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Quercetin and gallic acid mediated synthesis of bimetallic (silver and selenium) nanoparticles and their antitumor and antimicrobial potential

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ABSTRACT

In this study a synthetic approach for the stable, mono-dispersed high yielding bimetallic (Ag–Se) nanoparticles by quercetin and gallic acid is described. The bimetallic nanoparticles were synthesized at room temperature. Different reaction parameters (concentration of quercetin, gallic acid and Ag/Se salt, pH, temperature and reaction time) were optimized to control the properties of nanoparticles. The nanoparticles were characterized by various analytical techniques and their size was determined to be 30–35 nm. Our findings suggest that both the reduction as well as stabilization of nanoparticles were achieved by the flavonoids and phenolics. This study describes the efficacy of quercetin and gallic acid mediated synthesis of bimetallic (Ag–Se) nanoparticles and their *in vitro* antioxidant, antimicrobial (Gram-positive and Gram-negative bacteria) and antitumor potentials. The synthesized Ag–Se nanoparticles were used as anticancer agents for Dalton lymphoma (DL) cells and in *in vitro* 80% of its viability was reduced at 50 μg/mL.

1. Introduction

Nanoparticles have the wide ranges of applications in various fields from pharmaceuticals to medical diagnosis, physics to biology and therapeutics to biosensor development [1]. A large scale of research has been focused to control the size and shape of nanoparticles to reflect in their chemical, biological and optical properties [2]. Due to their unique properties (larger surface to volume ratio, absorption in the visible region), therapeutic potential in treating a variety of diseases including cancer is highly effective [3]. Reduction in particle size results in the increased therapeutic efficacy and reduced side effect. In the last decade, several pharmaceutical companies have got the approval of nano-formulation based drugs from the Food and Drug Administration (FDA) and other health institutes [4].

Silver and selenium nanoparticles have gained much attention towards the field of nanomedicine [5]. Selenium is already reported for the treatment of various types of cancer, however, the mechanism for anticancer activity is not fully understood [6]. Several hypothetical mechanisms have been proposed for their activity i.e. antioxidant protection, enhanced carcinogen detoxification, enhanced immune surveillance, inhibition of tumor cell invasion, inhibition of angiogenesis and modulation of cell proliferation, etc. [7]. Metal nanoparticles are synthesized by various physical, chemical and biological methods [8–15]. Even though the chemical methods involve a simple procedure, they employ reducing agents, such as sodium borohydrate and other inorganic compounds which are highly toxic and hazardous to the environment [16].

The eco-friendly green synthesis involves formation of nanoparticles by means of phytochemicals or bio-molecular reduction with better control over the shape and size having no toxicity [17]. A variety of metal nanoparticles such as Ag, Au, Pd, Se, Cu and Pt has been synthesized using biological catalyst [17,18]. Biomolecules present in the plants such as proteins/enzymes, polysaccharides, alkaloids, flavonoids, terpenoids, phenolic compounds and vitamins are generally involved in the reduction, formation and stabilization of metal nanoparticles [19–22]. The synthesis of silver, gold and selenium NPs using biological route had been previously reported in literature [17,18,23,24].

A common flavonoid, quercetin is one of the flavonol family members that contains five hydroxyl groups in positions 3, 5, 7, 3′, 4′ and a carbonyl group in fourth position. The quercetin is an important natural free radical scavenger and easily forms complexes with many metals [25]. Flavonoid/phyto-chemicals are utilized for human health due to their biological and pharmacological activities including anticancer, anti-allergic, antioxidant, antiviral, anti-inflammatory and cardiovascular protection potentials [26]. Gallic acid, a naturally occurring plant polyphenol, is a strong antioxidant. Gallic acid prevents the rancidity induced by lipid

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peroxidation and is used in the processed food, cosmetics and food packing materials. The pharmacological activity was also reported by Yen et al. [27].

In the present study, bimetallic nanoparticles (Ag–Se NPs) have been synthesized using two phytochemicals such as quercetin and gallic acid. The nanoparticles were characterized and therapeutic potentials were investigated. The ultimate aim of the present study was to synthesize bimetallic nanoparticles using bioactive flavonoids and phenolics to find out their antioxidant, antimicrobial and antitumor potentials.

2. Materials and methods

2.1. Reagents and media

Silver nitrate ($AgNO_3$), sodium selenite (Na_2SeO_3), azino-bis-ethyl benzthiazoline-sulfonic acid (ABTS), Folin–Ciocalteau, concanavalin-A (Con-A), gallic acid, diphenyl-picryl hydrazine (DPPH), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], trolox and quercetin dihydrate were purchased from Sigma–aldrich (Bangalore, India). Fetal calf serum (FCS) was purchased from Invitrogen, Grand Island, NY, USA and culture medium (RPMI-1640) was purchased from HiMedia, Mumbai, India. All other chemicals were procured either from HiMedia, Mumbai, India or Super Religare Laboratory (SRL), Mumbai, India. Bacterial strains were obtained from Microbial Type Culture Collection, Institute of Microbial Technology – IMTECH, Chandigarh, India.

2.2. Synthesis of nanoparticles

Nanoparticles were synthesized following the previously reported method using plant extract with a little modification [8]. Various concentrations of quercetin and gallic acid were prepared for the synthesis of nanoparticles. Different dilutions of quercetin and gallic acid (10–1000 μL) from stock solution (10 mM) was prepared in water and further diluted to 100 mL with deionised water to make the reducing solution of different concentrations. The resulting solutions were reacted with a mixture of silver and selenium salt (0.5–5 mM) by incubating at 35 °C (200 rpm) in dark condition. Various reaction parameters for nanoparticle synthesis [quercetin and gallic acid (1–100 μM, each), metal salt concentration (0.5–5 mM), pH (3–11), temperature (25–65 °C), etc.] were optimized to increase the yield of the nanoparticles. pH of the reaction mixture was adjusted using 1 N NaOH and 1 N HCOOH.

2.3. Characterization of bimetallic nanoparticles (Ag–Se NPs)

The nanoparticles were purified by centrifugation and characterized using various techniques.

2.3.1. UV–Vis spectroscopic analysis

To analyse the UV–Vis absorption of Ag–Se nanoparticles, the samples were scanned on UV–Vis spectrophotometer (Hitachi U 3010 double beam) in the range of 300–700 nm with water as baseline. The stability of synthesized nanoparticles in solution form was determined using UV–Vis absorption in every 7 days for 3 months.

2.3.2. Size distribution and zeta potential

To calculate the particle size distribution and charge potential, a colloidal nanoparticle solution was used in a quartz cuvette (Zetasizer Nano Instrument, Malvern) and measurements were taken.

2.3.3. Transmission electron microscopy

TEM measurements were taken using FEI HR-TEM instrument operated at voltage 120 keV. The samples were prepared onto carbon coated copper TEM grids by drop coating of Ag–Se NPs diluted colloidal solution. The elemental analysis was performed to confirm the nanoparticle element through energy dispersive X-ray spectroscopy (EDX).

2.3.4. X-ray diffraction analysis

X-ray diffraction (XRD) analysis of dried powder of Ag–Se NPs was carried out on a XRD instrument (Bruker axs System, D8, Germany). The scanning was performed in the angular range of 2θ from 30° to 80°.

2.4. Determination of total flavonoid and phenolic contents

Nanoparticles were harvested by centrifugation (22,000*g*, 15 min) and washed thrice with deionised water to remove excess quercetin dihydrate and gallic acid. The total flavonoid content was determined by previously reported method using quercetin dihydrate as a reference standard [28,29]. Briefly, the Ag–Se NP samples (50 μg/mL) and quercetin dihydrate (50–500 μg/mL) were individually mixed in water. All the solutions (100 μL each) were individually mixed with 2% (100 μL) aqueous solution of anhydrous aluminium chloride. After 10 min incubation at room temperature, the absorbance of the reaction mixture was measured at 435 nm. The total flavonoid content was expressed as quercetin dihydrate equivalent (QDE %). All the experiments were performed in triplicate.

Using previously reported method [28,29], a sample of nanoparticle (50 μg/mL) and standard gallic acid 50 μL (50–500 μg/mL) were individually mixed with deionized water (3.95 mL) in a falcon tube (15 mL) followed by the addition of 2 N Folin–Ciocalteau's phenol reagent (250 μL). The resulting mixture was vortexed and incubated at room temperature for 10 min followed by the addition of sodium carbonate solution (20% w/v, 750 μL). Samples were vortexed again and kept at room temperature for 2 h. The absorbance was recorded at 725 nm. The total phenolic content was expressed as gallic acid equivalent (GAE %).

2.5. Antioxidant assays

ABTS, DPPH and MTT antioxidant assays were performed to determine the antioxidant activity of the nanoparticles. These assays were carried out according to the standard assay procedures reported in literature [28,29].

2.5.1. ABTS assay

ABTS liquid solution was mixed with aqueous solution of potassium per sulphate (2.45 mM) with a ratio of 2:1 (v/v). The mixture was allowed to keep in dark at room temperature for 8 h. In the resulted ABTS solution (190 μL), 10 μL Ag–Se NPs (50 μg/mL) and different concentrations of ascorbic acid (25–250 μg/mL, 10 μL each) were separately added. The experiments were performed in triplicate. The mixtures were then incubated for 6 min at room temperature and the absorbance was measured at 734 nm. The deviations in absorbance with respect to the negative control (ABTS solution without testing compound) was expressed as 100% free radicals and calculated as percentage scavenging. Ascorbic acid (25–250 μg/mL) was used to make the standard curve and also used as a positive control (50 μg/mL).

2.5.2. DPPH assay

An ethanolic solution of DPPH (200 μM) in 100 μL deionized water was used as negative control. The nanoparticle solution (50 μg/mL) and standard trolox (25–250 μg/mL) were separately