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Cytotoxicity and antimicrobial activities of green synthesized silver nanoparticles

S. Lokina [a], A. Stephen [b], V. Kaviyarasan [c], C. Arulvasu [d], V. Narayanan [a,*]

[a] Department of Inorganic Chemistry, School of Chemical Sciences, University of Madras, Guindy Maraimalai Campus, Chennai 600025, India
[b] Department of Nuclear Physics, University of Madras, Guindy Maraimalai Campus, Chennai 600025, India
[c] CAS in Botany, University of Madras, Guindy Maraimalai Campus, Chennai 600025, India
[d] Department of Zoology, University of Madras, Guindy Maraimalai Campus, Chennai 600025, India

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ABSTRACT

Bio-inspired silver nanoparticles are synthesized using *Malus domestica* (apple) extract. Polyphenols present in the apple extract act as a reducing and capping agent to produce the silver nanoparticles. UV–Visible analysis shows the surface plasmon resonance (SPR) absorption at 420 nm. The FTIR analysis was used to identify the functional groups responsible for the bio-reduction of silver ion. The XRD and HRTEM images confirm the formation of silver nanoparticles. The minimal inhibitory concentration (MIC) of silver nanoparticles was recorded against most of the bacteria and fungus. Further, MCF-7 human breast adenocarcinoma cancer cell line was employed to observe the efficacy of cancer cell killing.

1. Introduction

Nanotechnology is one of the promising fields for generating new applications in medicine. The metallic nanoparticles have been synthesized by various methods. The novel biosynthetic route using fruit and plant extracts has been proved superior to other methods. The silver nanoparticles have some distinctive properties like conductivity, catalytic activity, chemical stability and antimicrobial activity. The biosynthesis of silver and gold nanoparticles using geranium and neem leaf extracts was reported by Shankar et al. [1,2]. The green chemistry utilizes nontoxic chemicals and environmental friendly silver nanoparticles from abundantly available apple fruit extract. The proverb "an apple a day keeps the doctor away," addresses the health effects of the apple [3]. There is the evidence that apples possess phenolic compounds which may be cancer-protective and demonstrate antioxidant activity [4]. The important phenolic phytochemicals present in apple are quercetin, kaempferol, myricetin, epicatechin, and procyanidin B_2 [5]. The primary phenolic acid, chlorogenic acid is found in pulp and skin of an apple. When an apple is red in color, it is because of more anthocyanins. Epicatechin is the primary polyphenol present in apples. Recent research studies shows that polyphenols in the skin of an apple absorb the UV-B radiation and prevent it from damaging the photosynthetic cells in the apple skin like natural sunscreen [6,7]. In the apple seeds, 98% of the flavonoid phloridzin is found. Research suggests that apples may reduce the risk of prostate cancer, colon cancer and lung cancer. Compared to many other fruits and vegetables, apples contain relatively low amounts of vitamin C but are a rich source of other antioxidant compounds [8]. The polyphenols in apples can prevent spikes in blood sugar by various mechanisms. Flavonoids like quercetin can inhibit enzymes (alpha-amylase and alpha-glucosidase) which are involved in the breakdown of carbohydrates into sugar. The fiber content in apples reduces the cholesterol by preventing reabsorption, and they are bulky for their calorific content.

The modern nanotechnology has facilitated the production of silver nanoparticles with low toxicity to human and greater efficacy against bacteria. Nanoparticles are attractive alternative to antibiotics by showing improved activity against multi drug resistant bacteria. In general, silver ions can bind with a variety of negatively charged molecules like RNA, DNA and proteins. These biologically synthesized silver nanoparticles were found to be highly toxic against different multi drug resistant human pathogens. The various silver-based compounds and materials containing ionic

* Corresponding author.
E-mail addresses: vnnara@unom.ac.in, vnnara@yahoo.co.in (V. Narayanan).

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silver (Ag^+) [9,10] or metallic silver (Ag^0) [11] have been synthesized and shown to exhibit antimicrobial activity against various bacteria. The inactivation of bacteria is associated with nanoparticles concentration [12], nanoparticle shape, bacterial type [13,14], the presence of Ag^+ and nanoparticle size [15–17]. The bacterial growth at a given concentration of silver has been found to be dependent on the initial number of cells. The antibacterial efficacy of silver nanoparticles increases because of their larger total surface area per unit volume [18].

The effect of antimicrobial activity of the silver nanoparticles was observed in *Staphylococcus aureus*, *Citrobacter koseri*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*. The minimum inhibitory concentration was determined by Resazurin microtitre assay (REMA). DNA and bacterial membrane proteins possess sulfur and phosphorus compounds. Silver has higher affinity to react with these compounds [19].

Cancer is a major health problem and it arises from one single cell. The transformation of a normal cell into a tumor cell occurs in a multistage process and the changes are due to the interaction between genetic factors of a person and external agents of three categories like physical carcinogens, chemical carcinogens and biological carcinogens. In worldwide, each year 7.6 million people die from cancer. According to WHO, if it continue rising without any immediate action, 13.1 million people may die in 2030. Tobacco use, alcohol use, lack of physical activity, low intake of fruit and vegetable are some of the important risk factors, the reason for 30% of worldwide cancer deaths. Diagnosis of tumors in the human body was very difficult [20,21] at their earlier stage and there was a search of new treatment for treating this deadly disease. Radiotherapy, chemotherapy and surgery are some of the cancer treatments which are used to improve the patient's life. Recently, nanoparticles are also used to overcome this problem. The nanoscale devices can easily enter the cells and they made an interaction with DNA, proteins, enzymes and cell receptors. The nanoparticles can detect the cancer disease in a very small volume of cells or tissue [22,23]. In this study we focused on the cytotoxicity of silver nanoparticles on cultured MCF-7 cell line using different concentrations. It is the acronym of Michigan Cancer Foundation – 7 (Cancer Institute in Detroit). MCF-7 cell line was first obtained from the breast tissue of a 69-year old (Caucasian woman) Frances Mallon. Her cells only gave a current knowledge about breast cancer [24]. Prior to MCF-7, it was found very difficult for cancer researchers to obtain a mammary cell line. They are useful for *in vitro* breast cancer studies because the cell line has retained about the mammary epithelium. They enhance the ability of the MCF-7 cells to process estrogen via estrogen receptors in the cytoplasm of the cell. MTT (3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide) method was used to assess the antiproliferative effect [25].

In the present study, we wish to report for the first time green synthesis of silver nanoparticles using the extract of apple (*Malus domestica*). The optical absorption spectrum of green synthesized silver nanoparticles is recorded by using UV–Visible spectrophotometer. Morphological characterizations are performed using XRD, SEM and TEM. The spherical shaped silver nanoparticles showed its toxicity against MCF-7 human breast adenocarcinoma cancer cell lines. They showed excellent antimicrobial effect to inhibit different pathogenic bacteria and fungus.

2. Results and discussions

2.1. UV–Visible spectroscopy

The apple extract was mixed with the aqueous solution of silver nitrate. The color change from yellow to black indicates the formation of stable silver nanoparticles. Fig. 1a shows the optical photograph of pure apple extract (a) and after addition of silver ions to apple extract (b). The free electrons of silver nanoparticles give rise to a surface plasmon resonance absorption band [26–28]. The synthesis of silver nanoparticles was evaluated at different contact time by taking absorbance using UV–Vis spectroscopy. It was noted that the sharpness of the absorption peak increases with increase of time. The absorbance of pure apple extract was taken at the beginning and after the addition of 0.01 M $AgNO_3$ to apple extract, the spectra was recorded every 60 min of time interval. At the initial stage, there was no characteristic plasmon resonance peak. The surface plasmon resonance band starts to occur at 420 nm [29,30] at the infinite time which indicates the formation of spherical silver nanoparticles (Fig. 1b). Jain et al. reported the similar results for the synthesis of silver nanoparticles [31].

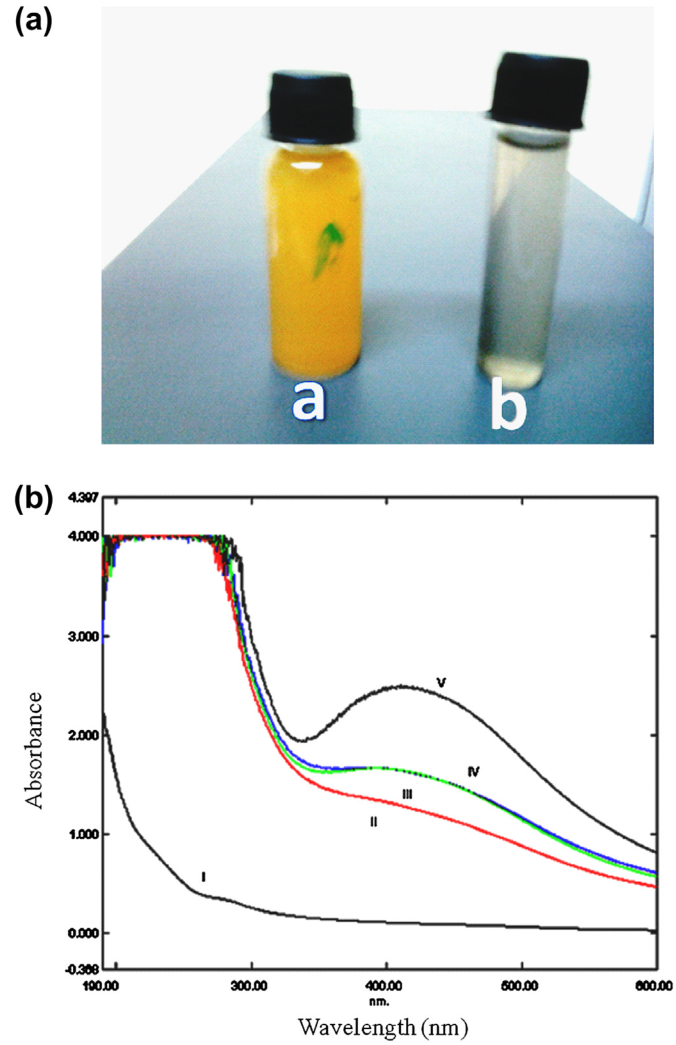

Fig. 1. a: (a) Pure apple extract (b) After the addition of $AgNO_3$ to apple extract. b: UV–Vis absorption spectrum of silver nanoparticles synthesized from apple extract, **I** – Pure apple extract, Immediately after the addition of $AgNO_3$, **II** – 1 h, **III** – 2 h **IV** – 3 h, **V** – Infinite time.

2.2. FTIR spectral analysis

FTIR measurements were used to identify the potential functional groups of the biomolecules present in the apple fruit extract. FTIR spectrum shows absorption bands at 3498, 2925, 1624, 1384,