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Cytotoxic activity of biosynthesized Ag Nanoparticles by *Plantago major* towards a human breast cancer cell line

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Abstract Silver nanoparticles were synthesized by bio-inspired route, a cost effective and fast synthesis method. Structural and morphological characterization of nanoparticles was performed by UV–visible absorption spectroscopy, Fourier transform infrared spectroscopy, X-ray diffraction and transmission electron microscopy. The cytotoxic activity of both nanoparticles and *Plantago major* extract containing nanoparticles against a human breast cancer cell (MCF-7) was studied in vitro. MTT (3-4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays were performed using various concentrations of *P. major* extract (alone) and extract containing AgNPs ranging from 0.5 to 2.5 µg/ml. Data analysis showed significant level of cytotoxic activity. The potential cytotoxicity of silver nanoparticles in the treatment of breast cancer is discussed.

Keywords Cytotoxic · Breast cancer · *Plantago major* · MTT · Biosynthesis AgNPs

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1 Introduction

Synthesis of nanoparticles (NPs) has been one of the branches of technology that exhibits a fast development in the area of nanotechnology (Hashemi et al. 2016; Mahmoudvand et al. 2014; Abedi et al. 2011; Sattarahmady et al. 2013, 2015; Khatami et al. 2017a). Specifically, major attention has been paid to investigate different controlled shape, size and dispersion degree of NPs which focused on bio-inspired methods (Shimoshige et al. 2017; Zare et al. 2017; Hamedi et al. 2017a; Mortazavi et al. 2017; Khatami et al. 2017b; Singh et al. 2016a, 2017a; Ahn et al. 2017; Beitollahi et al. 2016; Jahani and Beitollahi 2016; Seddighi et al. 2017). Among NPs, technology for the synthesis of silver nanoparticles (AgNPs) can be highlighted, due to its important activities such as anti-microbial, anti-leishmania activities and optical property (SudhaLakshmi 2011; Moradi et al. 2016; Hamedi et al. 2016, 2017b; De Sio et al. 2015; Meymandi et al. 2010; Singh et al. 2016b; Markus et al. 2016; Niroomand et al. 2017). Several methods have been used in the synthesis of NPs such as physical, chemical and biological methods (Bankara et al. 2010; Balasooriya et al. 2017; Nejad et al. 2017; Ghodselahi et al. 2014, 2015; Moussavi et al. 2014; Samadi et al. 2015; Beitollahi and Nekooei 2016). Among these methods, chemical method is simple, but reducing agents in it such as Citrate, Polyvinylpyrrolidone, Borohydride and other inorganic compounds are very toxic and are not appropriate for medical purposes (Kemp et al. 2009; Khodashenas and Ghorbani 2016; Moghaddam et al. 2017; Ahmadrajabi et al. 2016; Sobhanipoor et al. 2017). On the other hand, the nanoparticle produced by biological method is more valuable than the one produced by chemical method due to the non-existence of organic toxic residuals, production of minimum wastages, high volume of production and repeatability (Soshnikova et al. 2017).

Regardless of wide applications of AgNPs, there is a serious lack of information regarding their biological influences on human cells (Singh et al. 2017b). The toxic potential of biological synthesis of AgNPs on human breast cancer cell (MCF-7 cell line) has been evaluated. Breast cancer is one of the most widespread malignant cancers in women all over the world and is the most important cause of death arising from cancer among women (Malorni et al. 2006; Castro-Aceituno et al. 2016). Some researchers have reported the effects of plant extracts on cancer cells including breast cancer. Herbal compounds as anti-cancer agents were used for the first time by Hartwell et al. in the late 1960's. They used Podophyllotoxin and its derivatives as anti-cancer agents (Srivastava et al. 2005). The plant used in this research is fleawort (*Plantago major* L.) from the family of Plantaginaceae which has various medicinal purposes (Samuelsen 2000a). This plant is considered as an important medicinal plant due to the presence of compounds such as phenol, flavonoids, alkaloids, terpenoids and Vitamin C (Samuelsen 2000b). Traditionally, *P. major* has played a role in the treatment of cancer due to the presence of high amounts of phenolic compounds and flavonoids. Kanda et al. studied 8 types of flavonoids of *P. major* and indicated that Luteolin-7-O-β-glucoside is the major compound of flavonoids in most species of *P. major* (Kanda et al. 1994). Results of researches by Galves et al. showed that Luteolin-7-O-β-glucoside has a strong anti-cancer activity in inhibiting breast adenocarcinoma (Galvez et al. 2003).

In the light of the above cited results, the present study attempted to design a green, high cost/benefit ratio and non-toxic method for synthesizing AgNPs using seed extract of *P. major* for the first time. Both the anti-cancer properties of plant extract and biosynthesized AgNPs on MCF-7 cancer cells were investigated.

2 Materials and methods

2.1 Preparation of the extract

The seeds were washed under running tap water. The clean seeds were powdered. 20 grams of obtained powder was added to an Erlenmeyer flask containing 100 ml of boiling deionized water and was boiled for 15 min. Next the extract was filtered by Whatman filter paper No. 1.

2.2 Synthesis of silver nanoparticle

Briefly, 0.1 M aqueous solution of silver nitrate ($AgNO_3$) was prepared. A 10 ml of *P. major* extract was added into 20 ml of aqueous solution of 1 mM silver nitrate for reduction of Ag^+ ions and was incubated overnight at room temperature. The resultant yellowish brown solution indicates the formation of AgNPs (Khatami et al. 2016a).

2.3 UV–vis spectra analysis

The AgNPs formation was monitored using UV visible spectrophotometry. Absorption wavelength was studied between the range of 200–700 nm.

2.4 Powder X-ray diffraction (XRD)

The red solid product was separated by repeated centrifugation at 14,000 rpm for 10 min followed by re-dispersion of the agglomerate of AgNPs into deionized water three times. After this procedure, the remaining sediment was dried at 30 °C for 48 h. Panalitical, X Pert Pro, from Netherland was used for XRD analysis. XRD analysis was also applied to determine the particle size using Scherer's formula:

$$d = \frac{k\lambda}{\beta \cdot \mathrm{Cos}\,\theta_{\mathrm{max}}}$$

where, d is the average crystal size, is the X-ray wavelength (0.1541 nm), β is the full-width at half-maximum (FWHM) and θ is the diffraction angle (Azizi et al. 2016).

2.5 TEM analysis

TEM (Carl ZIESS) was using to determine the size, shape and distribution of biosynthesized AgNPs (Darroudi et al. 2014).

2.6 FTIR

FTIR was performed before and after the biosynthesis of AgNPs to detect the possible functional groups in biomolecules present in the plant extract (Khatami et al. 2016b).

3 Anti-cancer test

3.1 Treatment of cancer cells by the extract

Twenty-four hours after seeding the cells in 96-well microtiter plates, the medium was changed and the cells were treated with doses of 1–15 µg/ml of AgNPs or of the extract and incubated for 24 h (Alishah et al. 2017; Oh et al. 2017). The MTT assay was performed as previously described (Castro-Aceituno et al. 2016).

Data were subjected to One-way Analysis of Variance to determine the significance of individual differences at $p < 0.05$ level. Significant means were compared by Duncan's multiple range test. All statistical analyses were conducted using SPSS statistical software package (SPSS, Version 10.0, Chicago, USA).

4 Results and discussion

The visual inspection of extract containing synthesized AgNPs showed a clear change in color from yellowish to reddish brown. This color evolution indicates the formation of metallic AgNPs. The Ag^0 nanoparticle was synthesized through the reduction process of Ag^+ ions using plant extract of Plantago as a major reducing agent to synthesize silver nanoparticles (Fig. 1).

The UV–visible absorption peak of *P. major* extract treated with silver nitrate is detected at around 432 nm indicating the formation of AgNPs (Fig. 1). Spectrum of the mixture containing seed extract of *P. major* treated with silver nitrate was measured at different times. It was observed that AgNPs are present for more than 4 months, in samples with the concentration of 5 mM of silver nitrate.

XRD analysis showed four distinct diffraction lines positioned at 38.03°, 44.38°, 64.54° and 77.64°, which were indexed to (*hkl*) planes 111, 200, 220 and 311, respectively, characteristic of metallic silver with cubic face-centered symmetry (Fig. 2). The unassigned diffraction line at $2\theta = 54.6°$ in Fig. 2 is thought to be related to crystalline or semi-amorphous organic phases. The average size of the AgNPs was calculated using Scherer's formula being equal to 26 nm.

TEM image of the AgNPs showed that particles are spherical in shape with an average size ranging from 1 to 30 nm (Fig. 3). Given that the applications for AgNPs are highly dependent on the shape, size, and average particle size distribution of the particles, the strategy of using seed

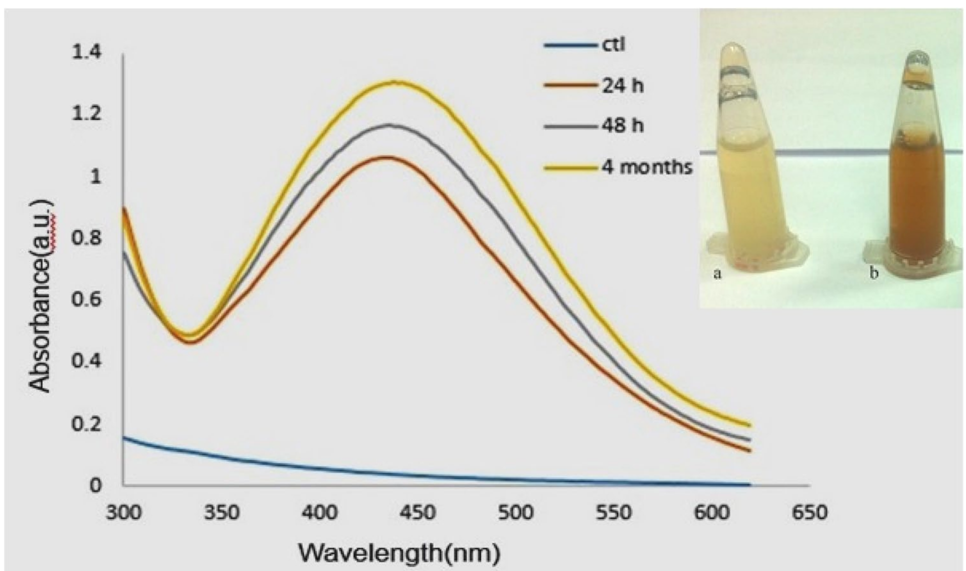

Fig. 1 UV–Vis absorption spectrum of biosynthesized AgNPs at different times

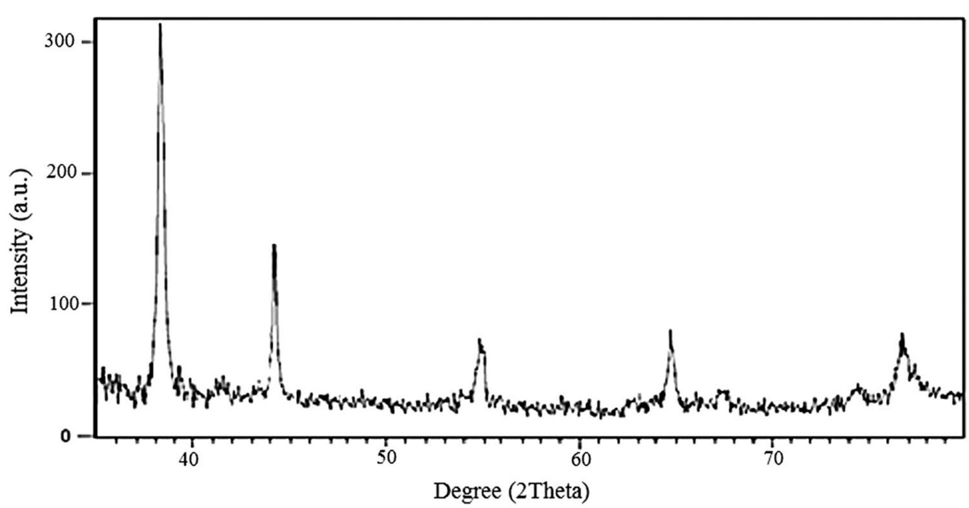

Fig. 2 X-ray diffraction pattern of biosynthesized AgNPs using *P. major* extract

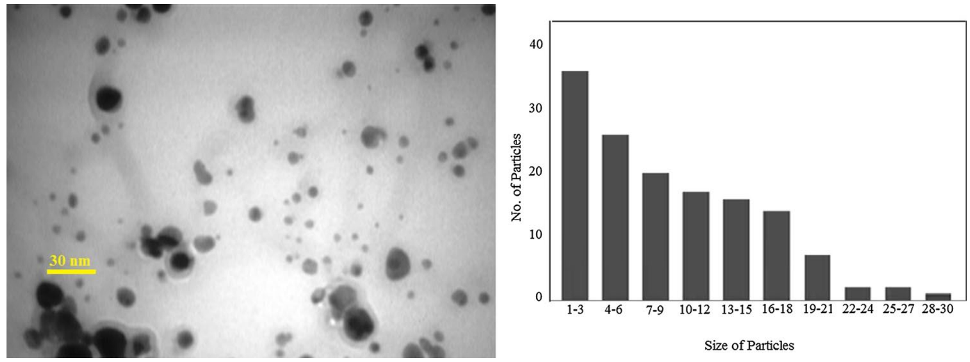

Fig. 3 TEM image and histogram of the biosynthesized AgNPs

extract of *P. major* seems promising. Therefore, in this study for the first time, we have reported the simple and facile green synthesis of AgNPs using seed extract of *P. major*.

FTIR spectrum shows absorption bands at 3422, 2921, 2856, 1743, 1631, 1450, 1377, 1240, 1043 and 596 cm^{-1} indicating the presence of capping agent with the nanoparticles (Fig. 4).

According to Fig. 4, the bands centered at 3422, 2921, 1743, 1631 and 1450 cm^{-1} in spectra are assigned to O–H, C–H, C–C, C–N and N–H, respectively. This assignment suggests a possible role of alcohol, phenol and proteins in the synthesis of AgNPs (Bankar et al. 2010).

The effects of different doses of seed extract of *P. major* and seed extract of *P. major* containing silver nanoparticles on cellular survival of MCF-7 breast cancer cells have been investigated. First, we examined the effect of seed extracts of *P. major* on MCF-7 cells survival using the MTT test. Twenty-four hours after seeding the cells into 96 well microliter plates, the effect of 1.0, 1.5, 2.0, 2.5, 5, 10 and 15 µg/ml of *P. major* extract on cell growth was analyzed. Extract concentrations of 1.0, 1.5 and 2 µg/ml did not show a considerable toxic effect on MCF-7 cancer cells, however, a significant toxic effect was detected with doses from 5 to 15 µg/ml. With the latter dose the toxic effect was highest (Fig. 5). Further analysis of the organic compounds and of their concentrations present in the extract of *P. major* seeds are needed. The results obtained by treating the MCF-7 cancer cells with extracts containing silver nanoparticles were quite different. We observed a toxic effect with concentrations of 1.5 µg/ml.

Krishnaraj et al. (2016) and Kulandaivelu et al. (2014) also studied the toxic effects of AgNPs on the MCF-7 cancer cell line. In those studies, however, AgNPs had significant cytotoxic effects at concentrations of 100 µg/ml (Krishnaraj et al. 2016; Kulandaivelu et al. 2014). Sonbaty studied the cytotoxic effect of fungi-synthesized AgNPs (8–20 nm) using *Agaricus bisporus* on MCF-7 breast cancer cell and reported its LD_{50} at 50 µg/ml (El-Sonbaty 2013), In addition, Murugan et al. (2016) reported that AgNPs IC_{50} is 60–80 µg/ml on MCF-7. Therefore, the anti-cancer activity

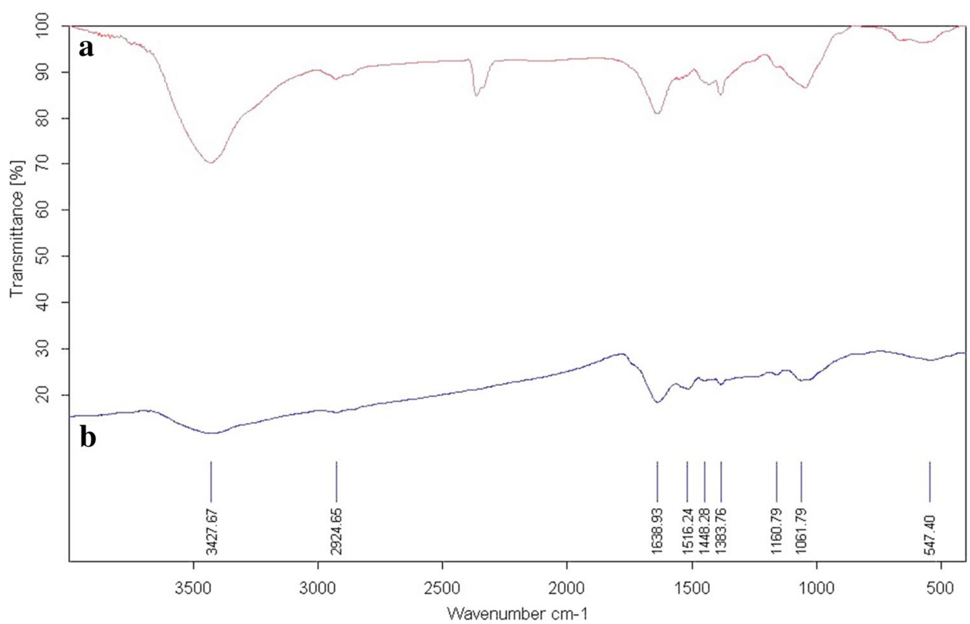

Fig. 4 FTIR spectra of biosynthesized AgNPs using *Plantago major*

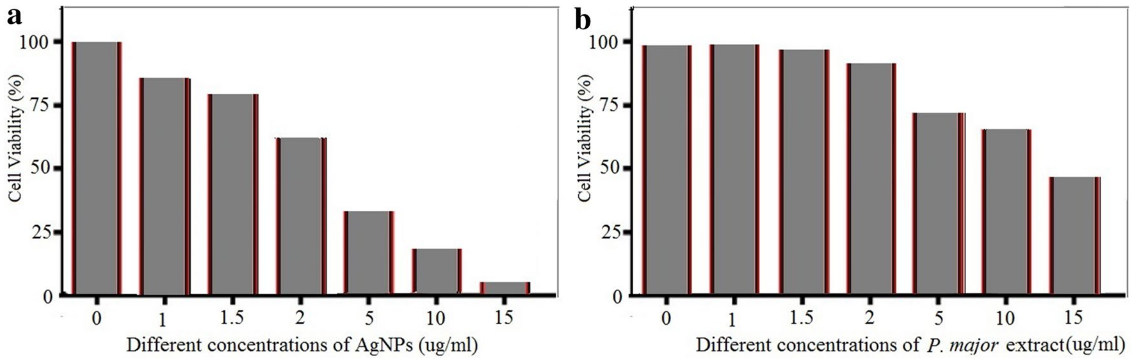

Fig. 5 Biosynthesized AgNPs using *Plantago major* reduce cell viability in MCF7 cell line. Cell viability was measured by MTT assay. Data are representative of three. **a** At different concentrations of *P. Major* extract containing silver nanoparticles. **b** At different concentrations of *P. Major* extract alone

of biosynthesized AgNPs using *P. major* observed in the present study was greater than that observed in the above cited studies. Higher anti-cancer activity of biosynthesized AgNPs using *P. major* can be especially useful in developing green synthesized NPs with greater anticancer potential.

5 Conclusion

Green synthesis of silver nanoparticles using seed extract of *P. major* plant was achieved. The UV–visible absorption peak of biosynthesized AgNPs is detected at around 432 nm. XRD analysis showed four distinct diffraction lines positioned at 38.03°, 44.38°, 64.54° and 77.64°, which were indexed to planes 111, 200, 220 and 311, respectively. Cytotoxic and anti-cancer effect of *P. major* seed extract on MCF-7 cancer cell was determined. Both the extract and the AgNPs exhibited a cytotoxic effect on the MCF-7 breast cancer cells. The biosynthesized AgNPs showed a stronger anticancer effect than that showed in similar studies performed.

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Author contributions The authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors confirm that this article content has no competing interests.

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