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Article

Antioxidant activity of phytosynthesized biomatrix-loaded noble metallic nanoparticles

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ABSTRACT

Biofabrication of noble monometallic platinum nanoparticles (Pt-NPs) and bimetallic gold–silver nanoparticles (AuAg-NPs) using aqueous extract of *Delonix regia* is presented here. Antioxidant activity of biomatrix-loaded metallic nanoparticles is estimated for scavenging of two model radicals *i.e.*, 2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt and 1,1′-Diphenyl-2-picrylhydrazyl. Broad spectral continuum spanning from visible to ultra-violet region (Pt-NPs: 30 min) and broad high intensity absorption peak around at 500 nm (AuAg-NPs: 10 min) in two different UV–Visible spectra confirmed the biofabrication. Nanoparticles fabricated with distorted spherical shape and crystalline face-centred-cubic geometry. Strong signal around at 2.10 keV (pure-phase platinum) and typical X-ray peaks observed at 2.20 and 3 keV suggested, co-existence and alloying interaction of Au and Ag in AuAg-NPs. ζ potential (−15.2 mV: Pt-NPs and −13.9 mV: AuAg-NPs) values suggested surface adsorption of polyphenolic compounds to provide stability. Nanoparticles exhibited pronounced antioxidant activity against free radicals through their electron/hydrogen transfer ability.

1. Introduction

Various noble metal nanoparticles have attracted a significant attention because of their size and shape dependent specific properties [1]. During recent years phytosynthesized noble metal nanoparticles and their applications have drawn interest of various researchers in the fields of biomedical science [2]. Phytosynthesis is an eco-benign, convenient, safe, cost-efficient and rapid method for synthesis of biocompatible metal nanoparticles. Use of metal nanoparticles as an antioxidant is one such application which has attracted significant attention of researchers. It is well known that antioxidant plays a crucial role for proper running of various bio-systems by scavenging various harmful radicals, which cause oxidative stress to various cellular components. Free radicals are by-products of normal metabolic reactions of biological systems. In living organism like humans, free radicals are known to be responsible for the development of many pathological conditions ranging from cancer to cardiovascular disease, Alzheimer's disease, atherosclerosis, diabetes and aging [3–5]. Besides this, free radicals are also responsible for oxidative deterioration of food materials. Effective scavenger of free radicals may serve as potent compounds to treat free radical mediated diseases as well as for food material preservation [6,7]. Till now, various research groups are involved generally in the exploration of antioxidant potential of various bio-resources. However, recently free radical scavenging activity of nanomaterials is also reported in various *in-vivo* and *in-vitro* systems [8–12].

Theoretically, fabrication of large number and variety of nanostructure material is possible through various chemical and physical routes. Most of these strategies require high energy and toxic chemicals. Thus, these methods are potentially dangerous to various biological systems and hazardous to the environment [13]. Therefore, biofabrication approaches are more economical, convenient and eco-benign and better substitutes for the classical procedures due to availability of various biological entities, rich biodiversity and easy availability [14]. Biosynthesis methods have developed as a highlight of the intersection between biotechnology and nanotechnology. Thus, these methods have been received increasing attention in the last decade. Biofabrication of metallic nanoparticles is an eco-friendly approach without the use of toxic, harsh and expensive chemicals and safe for human therapeutic use. Nowadays, because of their non-pathogenic and nil toxic profile, use of different medicinal plants extract, is gaining importance for biofabrication of high quality nanostructures with sustainable commercial viability. Use of eco-friendly and biocompatible nanostructure materials is also important in terms of their biomedical utility. Recently, Suriyakalaa *et al.* have shown the strategy for biofabrication of biocompatible nanoparticles and their utility as a hepatocurative agent [15].

In the present study *Delonix regia*, an ethno-medicinal plant of the *Caesalpiniaceae* family is selected for biofabrication. The bio-reducing ability of *D. regia* is recently reported for the synthesis of monometallic palladium [16], gold [17] and bimetallic gold–palladium nanoparticles

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[18] in our previous studies. Biofabrication of silver nanoparticles is also stated earlier using one another plant from *Delonix* genus (*Delonix elata*) [19]. Thus, the present study is focused on the exploration of the bio-reducing potential of *D. regia* for the synthesis of noble monometallic platinum nanoparticles (Pt-NPs) and bimetallic gold–silver nanoparticles (AuAg-NPs). Till now, biofabrication of nanoparticles has been focused mainly on the fabrication of noble monometallic nanoparticles of silver, gold and palladium. Albeit, platinum is also in the category of noble metal, but the biofabrication of platinum nanoparticles (Pt-NPs) has not been explored to the same extent as that of Au and Ag [20]. Very few reports are available for biofabrication of Pt-NPs [13]. Similarly, despite of the significant potential of alloy bimetallic nanoparticles in various domains due to bi-functional or synergistic effects, biofabrication of bimetallic nanoparticles has been explored very less, compared to monometallic nanoparticles [18]. Therefore, the present study is dedicated for the fabrication of Pt-NPs and AuAg-NPs using widely available bio-resource *D. regia*. Biomedical utility of both nanoparticles is further explored based on their antioxidant potential. Two model radicals *i.e.*, (2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical ($ABTS^{\bullet+}$)) and (1,1′-Diphenyl-2-picrylhydrazyl radical (DPPH•)) have been utilized for assessment of the antioxidant potential of biofabricated nanoparticles.

2. Experimental

2.1. Materials

Leaves of *D. regia* were collected from the college campus of S.V.N.I.T., Surat, Gujarat. Potassium persulfate, ABTS, ethanol and methanol were procured from Germany (Merck, Darmstadt). Folin–Ciocalteu's reagent, quercetin, bovine serum albumin, gallic acid and DPPH• were obtained from Germany (Sigma-Aldrich, Steinheim). Gold chloride trihydrate, silver nitrate, chloroplatinic acid hexahydrate, ascorbic acid, potassium phosphate, potassium ferricyanide, trichloroacetic acid, ferric chloride, sulfuric acid and sodium phosphate were acquired from India (Himedia, Mumbai). Ammonium molybdate was purchased from India (Merck, Mumbai). Sodium carbonate, aluminium chloride, and potassium acetate were bought from India (Qualigens, Mumbai). Double distilled deionized water was collected from Elix Millipore system.

2.2. Preparation of D. regia leaf extract

To prepare the extract of *D. regia* leaves, 30 g of *D. regia* leaves, was added to 120 ml of deionized water in round-bottom flasks of 500 ml capacity. The mixture was then heated for 10 min at 60 °C. The resultant extract was filtered with Whatman filter paper no. 40. Thereafter, filtrate was used for further experiments.

2.3. Biofabrication of nanoparticles

Aqueous extract of fresh leaves of *D. regia* was prepared according to the procedure described in literature [16]. In a typical reaction, about 400 ml of 1×10^{-3} mol·L^{-1} chloroplatinic acid hexahydrate solution was mixed with 100 ml aqueous leaf extract of *D. regia*. Thermal assisted reduction of chloroplatinic acid hexahydrate was carried out by keeping this reaction mixture in a sealed flask for 30 min (at 90 °C) on a rotary shaker (500 r·min^{-1}).

Similarly, biofabrication of AuAg-NPs was started by the addition of a well-mixed 800 ml aqueous solution of gold chloride trihydrate (400 ml; 1×10^{-3} mol·L^{-1}): silver nitrate (400 ml; 1×10^{-3} mol·L^{-1}) with 100 ml aqueous extract of fresh leaves of *D. regia*. Spontaneous reduction of a mixture of salt solutions was carried out by keeping this reaction mixture in a sealed flask for 10 min on a rotary shaker (500 r·min^{-1}).

Both reduced nanoparticle solutions were kept for sonication (10 min) to separate nanoparticles from the bioorganics of leaf extract followed by repetitive centrifugation at 15,000 r·min^{-1} for 10 min for nanoparticle recovery. After centrifugation, pellets were washed with double distilled deionized water to remove the impurities followed by drying at 60 °C for 2 h. Control reactions were performed with double distilled deionized water instead of leaf extract under the same reaction condition.

2.4. Calorimetric assay for total phenolic acid (TPA), total proteins (TP), total flavonoids (TF), reducing power capacity (RPC) and total antioxidant capacity (TAC)

The concentrations of TPA, TF and TP in *D. regia* leaf extract were determined by Folin–Ciocalteu's, aluminium chloride, and Bradford protein calorimetric methods respectively [16]. The quantities of TPA, TF and TP were stated in gallic acid equivalent (GAE), quercetin equivalent (QE) and bovine serum albumin equivalent (BSAE) respectively. The quantity of these bioorganic compounds was expressed in mg of respective equivalent per gram of fresh weight (fw) of leaves.

The RPC of *D. regia* leaf extract was determined by following the procedure explained by Oyaizu with slight modifications [21]. In a typical reaction, 0.5 ml sample of *D. regia* leaf extract was mixed with 2.5 ml of potassium ferricyanide (1%) and 2.5 ml of phosphate buffer (0.2 mol·L^{-1}; pH 6.6) followed by incubation for 20 min (50°°C). Then, 2.5 ml of trichloroacetic acid solution (10%) was mixed with the above mixture. This mixture was kept for centrifugation at 3000 RPM (10 min). Then, 2.5 ml of distilled water and 0.5 ml of ferric chloride solution (0.1%) were mixed with 2.5 ml of supernatant solution (obtained after centrifugation). The quantity of iron-(II)-ferricyanide complex formation in the reaction mixture was measured by the formation of Perl's Prussian blue color. The absorbance value of this reaction solution was measured at 700 nm against a blank. A typical blank solution contains an equivalent volume of double distilled water, instead of leaf extract. Quantification of RPC was done on the basis of standard curve of ascorbic acid (AA) and it was stated in mg of ascorbic acid equivalent (AAE) per gram of fw of leaves.

The TAC of *D. regia* leaf extract was assayed following the procedure of Prieto *et al.* with few alterations [22]. A 0.2 ml aliquot of aqueous extract of *D. regia* was mixed with 2.0 ml of reagent solution (1:1:1 ratio of 0.6 mol·L^{-1} sulfuric acid, 28 mmol·L^{-1} sodium phosphate and 4 mmol·L^{-1} ammonium molybdate) in a capped sample vial followed by incubation in a water bath at 95 °C (90 min). This solution was further cooled to room temperature. The amount of green phosphate/Mo (V) complex formation in the reaction mixture was measured at 695 nm against a blank. A typical blank solution consisted 2.0 ml of reagent solution with 0.2 ml of the double distilled water. Quantification of TAC was done on the basis of standard curve of AA and it was stated in mg of AAE per gram of fw of leaves.

2.5. In-vitro antioxidant assay of biofabricated nanoparticles

The antioxidant activity of biofabricated nanoparticles was evaluated by measuring their capability to scavenge synthetic stable radical of DPPH• and $ABTS^{\bullet+}$ using the method given by Serpen *et al.* with minor modifications [23].

For DPPH• scavenging reaction, nanoparticle samples of 2.5, 5, 10, 15, 20 and 30 mg were mixed with 4.0 ml of DPPH• solution (400 mmol·L^{-1} in 80% vol% ethanol) in different Eppendorf tubes followed by ultrasonication for 30 min to allow the surface reaction of nanoparticles with DPPH•. Then reactions were quenched by removing the nanoparticles from reaction mixtures. For this different reaction mixtures were centrifuged at 10000 r·min^{-1} (2 min) followed by filtration using a 0.1 μmol·L^{-1} membrane filter. The absorbance of different filtrates was then measured at λ_{max} (521 nm) to understand the scavenging activity of biosynthesized nanoparticles for DPPH•. DPPH• is a stable radical and its scavenging reaction usually takes a longer time when compared with $ABTS^{\bullet+}$ [23,24]. Therefore, all investigations were performed after 30 min of reaction.

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