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The antioxidant and catalytic activities of green synthesized gold nanoparticles from *Piper longum* fruit extract

*Jayachandra Reddy Nakkala, Rani Mata, Sudha Rani Sadras**

Department of Biochemistry and Molecular Biology, School of Life Sciences, Pondicherry University, R. Venkataraman Nagar, Kalapet, Pondicherry 605 014, India

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ABSTRACT

In this study, we tested successfully a green method for the synthesis of gold nanoparticles using *Piper longum* fruit extract (PLFE) along with data on their *in vitro* antioxidant and catalytic activities. The formation of *P. longum* gold nanoparticles (PLAuNPs) was confirmed by UV–visible spectroscopy. The average size of the PLAuNPs was 56 nm as confirmed by the DLS particle size analyzer. The TEM-EDX revealed that PLAuNPs were spherical in shape and contained metallic gold. FTIR analysis indicated that the phenolic groups present in PLFE were involved in the reduction and capping of PLAuNPs. The thermal stability of PLAuNPs was analyzed by the TGA. PLFE and PLAuNPs exhibited moderate free radical quenching ability when analyzed by various *in vitro* antioxidant assays. The catalytic activity of PLAuNPs against four organic dyes namely methyl blue, methyl red, crystal violet and acridine orange was tested, which resulted in respective degradation of 65, 28, 39 and 34% after 28 h of mixing. Such green nanoparticles with potent catalytic properties might be useful in clearing the toxic dyes in industrial effluents.

1. Introduction

Synthetic dyes are a cause of concern owing to their potential to trigger significant environmental pollution. They represent the major group of organic compounds released from paper, leather, textile, food, pharmaceutical and cosmetic industries (Kulkarni et al., 1985; Zollinger, 1987). Azo dyes have been known to possess carcinogenic property (Chung and Cerniglia, 1992; Kusic et al., 2006). These dyes resist microbial degradation as well as physico-chemical treatments when released in high concentrations in industrial effluents. Thus, there is a growing need for developing novel methods in treating these dyes by using green synthesized metal nanoparticles (Geoprincy et al., 2013). Metal nanoparticles such as gold and silver are identified as effective catalysts due to their ability to transfer electrons between the donor and the acceptor–electron relay system (Gupta et al., 2011). In recent years, benign synthesis of nanoparticles by using plant extracts or microorganisms such as bacteria, fungi, algae have gained greater attention in nanotechnology (Thakkar et al., 2010). The advantage of green method over the other traditional methods are based on the natural availability of the reducing compounds, simple methodology, rapid formation of nanoparticles with high stability, eco-friendly nature and cost-effective. Both the reduced metals and the capped phyto-constituents (present in the extract) acts synergistically in different applications like catalytic degradation of organic dyes, antioxidant, anticancer, antibacterial and wound healing. Among these biological methods, plant mediated synthesis has more advantages over the microbial methods, as it overcomes the tedious processes of culture maintenance along with the extremely

* *Corresponding author*. Tel.: +91 9443768726; fax: +914132655734.
E-mail address: dr.ssrlab@gmail.com (S.R. Sadras).
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reduced reaction time required for preparation of nanoparticles (Bar et al., 2009; Song and Kim, 2009). Recently, metal oxide nanoparticles were also synthesized using extracts of *Aspalathus linearis* (Diallo et al., 2015), *Agathosma betulina* (Thema et al., 2015), *Hibiscus Sabdariffa* flower (Thovhogi et al., 2015), and *Callistemon viminalis* (Sone et al., 2015).

The catalytic activity of gold nanoparticles synthesized from plant extracts has been reported recently with their ability to reduce methylene blue dye (Das and Velusamy, 2014; Suvith and Philip, 2014). The *Piper longum* fruit has been widely used in traditional medicines to treat many diseases (Kumar et al., 2011). Various parts of *P. longum* are recognized to contain phytoconstituents like piperine, piperlongumine, sylvatin, sesamin, pipermonaline, and piperundecalidine.

In this study, gold nanoparticles were synthesized using the aqueous extract of *P. longum* fruit (PLFE). The synthesized gold nanoparticles (PLAuNPs) were characterized by UV–visible spectroscopy, transmission electron microscopy-energy dispersive X-rays (TEM-EDX), Brunauer–Emmett–Teller (BET) surface area analyzer, fourier-transform infrared spectroscopy (FT-IR), dynamic light scattering (DLS) particle size analyzer and thermogravimetric analysis (TGA). The PLAuNPs and PLFE were evaluated for their *in vitro* free radical quenching ability by DPPH, nitric oxide, super oxide and hydrogen peroxide scavenging assays. The catalytic efficiency of PLAuNPs was tested against four organic dyes—methylene blue, methyl red, crystal violet and acridine orange.

2. Materials and methods

2.1. Materials

$HAuCl_4$ and potassium bromide (KBr) were purchased from Sigma-Aldrich, Bangalore, India. Rutin, DPPH, nitro blue tetrazolium (NBT), NADH (nicotinamide adenine dinucleotide), methylene blue, methyl red, crystal violet and acridine orange (AO), were purchased from Hi-Media. *P. longum* fruit was purchased from local market, Puducherry, India. Double distilled water was used for all the experiments. All other chemicals used in this study were of analytical grade.

2.2. Extract preparation

Dried 1% (w/v) *P. longum* fruit power was added to 100 ml double distilled water in 250 ml Erlenmeyer flask. The mixture was heated by using magnetic heating stirrer at 60 °C for 10 min and the solution was filtered by Whatman No.1 filter paper, then it was stored at 4 °C for further use.

2.3. Green synthesis of PLAuNPs

10 ml of PLFE was added to 10 ml of $HAuCl_4$ (1 mM) and incubated at room temperature for 5 h in the dark. The PLAuNPs formed in the reaction mixture was collected by repeated centrifugation at 18 000 rpm for 20 min subsequently air dried at room temperature and used for further characterization.

2.4. Characterization of PLAuNPs

The PLAuNPs was analyzed by means of UV–visible spectroscopy (UV-1700 Shimadzu) in the range from 400 to 700 nm at regular time intervals. The morphology and elemental composition of PLAuNPs were analyzed by TEM-EDX for which the sample was prepared by dropping a small amount of PLAuNPs in powder form over a carbon coated copper grid. The extra amount of the sample was removed by using blotting paper and the film on the TEM grid was allowed to dry for 5 min under mercury lamp. The X-ray diffraction pattern of PLAuNPs is obtained on XPERT-PRO diffractometer using Cu ka radiation with nickel monochromator in the range of 2θ from 10° to 80°. The surface area of the PLAuNPs sample was analyzed by using BET surface area analyzer (Micromeritics Gemini VII). FT-IR (Thermo Nicolet Nexus 670 spectrometer) was used to determine the functional groups present in PLFE that were involved in the formation of PLAuNPs and the spectrum was recorded at 4000–400 cm^{-1}. The DLS particle size analyzer was used for determining the size and distribution of the PLAuNPs. The thermal stability of the PLAuNPs was assessed by using TGA.

2.5. In vitro antioxidant assays

2.5.1. DPPH assay

DPPH radical scavenging assay for PLFE and PLAuNPs was performed according to the method (Choi et al., 2002). In this test, 3 ml of DPPH (0.1 mM) was separately mixed with a 1 ml solution of PLFE and PLAuNPs containing different concentrations (100 to 600 μg/ml) and the mixture was incubated in the dark for 15 min. After incubation, the absorption of the sample was measured by using UV–visible spectrophotometry at 517 nm against methanol as blank. In this experiment DPPH with methanol was used as control and rutin was used as standard. The percentage of inhibition was calculated by the following formula:

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

2.5.2. Superoxide radical scavenging assay

Superoxide radical quenching activities of PLFE and PLAuNPs were evaluated on the basis of inhibition of NBT reduction (Nishikimi et al., 1972). In this method, 1 ml of the reaction mixture containing different concentrations of PLFE and PLAuNPs (50 to 500 μg/ml), phosphate buffer (100 mM, pH 7.4), NADH (468 μM), NBT (156 μM) and PMS (60 μM) was incubated for 5 min and formation of the purple colored formazan was measured at 560 nm. Rutin was used as a positive control in this assay.

2.5.3. Nitric oxide radical scavenging assay

The nitric oxide scavenging activities of PLFE and PLAuNPs were determined according to the GriessIllosvoy reaction (Garatt, 1964). Briefly, nitric oxide radicals generated from sodium nitroprusside (10 mM) were added to different concentrations of PLFE and PLAuNPs (50–500 μg/ml) in phosphate buffer saline (pH 7.4) and incubated for 90 min. After incubation, Griess reagent was added and the absorbance was measured at 540 nm. Rutin was used as a positive control.

2.5.4. Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging activity of PLFE and PLAuNPs was performed based on the method described by (Patel et al., 2010). In this assay, 300 μl of PLFE and PLAuNPs with different concentrations of samples (10–60 μg/ml) were added to 600 μl of H_2O_2 solution (100 mM, in PBS) and the absorbance was measured at 230 nm with separate blank reagent for each concentration. Rutin was used as standard in this assay. The