusion: The progress of the work done provides newer insights for the future development and formulation of a stabilized metal nanoparticle immuno-conjugate drug by linking cancer antibodies (both polyclonal & monoclonal), intending to create a force multiplier effect on to the targeted cancer cells.

Keywords: Flavonoid; Nanoparticles; Antioxidant; Cytotoxicity; Therapeutics

Abbreviations:

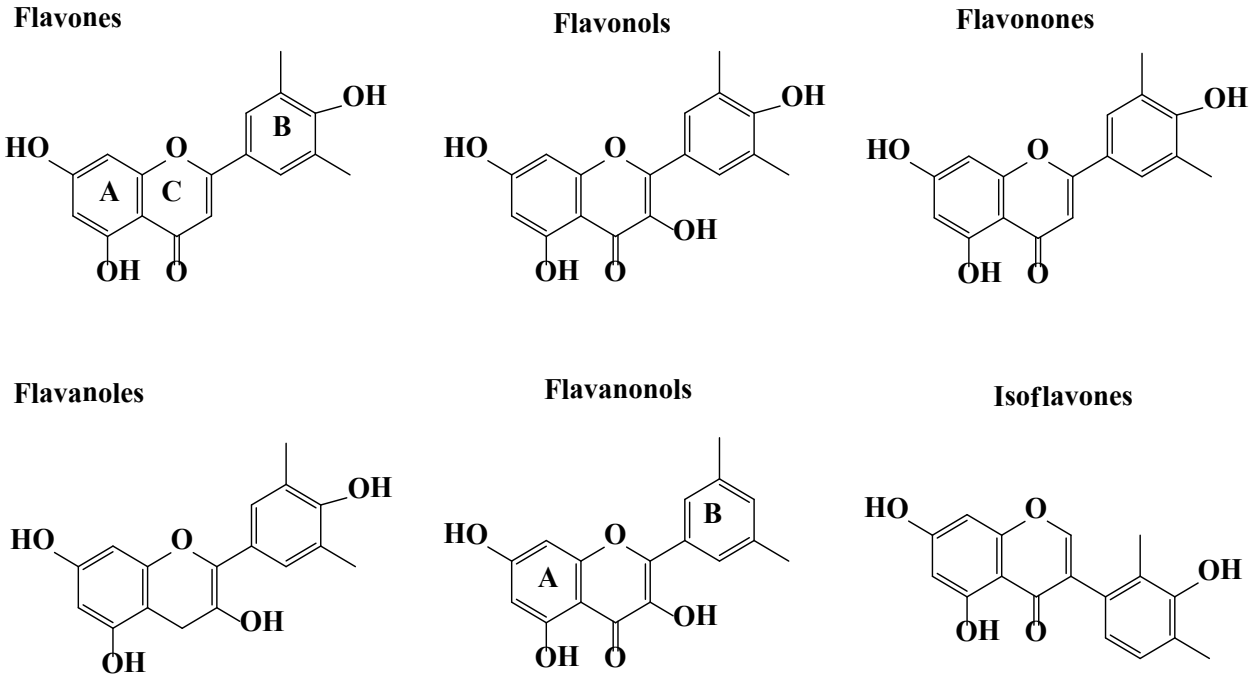
DHF	:	3,6 dihydroxyflavone
DLS	:	Dynamic Light Scattering
DPPH	:	2, 2 diphenyl 1,1 picryl hydrazyl ion
AuNp/GNP	:	Gold Nanoparticles
Lyc	:	Lycopene
ROS	:	Reactive Oxygen Species
Se.Me.Sc	:	Selenium Methyl Selenocysteine

Introduction

The Reactive Oxygen Species (ROS) is generally responsible for all tissue related injuries in the body and the generation of diseases like cancer. The body is continuously facing exposure to all possible types of free radicals and ROS, both from exogenous and endogenously generated sources, that generates oxidative stress, modulating apoptosis and leads to several types of cancers [1,2]. The multifaceted phytochemical-based green nanotechnological approach has been proven productive for the generation of stabilized and non-toxic nanoparticles that can have further utilization for different biotic applications both diagnostic and therapeutic purposes [3-4]. The presence of different cancer defending phytochemicals in different plant species and their utilization in developing tumor-specific nanoparticles provides an outstanding opportunity not only for their designing and development but also in the treatment of cancer and other related diseases, linking them through specific anticancer bioagents [5-7].

Chemically flavonoids are fifteen carbon compounds with two benzene rings A & B, interconnected by a heterocyclic pyran ring (C). They can be categorized into different classes like flavones (e.g., luteolin, apigenin), flavonols (e.g., myricetin, quercetin, kaempferol), flavanones, (e.g., naringenin, hesperidin) isoflavones, etc. All related classes of flavonoids vary in their level of oxidation and also on the substitution motif of pyrene ring. However, the individual compounds belonging to the same class differ only in the pattern of substitution of (A) & (B.) ring [8,9].

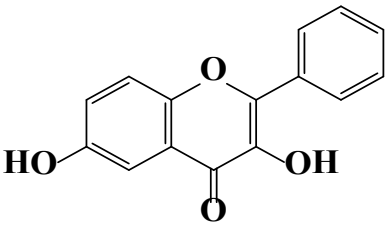
Chemical structures of the flavonoid family



The biological and metabolic activity of a flavonoid is closely related to its configuration, the total number of hydroxyl groups present, and also on the pattern of substitution of a functional group on its nuclear structure. The hydroxyl group present on flavonoid regulates their antioxidant activity either by scavenging of free radicals or by chelation of metal ions [10,11]. The hydroxyl configuration on the 'B' ring is mainly responsible for determining the scavenging activity of the ROS and RNS. Flavonoids inhibit enzymes involved in ROS generation i.e. glutathione S-transferase, microsomal monooxygenase, NADH oxidase, etc. Lipid peroxidation, which is one

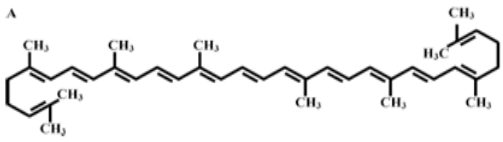
of the common consequences of oxidative damage, flavonoid helps in protecting lipids against oxidative stress by a different mechanism. The literature reported demonstrates that a flavonoid with an unsaturated 2, 3 bonds conjugated with 4-oxo are the most potent antioxidants [12,13].

3,6 Dihydroxyflavone: 3,6 dihydroxyflavone (3,6 DHF) is reported to be a promising anticancer compound with powerful antioxidant activity and strong cytotoxicity against breast cancer cells. Such unique properties of this compound make it attractive in developing it as a potent anticancer nano-drug material. However, there is still uncertainty on the effectiveness of its properties *in-vivo* and also its chemopreventive activities against mammary carcinogenesis, due to limited literatures available [14,15].



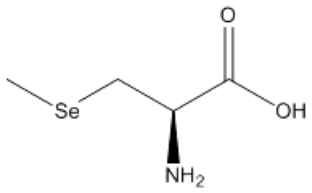
3, 6 dihydroxy Flavone

Lycopene, a powerful antioxidant and a member of the carotenoid family is mostly found in tomatoes and grape extract products. It has a strong singlet-oxygen quenching capacity of about 50 and 100 times higher than that of β-carotene and vitamin E, respectively. Lycopene is a promising anticancer, anti-inflammatory, neuroprotective, and antiproliferative agent [16-18]. The protective effect of antioxidants is mainly exerted by decreasing the abnormal cell division and by reducing oxidative damage to DNA. Therefore, the detrimental effects of ROS in the body can effectively be prevented and regulated by the protective function of an antioxidant mechanism, which successfully repairs the damage caused primarily to enzymes like glutathione, melatonin, and secondarily to antioxidant vitamins. The present study emphasizes on the antioxidant and immunity-related activities of the lycopene against diverse cancer cells, both single and in combination [19,20].



Structure of Lycopene

Selenium compounds are selectively among those few agents that have an alarming effect on morbidity and mortality of cancer cells, especially in inhibiting multiple mammary tumors and carcinoma of skin [21-22]. Selenium methyl selenocysteine, a new organic selenium compound has been reported to have greater potential as a chemopreventive agent, compared to several other previously employed selenium compounds [23,24]. Se-methyl selenocysteine is considered as a more efficacious chemopreventive agent than others because of the fact it belongs to that series of selenium compounds which directly enters into the methylated pool rather than those selenium compounds that metabolized through the H_2Se pool [25,26].



Structure of selenium methyl selenocysteine

The present study has been designed to evaluate the anticancer, antiproliferative, antioxidative, and enzyme modulated effects of flavonoids and other dietary chemopreventive agents including lycopene (antioxidant), Se-methyl selenocysteine (sensitizer), on different cancer cell lines. Their combinational treatments with gold nanoparticles were evaluated to test for synergistic interactions in the inhibition of cancer cell growth [27-30].

Material and Methods

Materials

All the chemicals and reagents purchased are of analytical grade quality and are detailed as follows: Auric chloride ($HAuCl_4.3H_2O$), target flavonoid (3,6 dihydroxyflavone), and ascorbic acid (as reference compound) were purchased from Sigma Aldrich chemicals. Selenium-methyl selenocysteine (as sensitizer) was purchased in the form of Se-methyl selenocysteine capsules-200mcq from Life Extension company, and lycopene (an antioxidant) was purchased from Sigma (Analytical grade-75051). Hydrogen tetra chloro-aurate ($HAuCl_4.3H_2O$), a hygroscopic solid was purchased from Sigma- Aldrich in one gram quantity and used the entire bottle of 1g $HAuCl_4.3H_2O$ in 250 ml distilled water to make a 10 mM stock solution of gold (III) and stored in a brown bottle. We diluted 25 ml of this stock solution in 250 ml to make 1mM concentration, used in our subsequent experimental trials. Other general chemicals used for evaluating the antioxidant/ free radical scavenging activity: Dimethyl sulphoxide (DMSO), Millipore Q-water DPPH (2,2 di-phenyl 1,1 picryl hydrazyl), phosphate buffer, methyl alcohol, hydrogen peroxide, and others, obtained from the central research laboratory of chemical science department of the institute or harvested locally.

Preparation of gold nanoparticles using targeted flavonoid 3,6 dihydroxyflavone as a reducing and stabilizing agent

10 mg of a targeted flavonoid (3,6 dihydroxyflavone) was taken in a beaker and dissolved with 10 ml of dimethyl sulphoxide solution. The solution was allowed to stir for 15-20 minutes at 25 °C followed by continuous addition of 10 ml auric chloride solution ($HAucl_4.3H_2O$; molarity-1mM). The change in color of the solution from pale yellow to brown red, indicates the formation of gold nanoparticles. The above reaction mixture was then stirred further for an additional 45 minutes at 25 °C to stabilize the synthesized nanoparticles and for preventing their agglomeration. All the characterization and stability related measurements of the biosynthesized particles were carried out adopting standard procedures [31-32].

Ultraviolet-visible analysis and *in-vitro* stability study

The successful biogenesis of nanoparticle was analyzed by the ultra-violet visible analysis method. The change in color of the resulting solution from pale yellow to thin red upon addition of auric chloride solution ($HAucl_4.3H_2O$; 1mM conc), generates a broad-spectrum peak in the range of 430-525 nm of the UV-spectra indicating the mono-dispersive nature of the biosynthesized particles. Similarly, another peak at a higher range indicates the signs for the aggregation of some of the particles. This was further ascertained from the data analysis of particle size analyzer or DLS (Dynamic light scattering, Malvern (Figure 1). The advent of the thin red color of the particles is mainly because of the phenomenon of surface Plasmon resonance, which arises due to the interaction of free electrons by the electromagnetic field. The variation in absorbance peak depends upon the size and dimensions of biosynthesized particles, which in turn can be related to change in concentration or pH of the medium (Figure 2A and 2B). The stability of the conjugated product gold nanoparticle embedded 3,6 dihydroxy flavone was monitored at the different pH of phosphate buffer, in human serum albumin (1%) and bovine serum albumin (1%) for an hour. Only a minimal shifting difference of plasmon wavelength was observed in all possible concentrations of the reaction mixture, confirming greater stability of the conjugated product in different biological fluid mediums at different pH value.

Figure 1: Particle size analyzer (DLS) nanoparticle size report with graph (Malvaren).

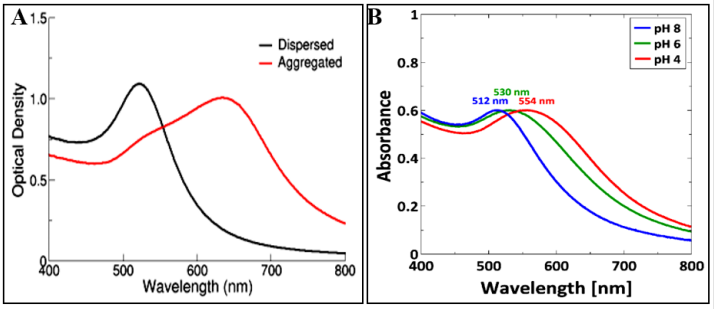


Figure 2: (A) Spectral peaks at two different levels indicating both dispersive and aggregated phase of biosynthesized nanoparticles **(B)** The variation in absorbance peak of biosynthesized nanoparticles with the change in concentration or pH of the medium.

XRD Analyses

The XRD analyses of the target flavonoid 3,6 dihydroxyflavone do not give any characteristic peak, confirming the amorphous nature of the flavonoid. However, in the case of nanogold conjugated 3,6 dihydroxyflavone three peaks of Au were observed at (111), (200), and (220) located at (2q) 37.20°, 46.73°, 63.18°, respectively, thus confirming the crystalline nature of the gold sample (Figure 3).

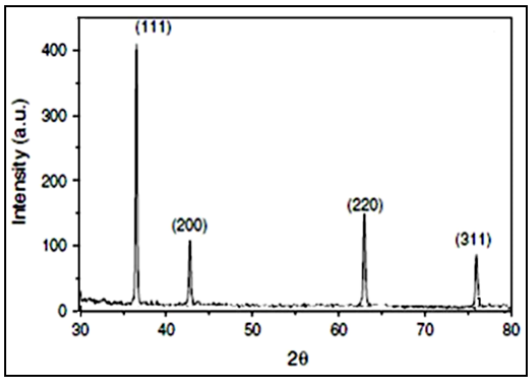


Figure 3: XRD pattern of gold nanoparticle conjugated 3,6 dihydroxyflavone with characteristic peaks, confirming the crystalline nature of the gold phase.

Statistical analysis

All the experimental tests were performed in triplicate and the data expressed as a mean of ±SD. The data obtained as percent inhibition is expressed in respect to the control and the statistical analyses were carried out using ANOVA or one-way variance analyses. $p \leq 0.05$ was considered statistically significant.

Results

Estimation of the antioxidant (free radical scavenging activity) of gold nanoparticles with a target flavonoid and other dietary supplements

The antioxidant and free radical scavenging activity of flavonoid embedded gold nanoparticles and selected dietary supplements was evaluated both single and combinational using DPPH (2, 2 diphenyl 1,1 picryl hydrazyl ion), hydrogen peroxide, hydroxyl radical by scavenging assays.

DPPH (2,2 diphenyl 1,1 picryl hydrazyl ion) radical scavenging activity

The estimation for the free radical scavenging activity of gold nanoparticle embedded target flavonoid (3,6, dihydroxylflavone), antioxidant (lycopene), selenium methyl selenocysteine (both single and combinational) was studied and determined using the DPPH assay [33]. A dilution series ranging from (10-100 µg/ml) of the reaction mixtures with different phytochemical constituents were allowed to incubate with an alcoholic solution of DPPH (2 ml of 0.2 mM conc). The content of the reaction mixture was forcibly mixed and allowed to sustain at least for 45 min at room temperature. The absorbance measurement was taken at 520-535 nm range. Ascorbic acid being an ideal was used as a reference compound. The percentage depletion of the concerned radical was then estimated by applying the formula (C-T/C) x 100, where C is the absorbance of the control (reference), and T is the test sample.

The radical scavenging activity of different chemical compounds was studied at different molar concentrations exhibiting its dose-dependent nature. The experimental data analysis from Table 1 indicates that at a higher value of concentration (100µg/ml) all the individual photochemical shows the maximum percentage of inhibition. 3,6 dihydroxyflavone (65.4%), lycopene (70.26%), and selenium-methyl selenocysteine (46.28%) against the standard reference compound ascorbic acid (98.2%). However, when tested with the triplet combination of the above three in a ratio (1:1:1), a significant rise in percentage inhibition (74.9%) at the same concentration level was observed. Similarly, DHF embedded gold nanoparticle showed (68.8%) inhibition as compared to native 3,6 dihydroxyflavone which individually showed (65.4%) inhibition and with the inclusion of all selective dietary supplements with 3,6 dihydroxylflavone embedded gold nanoparticles an overall enhancement of (26.65%) in the antioxidant activity was observed (Figure 4).

	(%) Percentage inhibition						
Conc (µg/ml)	**DHF**	**Lycopene (Lyc)**	**Selenium methyl selenocysteine (Se. MeSc.)**	**GNP + DHF**	**DHF+ Lyc + Se.Me.Sc**	**GNDHF+ Lyc + SeMe.Sc**	**Vit C**
100 µg/ml	65.40±1.2	70.26±1.3	46.28±0.6	68.80±1.3	74.90±1.4	87.30±1.2	98.20±1.6
75 µg/ml	56.26±0.9	64.50±1.1	44.30±0.8	62.20±1.2	70.80±1.3	84.50±1.0	97.46±1.2
50 µg/ml	48.60±0.7	56.60±0.8	39.30±0.5	52.54±0.9	66.40±0.8	72.46±0.8	89.45±1.4
25 µg/ml	32.80±0.5	47.20±0.6	31.5±0.6	43.75±0.7	52.70±0.6	66.76±0.6	84.30±1.3

Table 1: Percentage inhibition of DPPH radical showing dependency on selective dietary supplements (both single and combinational) against reference ascorbic acid mean ± SD (n=3).

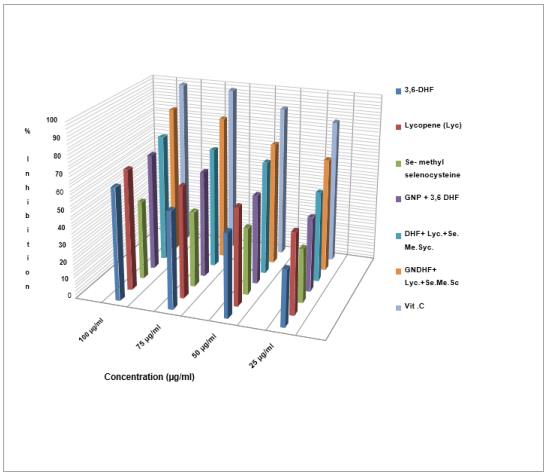


Figure 4: Chart showing comparative percentage inhibition of **DPPH** radical with selective dietary compounds (both single and combinational) against reference ascorbic acid).

Hydrogen peroxide (H_2O_2) scavenging activity

The H_2O_2 scavenging ability of dietary compounds can be linked with their tendency of donating electrons to H_2O_2, thus neutralizing it to water [34]. The free-radical scavenging activity of all the selective dietary compounds under study (both single and combinational) generally increases with an increase in their concentration. The variation in percentage inhibition for H_2O_2 radical activity with the change in concentration is shown in Table 2.

	(%) Percentage inhibition						
Conc (µg/ml)	**DHF**	**Lycopene (Lyc)**	**selenium methyl selenocysteine (Se.MeSc.)**	**GNP+ DHF**	**DHF+ Lyc + Se. Me.Sc.**	**GNPDHF+ Lyc+ Se.MeSc.**	**Vit C**
100 µg/ml	59.30±1.1	60.65±0.9	39.80±0.6	69.88±1.4	70.15±07	81.80±1.2	95.10±1.4
75 µg/ml	51.80±0.9	56.50±0.7	35.20±0.5	61.64±0.7	64.20±1.1	76.50±0.8	92.40±1.0
50 µg/ml	45.30±0.7	42.20±0.7	27.1±0.6	57.80±0.9	60.82±0.9	64.58±1.1	87.50±1.3
25 µg/ml	27.30±0.5	31.3±0.6	19.20±0.3	40.20±0.7	44.65±0.8	56.25±0.8	85.30±1.1

Table 2: Percentage inhibition of H_2O_2 radical showing dependency on selective dietary supplements (both single and combinational) against reference ascorbic acid mean ± SD (n=3).

The data reveals the percentage of inhibition of different components tested individually. 3,6 dihydroxyflavone (59.30%), lycopene (60.65%), and selenium methyl selenocysteine (39.80%) against standard ascorbic acid (95.10%) at a concentration of 100 µg/ml. However, the triplet combination of the above three exhibits maximum inhibition of (70.15%) indicating a rise of 16.9% in inhibition rate. Similarly, nanogold conjugated 3,6 dihydroxyflavone along with all dietary supplements (lycopene and selenium methyl selenocysteine) showed a significant increase in inhibition percentage (81.80%) indicating an overall gain of (28.55%) enhancement in antioxidant activity (Figure 5).

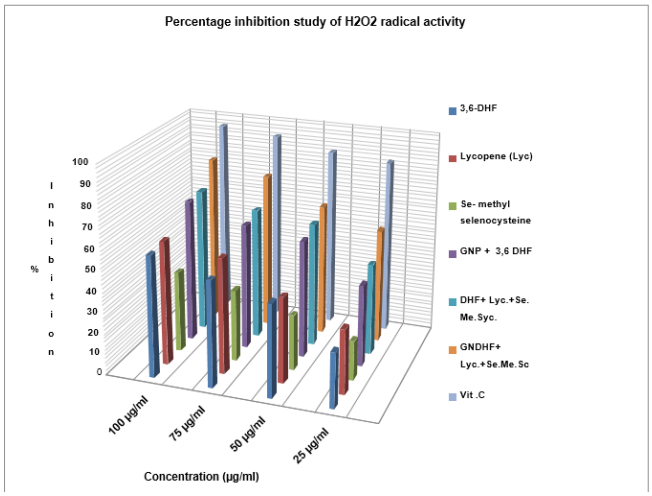


Figure 5: Chart showing percentage inhibition of H_2O_2 radical and its relationship with selective dietary compounds (single and combinational) against reference ascorbic acid.

Hydroxyl group free radical scavenging activity (Fenton assay):

The hydroxyl radical scavenging activity of the selected phytochemicals with gold nanoparticles was observed both singly and in combination using OH- radical scavenging assay (Fenton's reaction). The hydroxyl radical formed during Fenton's reaction is reported to be most reactive of all the reduced forms of dioxygen and is capable of damaging the biomolecules found in living cells. The reaction mixture in a dilution series ranging from 25 µl to 100 µl with different dietary phytochemical constituents was incubated with deoxyribose, H2O2, FeCl3 in a phosphate buffer saline (pH 7.4) and ending up the reaction using thiobarbituric acid, trichloroacetic acid and then heating it in boiling water bath for 20 min. The content was cooled, and absorbance of the mixture was measured at 530-540 nm range [35].

The hydroxyl OH- group radical scavenging activity with different selective phytochemicals was estimated both individually and in combination. The data obtained from Table 3 demonstrate that all the selective phytochemicals when tested individually show their dose-dependency nature. All the phytochemical constituents when tested individually at a concentration of 100 µg/ml show the maximum percentage inhibition data. 3,6 DHF (62.10%), lycopene (63.50%), selenium methyl selenocysteine (41.62%) against the standard ascorbic acid showing maximum percentage inhibition as (96.18%). The combinational result of the above three important phytochemicals when tested in combination (1:1:1), much improved results reported (70.63%) at the same concentration level of 100 µg/ml, indicating an average increase of (14.89%) in the percentage free radical inhibition activity. Similarly, experiments when carried out with a force multiplier combination of 3,6 DHF embedded gold nanoparticle along with all the selective dietary supplements, the percentage inhibition of (85.10%) was reported, indicating an overall rise of (29.36%) enhancement in antioxidant activity (Figure 6).

	(%) Percentage inhibition						
Conc (µg/ml)	**DHF**	**Lycopene (Lyc)**	**selenium methyl selenocysteine (Se-Me.Sc)**	**GNP +DHF**	**DHF+ Lyc + Se.Me.Sc**	**GNPDHF+ Lyc + Se.Me. Sc.**	**Vit C**
100 µg/ml	62.10±0.9	63.50±1.0	41.62±0.6	70.11±1.4	70.63±0.8	85.10±1.2	96.18±1.5
75 µg/ml	54.80±0.9	57.50±0.7	30.20±0.5	59.84±0.7	54.80±1.1	66.50±0.8	91.10±1.0
50 µg/ml	35.30±0.5	32.20±0.4	24.90±0.2	40.80±0.6	34.82±0.4	47.58±0.6	86.50±1.3
25 µg/ml	24.30±0.5	21.3±0.6	13.20±0.3	30.20±0.4	24.50±0.3	39.25±0.6	81.30±1.1

Table 3: Percentage inhibition of OH radical and its relationship with selective dietary supplements (single and combinational) *vs.* standard ascorbic acid mean ± SD (n=3).

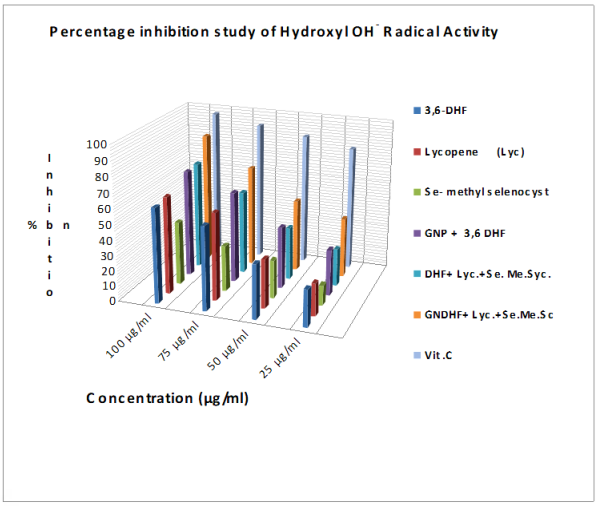


Figure 6: Chart showing Percentage inhibition of OH^- radical (single and combinational).

Cytotoxic study analysis

The investigation relating to the biocompatibility of 3,6 dihydroxyflavone embedded gold nanoparticles (AuNp-DHF) was carried out on prostate cancer PC3) and breast cancer (MCF7) cells using a colorimetric MTT cell viability assay [36]. The untreated PC3 and MCF7 cells and the cells treated with different series of dilution (10, 25, 50, 75, 100 µl) of different (AuNp-DHF) conjugates were subjected to MTT assay for cell viability determination. Only the cells that are viable after 24 h of exposure to the sample are capable of metabolizing a dye (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) producing a purple-colored precipitate, dissolved in detergent and analyzed spectrophotometrically at 570 nm. After 24 h post-treatment, both PC3 and MCF7 cells reported excellent viability even up to 100 µl concentration of (AuNp-DHF) solution (Figure 7). The results obtained demonstrate that the phytochemical potential of 3,6 dihydroxyflavone provides a non-toxic coating on AuNps. It is also important to observe that Gold (I) and Gold (III) exhibit varying degrees of cytotoxicity to a variety of cells and was significantly reduced with the presence of a flavonoid 3,6 dihydroxyflavone. This lack of toxicity provides a new opportunity for its much safer application in cancer imaging and therapy.

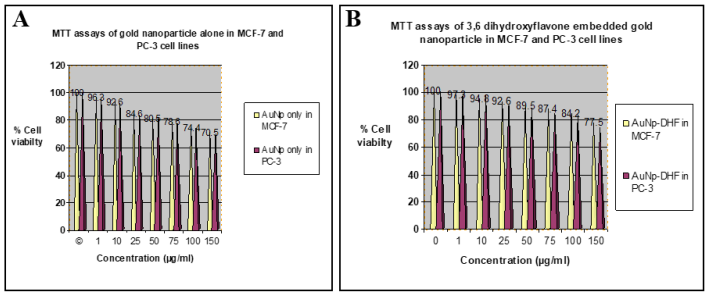


Figure 7: Cytotoxic Studies: MTT assay on the cell viability estimation of (A) non-conjugated gold nanoparticles and (B) 3,6 dihydroxyflavone embedded gold nanoparticles (AuNp-DHF-conjugate) tested with prostate cancer (PC3) and breast cancer (MCF7) cells, using solutions of different dilution series.

Assessment of antimicrobial activity of gold nanoparticle embedded targeted flavonoid with dietary supplements

The *in-vitro* antibacterial assessment of biosynthesized gold nanoparticles with 3,6 dihydroxy flavone was studied both as a test sample (T_1 and T_2) with the inclusion of selective dietary supplements like lycopene and Se-methyl selenocysteine tested against different bacterial strains. The percentage growth in the bacterial count was measured with two different test samples ($T_{1)}$ and (T_2), against the pure nutrient broth media. The zone of inhibition was examined by taking the Optical Density (OD) values. Different types of doses with different molar concentrations were tested to evaluate the dose determining effect. Low dose (1 µg/ml), medium dose (2 µg/ml), and high dose (3 µg/ml). The reading for the negative control (without any dose) of bacterial growth after 24 h observed was (70.2%), (69.3%), and (62.5%) for different bacterial strains: Staphylococcus aureus, E. coli, and Bacillus subtilis, respectively (Table 4).

Bacterial Strain	Negative Control (Broth + Inoculum)	% Growth (→Reduction) Sample T1 (DHF embedded GNP only.			% Growth (→Reduction) Sample T2 (DHF embedded GNP with other dietary supplements.)		
	Without dose	**Dose with low Conc**	**Dose with medium Conc**	**Dose with High Conc**	**Dose with low Conc**	**Dose with medium Conc**	**Dose with High Conc**
	(0 µg/ml)	**(1 µg/ml)**	**(2 µg/ml)**	**(3 µg/ml)**	**(1 µg/ml)**	**(2 µg/ml)**	**(3 µg/ml)**
Staphylococcus aureus	70.2±1.4	48.1±0.6	35.1±1.3	20.7±0.8	41.4±0.4	28.7±1.2	17.4±0.8
Escherichia coli	69.3±0.8	42.6±1.3	27.2±0.8	6.2±0.9	40.2±1.4	21.6±0.6	4.2±0.9
Bacillus subtilis	62.5±0.5	55.7±0.1	51.3±0.74	44.4±0.2	53.8±0.2	47.1±1.1	40.2±0.2

Table 4: Assessment of antibacterial effect on different bacterial strains (Staphylococcus aureus, Escherichia coli & Bacillus subtilis) for low concentration dose, medium, and high concentration doses of DHF embedded gold-nanoparticles against two different test samples (T_1) & (T_2).

From the experimental data observed, it can be concluded that for sample 1 (GNP+DHF), the maximum growth reduction of *S. aureus* which initially without any dose was (70.2%), gets reduced to (20.7%). In the case of *E. coli* from a maximum growth of (69.3%), it was reduced up to (6.2%) and in the case of *Bacillus subtilis*, the maximum initial uncontrolled growth reported was (62.5%) which gets reduced up to (44.4%) at high dose concentration of the test sample. Similarly, when this data was compared with that of sample 2 (GNPDHF + Lycopene + Selenium methyl selenocysteine), the maximum growth reduction for *S. aureus*, *E. coli* and *B. subtilis* was reported up to (17.4%), (4.2%), and (40.2%), respectively (Figure 8). The two test samples T_1 and T_2 when analyzed for their antibacterial effects showed a significant reduction in bacterial growth for all the selective strains. The same dose concentration of both the samples when compared, then the sample (T_1) containing DHF embedded gold nanoparticles only without antioxidant and sensitizer, observed to be less effective as compared to sample (T_2) which includes the presence of antioxidant lycopene and a sensitizer selenium methyl selenocysteine.

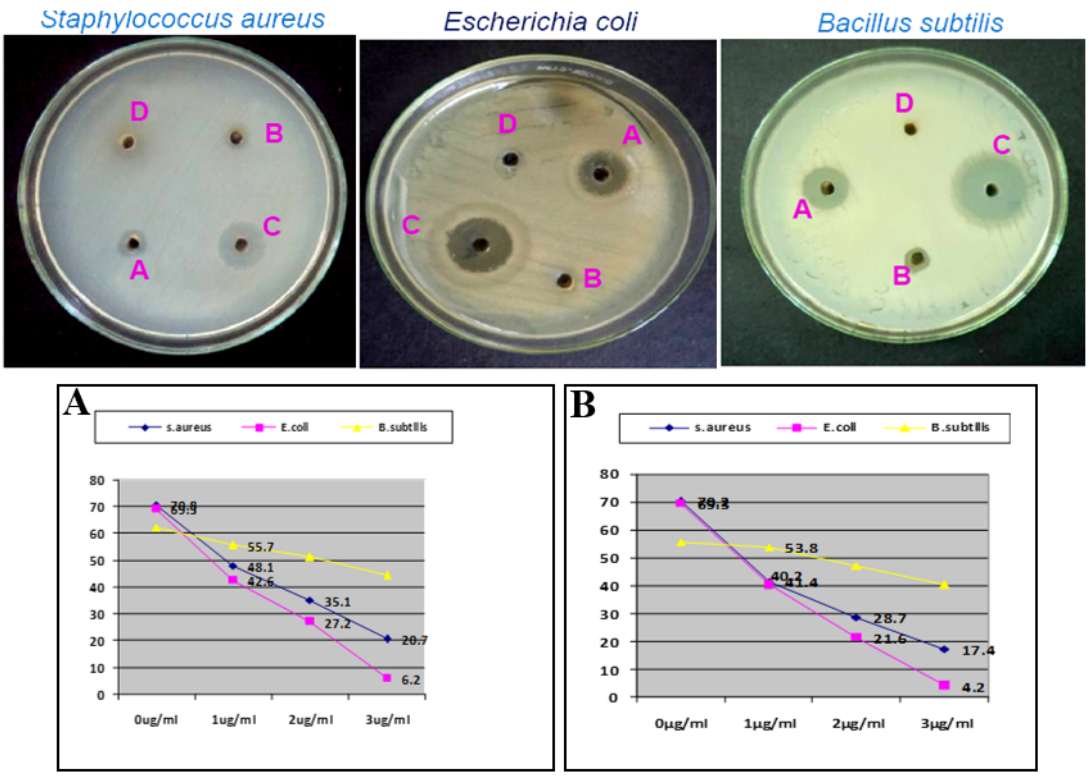


Figure 8: Percentage growth inhibition of different bacterial strains (*S. aureus, E. coli,* and *B. Subtilis*) with different concentration of doses - **A**) Sample T1 (DHF embedded GNP), **B**) Sample T2 (DHF-GNP with other dietary supplements like antioxidant & sensitizer).

Discussion

There is an intense interest in modifying existing drugs to improve pharmacokinetics, thereby reducing nonspecific side effects, and enabling higher dose delivery to target tissues. An important demonstration of the potential of multifunctional nanoparticles was their possible use as a drug delivery vehicle, as an active targeting agent, and as a therapeutic payload in various types of cancers. The present study initiates with an objective for synthesizing non-toxic and stabilized gold nanoparticles through a biological route using a target flavonoid 3,6 dihydroxyflavone together with an antioxidant lycopene and selenium methyl selenocysteine, a new organic selenium compound with much greater efficacy as a chemopreventive agent.

The polyphenolic groups present in flavonoids and lycopene serve the dual purpose, functioning as a reducing agent for gold ions as well as a stabilizing agent to produced shape-controlled gold nanoparticles. Moreover, these compounds display a remarkable spectrum of biological activities including those that might be able to influence processes that are dysregulated during cancer development. These include antioxidant, antimutagenic, anticarcinogenic, and modulation of enzymatic activities [37].

The biogenesis of stabilized gold nanoparticles was conducted successfully following the conditions as reported. The measurements relating to characterization and stability were carried out using standard procedures. The estimation of the average particle size of the flavonoid embedded gold nanoparticles was tested using a particle size analyzer (PSA-Malvern) available with us. The dynamic light scattering (DLS) study report indicated the formation of an average particle size range 50-60 nm with a polydispersity index (PDI) of 0.246, indicating some signs of aggregation of the particles. However, the use of sodium citrate has been recommended in some studies as a stabilizing agent to prevent aggregation of biosynthesized particles. Similarly, the use of folic acid as a ligand has been advocated for generating tumor specificity on the synthesized particles [38].

The XRD analyses of the test samples were analyzed in a testing lab (Sophisticated Analytical Instruments facility, Punjab University, Chandigarh, India). The intensity *vs.* 2-Theta data values obtained from the report, when analyzed showed three peaks corresponds to the values 111, 200, and 220 of Gold-nanoparticles at 2q = 38.88°, 42.93°, and 64.48°, respectively, confirming the crystalline nature of the gold phase.

The antioxidative analysis outcome of selective dietary compounds 3,6 dihydroxyflavone, lycopene, and selenium methyl selenocysteine was estimated using different free radical scavenging assays (DPPH, H_2O_2, OH) and it was observed that all selective dietary phytochemicals showed significant free radical scavenging activity and the triple combination of all the three in equimolar concentration in a ratio (1:1:1) showed remarkable enhancement in the antioxidant activity. Similarly, the antibacterial activity, when tested with two different test samples (T_1) & (T_2) of flavonoid fabricated gold nanoparticles (without and with the inclusion of lycopene & Se-methyl selenocysteine) when compared against different bacterial stains, the test sample (T_2, flavonoid fabricated gold nanoparticles with the inclusion of lycopene and Se-methyl selenocysteine) was reported to be much more competent in terms of its antibacterial nature.

Conclusion and Future perspective

Present study reports the formulation of a well-defined biosynthetic route for the formation of non-toxic, stabilized gold nanoparticles conjugated with a target flavonoid 3,6 dihydroxyflavone with potential anticancer properties. The inclusion of antioxidant lycopene and a sensitizer Se-methyl selenocysteine further enhanced the efficacy of reducing and stabilizing gold nanoparticles with much precise size and monodispersity, characterized by powerful antioxidative and antibacterial properties that might be able to influence those metabolic processes of the body that are dysregulated during cancer development [39]. The cytotoxic studies on different cell lines demonstrate an excellent cell viability result, which indicates the phytochemical potential of 3,6 dihydroxyflavone in providing a non-toxic coating on gold nanoparticles. This non-toxic nature of 3,6 dihydroxyflavone can be viewed as a new opportunity for its safe utilization in cancer diagnosis and therapy. The progressive success of the work done will help in providing future insights towards a new era of flavonoid based pharmaceutical agents that will pave the way for the development and formulation of phytochemical stabilized metal nanoparticles linking it to cancer antibodies (both monoclonal and polyclonal), with simultaneous enhanced biological activity and characterized anticancer properties for specific and selective targeting of cancer cells [40].

Highlights

- Present study was carried out to assess the impact of nanotech reinforcement of metal nanoparticles and the combinational effect of flavonoid and selective dietary supplements like antioxidant (lycopene) and sensitizer (selenium methyl selenocysteine) for enhancement in anticancer activity.
- The evaluation of the most potent combinational treatment effect of these anticancer agents with gold nanoparticles will lead towards the development of a new anticancer nano-drug that will likely be less toxic, safe, non-resistant, and more importantly poor-man friendly.

From the finding of the most potent phytochemical combinational nano-drug constituent further implementation of the idea to develop a nano immunoconjugate drug by linking cancer antibodies (both polyclonal and monoclonal antibody), to enhance the force multiplier therapy effect with much specific cancer cell target and tumor kill enhancement.

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