Journal of Molecular Liquids 212 (2015) 331–339

Contents lists available at ScienceDirect

Journal of Molecular Liquids

journal homepage: www.elsevier.com/locate/molliq

Studies on catalytic, antioxidant, antibacterial and anticancer activities of biogenic gold nanoparticles

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ARTICLE INFO

Article history:
Received 5 July 2015
Received in revised form 4 September 2015
Accepted 8 September 2015
Available online xxxx

Keywords:
Areca catechu
Gold nanoparticles
Catalytic degradation
Antioxidant activity
Antibacterial activity
HeLa cell lines

ABSTRACT

Biosynthesis of nanoparticles of precious metals has been attaining a surge of interest in recent years. In the present study, phytochemicals present in *Areca catechu* nut have been used for the synthesis of gold nanoparticles at 300 K, 373 K and under microwave irradiation of 2450 MHz. The synthesized nanoparticles have been characterized using UV–visible, TEM, XRD, and FTIR techniques. Perpetual changes in synthesis conditions are bestowed with appreciable morphological variation. An enhanced formation of monodispersed, spherical gold nanoparticles of size 13.7 nm could be obtained under microwave irradiation. XRD pattern confirms the crystalline nature of the as-synthesized nanoparticles. The biomolecules involved in the reduction and stabilization of nanoparticles have been identified using FTIR spectra. The catalytic efficiency of the synthesized gold nanoparticles of varying size distributions has been portrayed through the degradation of the organic pollutants, Methylene blue, Methyl orange, Eosin yellowish and 4-Nitrophenol. The observed size dependent catalytic activity may aid in the rapid elimination of industrial wastes leading to a green environment. The potential of the phytosynthesized nanogold in scavenging the harmful radical NO and the stable radical DPPH has been evaluated. In addition to its cytotoxic effect on HeLa cell lines, gold nanospheroids synthesized under microwave irradiation have been observed to exhibit an enhanced activity against a broad spectrum of bacterial pathogens as well.

1. Introduction

The rapidly growing field of nanotechnology has witnessed several synthesis strategies, ever since its emergence. However, the increased toxicity of metal nanoparticles (MNPS) synthesized using conventional methods [1–5] prompted scientists to search for a greener method. Biomediated synthesis of MNPS has gained wider acceptance over the years. Synthesis of MNPS using phytochemicals is more advantageous as it do not require elaborate processes. Due to their unique and tunable surface plasmon resonance, gold nanoparticles (GNPS) are of great interest to current researchers in nanotechnology. Besides extending their applications in homogeneous and heterogeneous catalysis [6–8], GNPS have made a significant mark in biological fields as well [9,10]. The fascinating properties of GNPS enable their extensive application as biomedicine, biosensor and as an indispensable component in many electronic and magnetic devices [11–13]. The synthesis of nanogold using extracts of plant species has always been recognized as one of the greener methods of preparation. Few notable works in this field include the use of extracts of *Azadirachta indica* [14], *Cymbopagon flexuosus* [15], *Cinnamomum camphora* [16], *Emblica officinalis* [17] and *Zingiber officinale* [18].

In this paper, the aqueous extract of areca nut has been used as the biological reductant of GNPS. Areca nut is the seed of the *Areca catechu* (Ac) tree cultivated in East Africa, South East Asia and the Pacific Islands. It is usually referred to as 'betel nut' chewed along with betel leaves. Areca nut constitutes carbohydrates, fats, proteins, crude fiber, polyphenols (terpenoids, flavonoids, and tannic acid), alkaloids and minerals. All the plant parts are considered to have therapeutic values. The ingredients present in areca nut have possible uses in cosmeceuticals, schizophrenia and psychosis. Phenolic components in this nut efficiently inhibit hyaluronidase activity. The alkaloid and polyphenols present in Ac enhance the healing of wounds owing to burns and skin graft surgery. Their use further extends to the treatment of constipation, dyspepsia, beriberi and oedema [19–21]. Easy procurement, non toxicity, cost effectiveness and bioactivity make areca nut feasible for use as a reducing agent.

Dyes widely used in textile and dyestuff industries are not readily biodegradable. In addition to being carcinogenic to various life forms, the deleterious effects of these hazardous materials cause severe environmental contamination. Traditional techniques such as flocculation, membrane separation and ultrafiltration are ineffective to decolorize organic dyes due to their stability and complex atomic structure [22,23]. In the present study, the catalytic degradation of Methylene blue (MB), Methyl orange (MO), Eosin yellowish (EY) and 4-Nitrophenol (4-NP) in the presence of the synthesized gold colloids have been carried out.

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http://dx.doi.org/10.1016/j.molliq.2015.09.013
0167-7322/

Highly toxic radicals in the biological system include reactive oxygen species (ROS), reactive nitrogen species (RNS), reactive chlorine species (RCS) and reactive sulfur species (RSS). Despite the presence of efficient endogeneous antioxidative defensive mechanisms in humans, a redox imbalance between radical production and antioxidant action due to drastic and perpetual changes in the exposed surroundings results in excessive radical production, accumulation and consequent oxidative stress leading to several neurodegenerative diseases. Ac nut is a good source of polyphenolic components, tannins and flavonoids [24]. Catechin, condensed tannin is a powerful antioxidant [25]. This justifies the use of aqueous extract of Ac nut for the synthesis of GNPS with radical scavenging activity. The antioxidant potential of the biogenic GNPS has been determined through Nitric oxide (NO) and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging activities.

The antibacterial efficacy of GNPS has always remained an established fact. In addition to their low toxicity, bactericidal and bacteriostatic effects of GNPS against a broad spectrum of bacteria make them excellent antimicrobial drug delivery vehicles [26]. In the present study, the potential of the phytosynthesized GNPS as an antibacterial against *Escherichia coli* (*E.coli*), *Klebsiella pneumonia* (*K.pneumonia*), *Pseudomonas aeruginosa* (*P.aeruginosa*), *Enterobacter sp.* (species) and *Staphylococcus aureus* (*S.aureus*) has been ascertained through agar well diffusion method.

Cancer is defined as a heterogeneous collection of cells that evolve in tumor microenvironment. In view of cancer diagnosis and treatment, GNPS have been perceived to be an imminent candidate for targeted drug delivery, which could be utilized to trace the path of cancer cells. It is obvious that most of the bioactive compounds are stupendous anticancer drugs that commonly inhibit the progression of cancer cells via apoptosis and inhibition of cell cycle [27,28]. In this study the synthesized GNPS have been screened for its anticancer effect against human cervical carcinoma cells (HeLa).

2. Experimental

2.1. Materials and methods

Dried Ac nuts are collected from local market, followed by thorough washing with demineralised water. 15 mg of the washed, finely powdered nuts of Ac are further boiled in 100 mL demineralised water for 5 min and filtered to get the aqueous nut extract. Chloroauric acid ($HAuCl_4$) purchased from Sigma-Aldrich is used as the source of Au^{3+}.

2.1.1. Synthesis of gold nanoparticles

Gold colloids are synthesized at constant concentration of the precursor solution and that of the reducing agent. The pH of the solution is set at 6. Temperature variation adopted for the synthesis includes 300 K and 373 K. To 30 mL of 2.5×10^{-4} M chloroauric acid, 10 mL of the aqueous nut extract is added at 300 K, with continued stirring for 5 min. The appearance of a stable violet color after 4–5 h indicates the formation of GNPS (colloid A). The experiment is repeated at 373 K to obtain red colored colloid B. Further, a mixture of the nut extract and precursor solution is irradiated with microwave radiation of frequency, 2450 MHz, for 1 min to get colloid C. Rapid formation of stable red colored gold colloid within 1 min of the addition of the nucleating agent, is attained in both the cases (colloid B and C). The synthesized colloids are found to be stable for two months.

2.2. Instrumentation

The UV–visible spectra of the synthesized GNPS are recorded on a Perkin Elmer Lambda-35 spectrophotometer in the wavelength range of 400–900 nm at a resolution of 1 nm. TEM images are obtained using a Tecnai G^2 30 transmission electron microscope. An XPERT-PRO diffractometer with Cu Kα radiation ($\lambda = 1.5406\ A^0$) is used to obtain the XRD pattern. FTIR spectra of GNPS and the extract are recorded using an IR prestige − 21 Shimadzu spectrometer by the KBr pellet technique.

2.3. Catalytic activity

The catalytic property of the biogenic gold colloids is evaluated by the reduction of organic dyes MB, MO, EY and 4-NP. 1 mL of 100 mM sodium borohydride ($NaBH_4$) is added to 1 mL of 1 mM organic pollutants (MB, MO and EY). Similarly, another mixture is prepared by mixing 1 mL of 5 mM 4-NP with 1 mL of 0.25 M $NaBH_4$. The former solution is made up to 12 mL while the latter is made up to 25 mL using demineralized water. This is followed by the stirring of both the mixtures for 10 min and addition of 2 mL of gold colloid A, to each mixture with stirring continued for 2 min. The catalytic degradation is monitored using UV–visible spectrophotometer at regular intervals of time. The experiment is repeated with colloids B and C.

2.4. In vitro antioxidant activity

The antioxidant potential of the phytosynthesized GNPS is studied through (1) NO scavenging activity and (2) DPPH assay.

2.4.1. NO scavenging activity

NO scavenging activity is measured spectrophotometrically. Sodium nitroprusside (5 mM) is mixed with methanol diluted samples and incubated at 25 °C for 30 min. The incubated solution is then diluted with an equal volume of Griess' reagent. The procedure is repeated for the blank solution. Gallic acid is chosen as the reference (R) [29]. The percentage inhibition of the reaction mixture with the addition of the antioxidant is A_{test} and that of the blank solution is $A_{control}$.

Percentage inhibition [30] is calculated using the equation,

$$\%\text{inhibition} = \left\{\frac{A_{control} - A_{test}}{A_{control}}\right\} \times 100 \quad (1)$$

2.4.2. DPPH assay

DPPH is a stable, purple colored free radical which turns yellow when scavenged. This property of the radical is exploited to exhibit the free radical scavenging activity of the GNPS. Different volumes (1.25–20 μL) of gold colloids (A, B, C) are made up to 40 μL with dimethyl sulfoxide (DMSO) and DPPH (0.1 mM) solution is added. At room temperature the reaction mixture is incubated in the dark for 20 min. After incubation, the absorbance measurements are made at 517 nm. The procedure is repeated for blank solution [30]. A decrease in absorbance with respect to blank solution, on addition of the antioxidant, indicates the degree of radical scavenging potential of the antioxidants. Ascorbic acid is taken as reference (R). The percentage inhibition of the radicals by the biogenic GNPS is determined using Eq. (1).

2.5. Antibacterial and anticancer studies

Agar-well diffusion method is adopted for the antibacterial studies of biosynthesized GNPS. Petri plates containing 20 mL Muller Hinton medium are seeded with bacterial strains such as *E.coli*, *K.pneumonia*, *P.aeruginosa*, *Enterobacter* and *S.aureus*. Well of approximately 10 mm is bored using a well cutter and 25, 50, and 100 μL of gold colloid C (14 mg in 1 mL DMSO) is added. The plates are kept incubated at 37 °C for 24 h. The antibacterial activity is measured in terms of diameter of the inhibition zone formed around the well with gentamycin as the positive control. (National Committee for Clinical Laboratory Standards. (1993a)).

HeLa cervical cancer cell line (NCCS Pune) is maintained in Dulbecco's modified eagles media (HIMEDIA) supplemented with 10%