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JOURNAL OF ENVIRONMENTAL SCIENCES
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Intracellular biosynthesis of Au and Ag nanoparticles using ethanolic extract of *Brassica oleracea* L. and studies on their physicochemical and biological properties

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ARTICLE INFO

Article history:
Received 8 April 2014
Revised 4 June 2014
Accepted 13 June 2014
Available online 27 January 2015

Keywords:
Biosynthesis
Broccoli extract
Gold
Silver nanoparticles
Antimicrobial

ABSTRACT

In this present study, we reported broccoli (*Brassica oleracea* L.) as a potential candidate for the synthesis of gold and silver nanoparticles (NPs) in green chemistry method. The synthesized metal nanoparticles are evaluated their antimicrobial efficacy against different human pathogenic organisms. The physico-chemical properties of gold nanoparticles were analyzed using different analytical techniques such as a UV–Vis spectrophotometer, Field Emission Scanning Electron Microscopy, energy dispersive X-ray spectroscopy, X-ray diffraction and a Fourier Transform Infrared spectrophotometer. In addition, gold and silver NP antimicrobial efficacy was checked by disc diffusion assay. UV–Vis color intensity of the nanoparticles was shown at 540 and 450 nm for gold and silver nanoparticles respectively. Higher magnification of the Field Emission Scanning Electron Microscopy image shows the variable morphology of the gold nanoparticles such as spherical, rod and triangular shapes and silver nanoparticles were seen in spherical shapes. The average spherical size of the particles was observed in 24–38 nm for gold and 30–45 nm for silver NPs. X-ray diffraction pattern confirmed the presence of gold nanoparticles and silver nanoparticles which were crystalline in nature. Additionally, the functional metabolites were identified by the Fourier Transform Infrared spectroscopy. IR spectra revealed phenols, alcohols, aldehydes (sugar moieties), vitamins and proteins are present in the broccoli extract which are accountable to synthesize the nanoparticles. The synthesized gold and silver NPs inhibited the growth of the tested bacterial and fungal pathogens at the concentration of 50 μg/mL respectively. In addition, broccoli mediated gold and silver nanoparticles have shown potent antimicrobial activity against human pathogens.

Introduction

In recent years, in order to enhance the growth of industrial and commercial sector, the nanotechnology has produced numerous value added products for daily life purposes. Nanotechnology is a field that incorporates life sciences, and has become enriched with activities in nanomaterial synthesis and energy production. The environmental friendly route synthesis of nanoparticles has

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http://dx.doi.org/10.1016/j.jes.2014.06.050
1001-0742/

abundant pharmaceutical applications such as treating and diagnosis of acute and chronic diseases (Shankar et al., 2004). Generally, physical and chemical methods have good exposure for the synthesis of nanomaterials in large quantities as well for specific size and shape (Noguez, 2007). However, these synthetic methods have an adverse effect on living things as a result of using strong chemicals as reductants and stabilizing agents. Accordingly, the development of novel environmentally benign methods for the biosynthesis of nanoparticles is widely explored for medical, pharmaceutical, electronic devices, solar energy and other commercial value added products. Nanoparticles have widespread application in different arenas due to their novel physicochemical properties (Kumar et al., 2011). According to that gold and silver nanoparticles have been used as cytotoxic drugs, antioxidant, antiinflammatory and antimicrobial agents (John et al., 2008). These advanced materials/metals such as gold, silver, copper, zinc, platinum and palladium nanoparticles have been used in diverse applications such as electronics, super capacitors, biolabelling and catalysis for chemical reaction (Park et al., 2011). The biosynthesis of nanoparticles using plant extracts is well designed, easy, single step reaction, to synthesize desired size and shape of the nanoparticles. However, the biosynthesis method does not use any reductants and stabilizing agents in the bioreduction steps (Klaus et al., 1999; Carlson et al., 2008). Recently, many bacteria biological substances such as, fungi, mushroom and plants are a greater effort to nanoparticles synthesis in green chemistry methods were reported, also the biological mediated nanoparticles can control various acute and chronic diseases (Becker, 1999). Broccoli (*Brassica oleracea* L.) vegetables belong to *Brassica* family. It contains a high amount of vitamins, antioxidants and anticarcinogenic compounds. Also, broccoli contain glucosinolates, a diverse class of sulfur- and nitrogen-containing metabolites (Nowack, 2010). Due to these compounds, broccoli has potential chemo protective properties and controlling various viral and bacterial diseases. Therefore, to our knowledge, this is the first scientific report on the use of broccoli extract to synthesize gold and silver nanoparticles in a non-hazardous way and monitor their biocide activity against various human pathogenic microorganisms.

1. Materials and methods

1.1. Preparation of ethanolic extract of broccoli

The healthy and cruciferous vegetable broccoli (family: *Brassicaceae*) was purchased from the commercial market at Kuantan, Malaysia. The plant materials were washed thoroughly using mild warm water to remove contamination from insects and fungal dust. The broccoli was cut into small pieces and dried at room temperature at shadow condition. The completely dried broccoli was powdered by using a mechanical grinder. A 1-g of broccoli fine powder was added with 200 mL of 90% ethanol and keep into a shaker at 37°C at 12 hr. The extract was filtered using a 0.22 μm membrane filter and the extract was stored at 4°C for further experimental analysis.

1.2. Biosynthesis of gold and silver nanoparticles

For the synthesis of desired size and shapes of AuNPs, AgNPs the various amount of broccoli extract used and also we optimized different physico-chemical parameters such as time (1, 2, 3, 6, 12 and 24 hr), metal ion concentration (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} mmol). The mixture solution was continuously monitored the product growth kinetics by UV–Vis spectrophotometer in the ranges of 300–700 nm. However, a color change occurred in the mixture solution, which indicates that reduction is occurring. After complete reduction, the synthesize medium was centrifuged at 12,000 rpm for 20 min. The pellet was collected and dried in an oven at 45°C. The biosynthesis reaction was attained without using any catalytic chemicals and polymer as a stabilizing and capping agent (Narayanan and Sakthivel, 2008).

1.3. Structural characterization of gold and silver nanoparticles

The UV–Vis spectra of gold and silver nanoparticles were recorded using a T80 UV–VIS spectrometer, PG instruments Ltd., Beijing, China with a resolution path of 10 nm. The field emission scanning electron microscope JEOL, JSM 7800F USA, and attached energy dispersive X-ray detector ware used and analysis the structure and composition of biosynthesized nanoparticles. The powder X-ray diffraction data of gold and silver nanoparticles were measured by XRD-Rigaku mini flux II, Japan with a built in nickel monochromator. The operating X-ray tube radiation used Cu Kα (λ = 1.5415 A°, 30 kV, 15 mA). The ICDD-40784 powder diffraction database was used and evaluate the nature of nanoparticles. IR spectra were obtained from FT-IR Perkin Elmer spectrum 100 CT, USA. The powder Au and Ag nanoparticle samples were prepared in thin pellets using potassium bromide (KBr). The sample was read at a 400–4000 cm^{-1} wavelength range with a resolution of 4 cm^{-1}.

1.4. Phytochemical characterization of ethanolic broccoli extract

To evaluate the presence of different phytochemicals such as phenolics, alkaloids, flavonoids, saponins, and ascorbic acid in the broccoli aqueous extract by standard method prescribed by Harborne, (1998) and Lewis and Ausubel, (2006).

1.5. Antibacterial assay for biosynthesized Au and Ag nanoparticles

The antibacterial activity of gold and silver nanoparticles was determined by using the agar disk diffusion method with minor modifications. Authentic microbial strains were cultured on nutrient agar medium (recommended by Himedia, India) for 24 hr at 37°C. A 1 mL authentic bacterial culture was taken and poured into a marked agar plate. The bacterial cultures were equally spread into the agar plate using a glass L rod. Besides, the prepared different concentrations of biosynthesized gold and silver nanoparticle antibacterial discs were placed into the bacterial cultured agar plate. After 24 hr incubation, the zone of inhibition was measured. The results were compared with the amphicillin which is a positive control. Experiments were carried out in triplicates to avoid the statistical errors.

1.6. *In vitro* antifungal assay of metallic Ag and Au nanoparticles

The gold and silver NPs effectively inhibited the growth of the fungal strains at the optimum concentrations compared with a standard drug. Potato dextrose agar plates were prepared according to the manufacture guidelines (NCCLS, 1998). The sterilized PDA plate was swabbed by selected fungal cultures on the surface of the plates. After that, the prepared gold and silver nanoparticle disks (50 μg/mL) were placed in the PDA