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GREEN BIOSYNTHESIS OF SILVER NANOPARTICLES USING *POMEGRANATE PEEL* AND INHIBITORY EFFECTS OF THE NANOPARTICLES ON AFLATOXIN PRODUCTION

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Abstract

In this work, pomegranate peel has been used as a natural and safe method for biosynthesis of silvernanoparticles. The synthesis of silver nanoparticles was confirmed using UV spectroscopy, which showed a peak around a wavelength of 437 nm. The morphology showed spherical and monodispersed nanoparticles with a size range between 5-50 nm. Using scanning electron microscopy (SEM) and transmission electron microscopy (TEM), X-ray diffraction (XRD) experiments revealed their crystalline nature. Active functional groups in the synthesized silver nanoparticles were determined using Fourier transform infrared (FTIR) spectrometers contained four bands at 3281.21 cm^{-1}, possibly indicating the participationof O–H functional group. The peak take place at 1,636.22 cm^{-1} may be pointed to C = N bending in the amide group or C = O stretching in carboxyl. Transfer in this peak (from 1,641 to 1,643 cm^{-1}) shown the possible role of amino groups or carboxyl in nanoparticle synthesis. The peaks at 431.95 and 421.28 cm^{-1} be related to AgNPs bonding with oxygen from hydroxyl groups which confirm the role of pomegranate peel as a reducing agent. Furthermore, we investigated effects of these nanoparticles on aflatoxin B1 production by the fungus *Aspergillus flavus*, isolated from hazelnut. The results found that aflatoxin production in all *A. flavus* isolates decreased with an increase in the concentration of silver nanoparticles. Maximum suppression of aflatoxin production was recorded at a nanoparticle concentration of 150 ppm.

Keywords: aflatoxin inhibition, characterization, green synthesis, pomegranate peel, silver nanoparticles

Introduction

Nanoparticles (NPs) are seen as a solution to many technological and environmental challenges. Green synthesis methods of NPs involve less chemicals and are, therefore, environment-friendly. Recently, some studies have been investigating antimicrobial and antioxidant production using nanoparticles synthesized from natural products (Nuamsetti *et al*., 2012, Hegazy *et al.*, 2015). Biosynthesis of silver nanoparticles (AgNPs) using plant parts is advantageous because they are eco-friendly, easily available and cost efficient (Shankar *et al.*, 2003, Song *et al.*, 2009, Narayanan *et al.*, 2008,Gan *et al.*,2012, Anjum *et al.*, 2016)**.**

There are different studies on synthesis of silver nanoparticles using plants such as sunflower, rice, sugarcane Mishra & Sardar, 2013), sorghum, maize (Choi *et al*., 2011)**,** neem (Shankar *et al*., 2004)**,** alfalfa (Gardea-Torresdey *et al*., 2003)**,** aloe (Chandran *et al*., 2006)**,** capsicum (Li *et al.*, 2013)**,** geranium (Vijayakumara *et al.*, 2013)**.** Fruit wastes have various phytochemicals and antioxidant compounds, such as gallocatechin (Someya *et al.*, 2002) and dopamine (Kanazawa & Sakakibara 2000), which are rich in free radicals (Kokila *et al.*, 2015, Ayaz *et al.*, 2016)**.** Many studies haveexploited using waste of plants such as banana peel (Ibrahim, 2015) orange, guava, lemon peel (Balashanmugam, 2014) for green synthesis of silver nanoparticles**.**

Pomegranate (*Punica granatum* L.), a fruit-bearing small tree, is native to Iran and the Mediterranean region and has been cultivated since ancient times (Meerts *et al.*, 2009, Shinwari *et al.*, 2011)**.** Applications of pomegranate fruit peels in synthesis of silver nanoparticles have been shown previously(Nisha *et al.*, 2015). The aim of this study was to synthesize silver nanoparticles using pomegranate peels and characterize them using ultraviolet–visible (UV) spectroscopy, Fourier transform infrared (FTIR) spectrometers, X-ray diffraction (XRD), scanning electron microscope (SEM) and transmission electron microscope (TEM).

Aflatoxins are carcinogenic and mutagenic secondary metabolites that are produced by fungi of Aspergillus species (Ahmad *et al.*, 2012). Major types of aflatoxins are B1, B2, G1, and G2 (Nisha *et al*., 2015). Aflatoxin B1 AFB1 is the most toxic aflatoxin, a known carcinogen affecting liver and possibly other organs (Jain *et al.*, 2009). In our study, we investigated inhibitory effects of the synthesized AgNPs on aflatoxin B1production by the fungus *Aspergillus flavus*.

Materials and Methods

Preparation of aqueous extract: The whole plant of *P. granatum* was collected locally from Kingdom of Saudi Arabia, Riyadh market. The peels of pomegranate were washed many times using distilled water, dried at 50˚C for 72 h in oven and ground to powder with a blender. 10 g of peel powder was added into 100 ml sterile distilled water, boiled for 15 min and filtered using Whatman filter paper (No. 1). Finally, the extract was sterilized using Millipore filters (0.2 µm).

Synthesis of AgNPs from plant extract:10 ml of pomegranate peel aqueous extract was mixed with 100 ml of silver nitrate (1mM) solution and incubated at 60-70ºC for 15-20 minutes. 1 mM silver nitrate solution, without addition of extract, was used as a control.

Characterization of AgNPs: The synthesis of AgNPs from *P. granatum* was verified using five techniques:

UV-visible spectroscopy: UV-Vis spectrophotometer (Victoria, Australia) was operated at wavelengths between 200 to 1000 nm to characterize synthesisof silver nanoparticles using pomegranate peel extract.

XRD measurements : The plant extract supernatant containing AgNPs was freeze-dried using a Heto Lyophilizer (Heto-Holten, Denmark). The powdered sample was analyzed by an X'pert PRO PANalytical diffractometer using CuKα radiation (k = 1.54056 Å) in the range of $20 \leq 2\theta \leq 80 \leq$ at 40 keV.

Fourier transform infrared (FTIR) : The changes in chemical bonds and composition were determined using (FTIR) Fourier transform infrared spectroscopy. After 3 days of incubation, the sample spectrum was recorded on the FTIR model Nicolet 6700 spectrometer with resolution of 4 cm^{-1} (Thermo Electron Corporation, USA). Measurements were performed in the range of 400– 4000 cm^{-1}.

Scanning electron microscopy (SEM): The morphology of AgNPs was studied using scanning electron microscope (SEM) JSM-7610F. Dried powder of the silver was placed on carbon-coated copper grid.

Transmission electron microscopy (TEM): The shape and size of the silver nanoparticles was determined using transmission electron microscopy -(JEM 1400 plus model with an acceleration voltage of 100 kV). Image J software was used for image analysis and particle size quantification.

Evaluation of AgNPs as inhibitors of aflatoxin B1 production: Three diverseconcentrations of AgNPs (50, 100 and 150 ppm) were added to SMKY medium (Sucrose 20 g, $MgSO_4.7H_2O$ 0.5 g, KNO_3 3 g, yeast extract 7 g) (Bankar *et al.*, 2010) and incubated with the toxigenic *A. flavus* at 25 ±2°C for 14 days. SMKY medium without silver nanoparticles was used as a control. The aflatoxin B1 was extracted using the filtrates after incubation (Abd El-Aziz, 2014) and analyzed B1using HPLC (Dubey *et al.*, 2010).

Results

UV-visible spectroscopy: After addition of $AgNO_3$ to pomegranate peel extract, the color of the mixture changed from yellow to dark brown (Fig. 1). Spectral analysis from UV-Vis spectroscopy showed that the solution peaked at an average wavelength of 437 nm after 72 h of reaction time, as shown in Fig.(2).

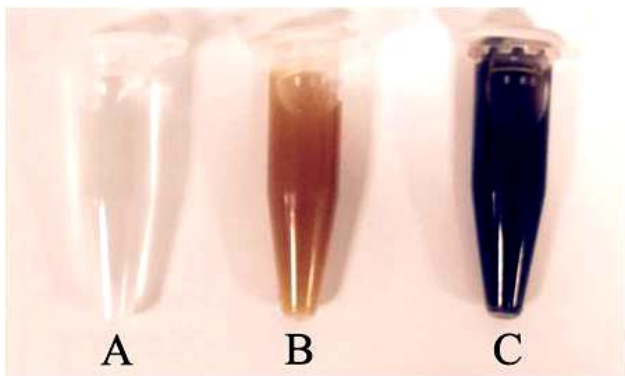

Fig.1 Picture of three eppendorf tube containing the aqueous solution of 10-3 M AgNO3 (A), Pomegranate peel Extract (B) and Synthesis of silver nanoparticles after addition A+B (c).

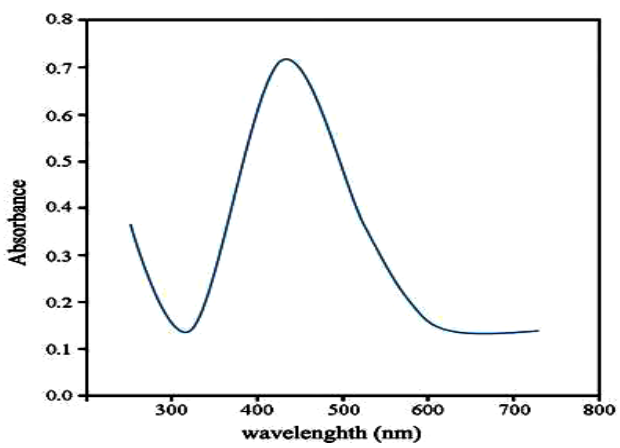

Fig. 2. The UV-Vis spectra recorded for the reaction of Pomegranate peel with AgNO3 solution

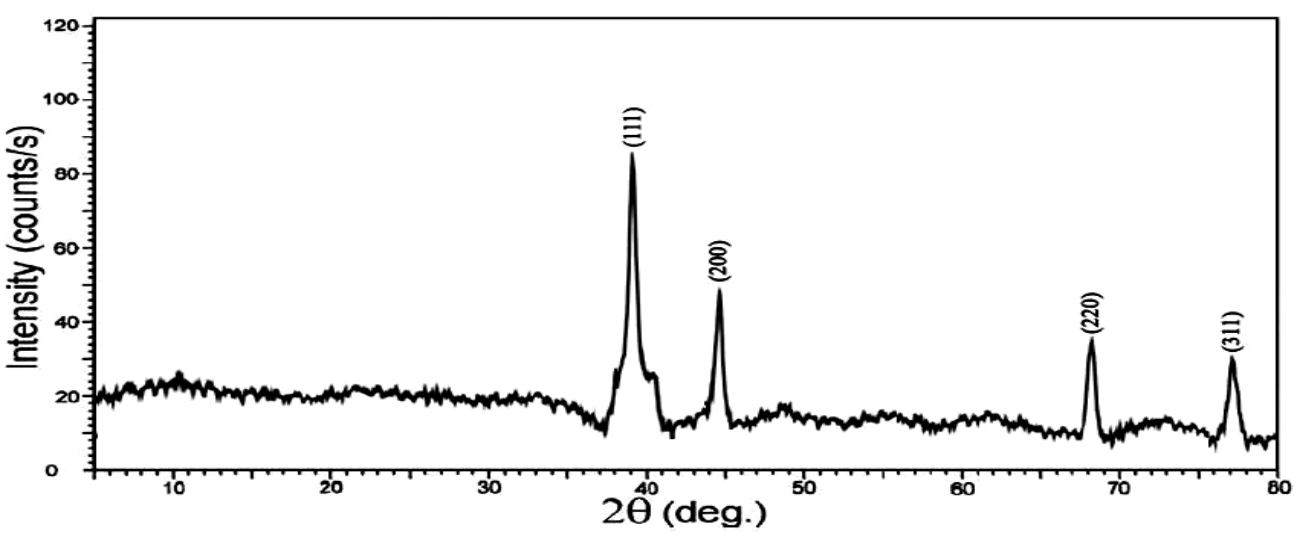

Fig. 3 Representative X-ray diffraction patterns of AgNPs synthesized by Pomegranate peel.

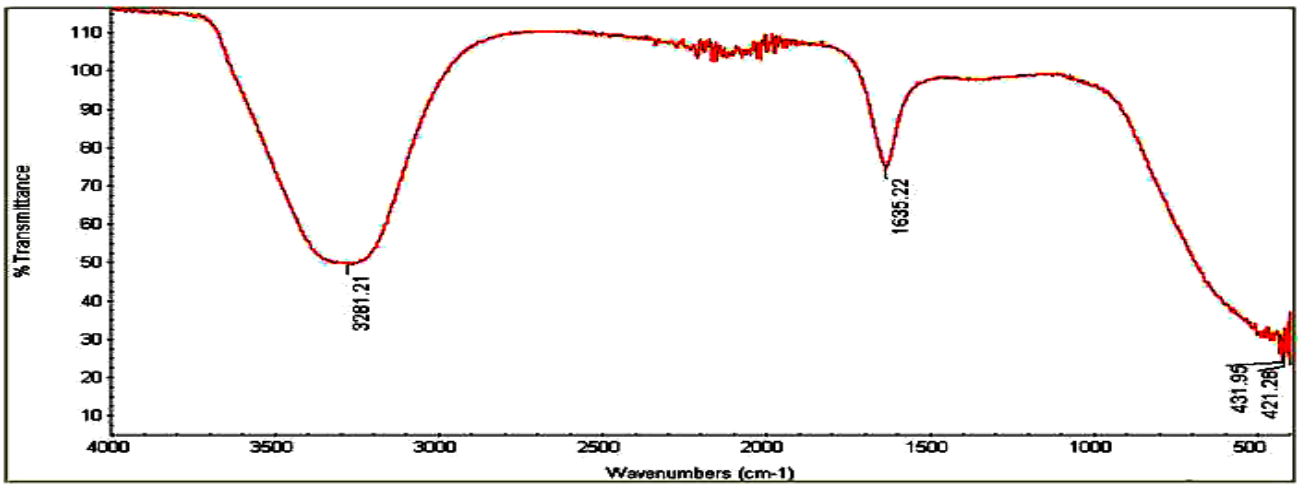

Fig.4. Fourier Transform Infrared Spectroscopy (FTIR) spectrum of Ag nanoparticles synthesized by reduction of Ag+ ions by Pomegranate peel.

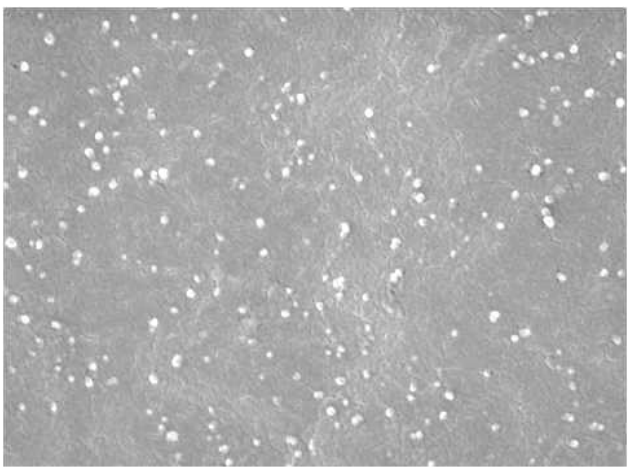

Fig. 5. Scan Electron Microscopy (SEM) images of synthesized silver nanoparticles by Pomegranate peel.

Fig. 6. Transmission Electron Microscopy (TEM) images of synthesized silver nanoparticles by Pomegranate peel.

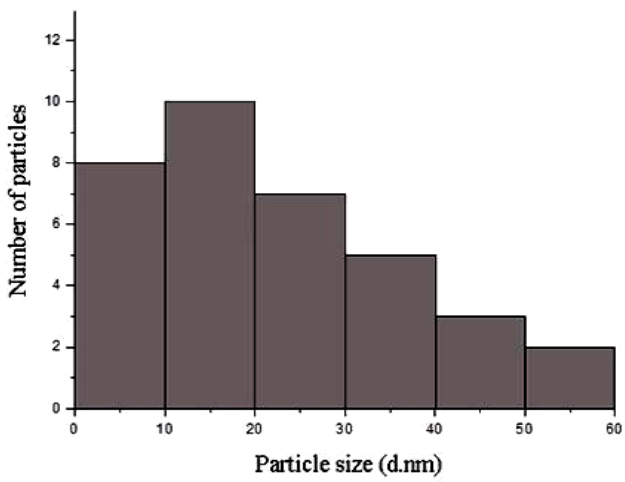

Fig. 7. A particle size distribution histogram of as synthesized silver nanoparticles determined from Transmission Electron Microscopy (TEM) images.

X-ray analysis :ntense XRD peaks were observed corresponding to the (111), (200), (220) and (311) planes at 2θ angles of 38.25, 44.31°, 68.31°, and 77.35°, respectively (Fig. 3).

FTIR spectroscopy:FTIR analysis was performed. Fig. (4) shows the FTIR spectrum of a freeze-dried powder of silver nanoparticles after 72 h of incubation. The FTIR spectrum contained four bands at 3281.21 cm^{-1} , 1635.22 cm^{-1} ,431.95 and 421.28 cm^{-1}.

Morphological characterization of AgNPs :SEM and TEM were used for morphological characterization of AgNPs. The SEM image in Fig.5 shows spherical shaped, highly dense silver nanoparticles. TEM micrographs (Fig. 6) show that the AgNPs are spherical and without agglomeration, the particle size histograms (Fig. 7) of silver nanoparticles show that the particle size ranges from 5 to 50 nm.

Evaluation of silver nanoparticles as anti-aflatoxigenic: Fig. 8 shows the effect of different concentrations of silver nanoparticles on the production of aflatoxin B1 and Fig. 9 shows HPLC analysis of the same. All concentrations of AgNPs led to a decrease in aflatoxin B1production, as compared to the control. A concentration of 150 ppm showed the maximum inhibitory effect with approximately 6-fold decrease in aflatoxin levels, as compared to the control.

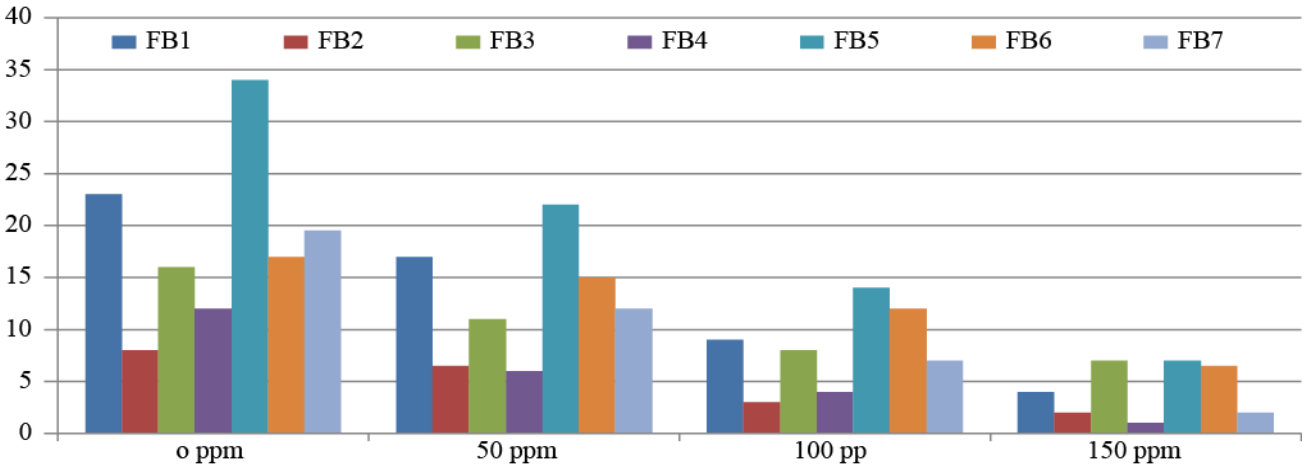

Fig. 8. Effect of different silver nanoparticles concentrations on aflatoxin B1 production by 7 isolates (FB1-FB7).

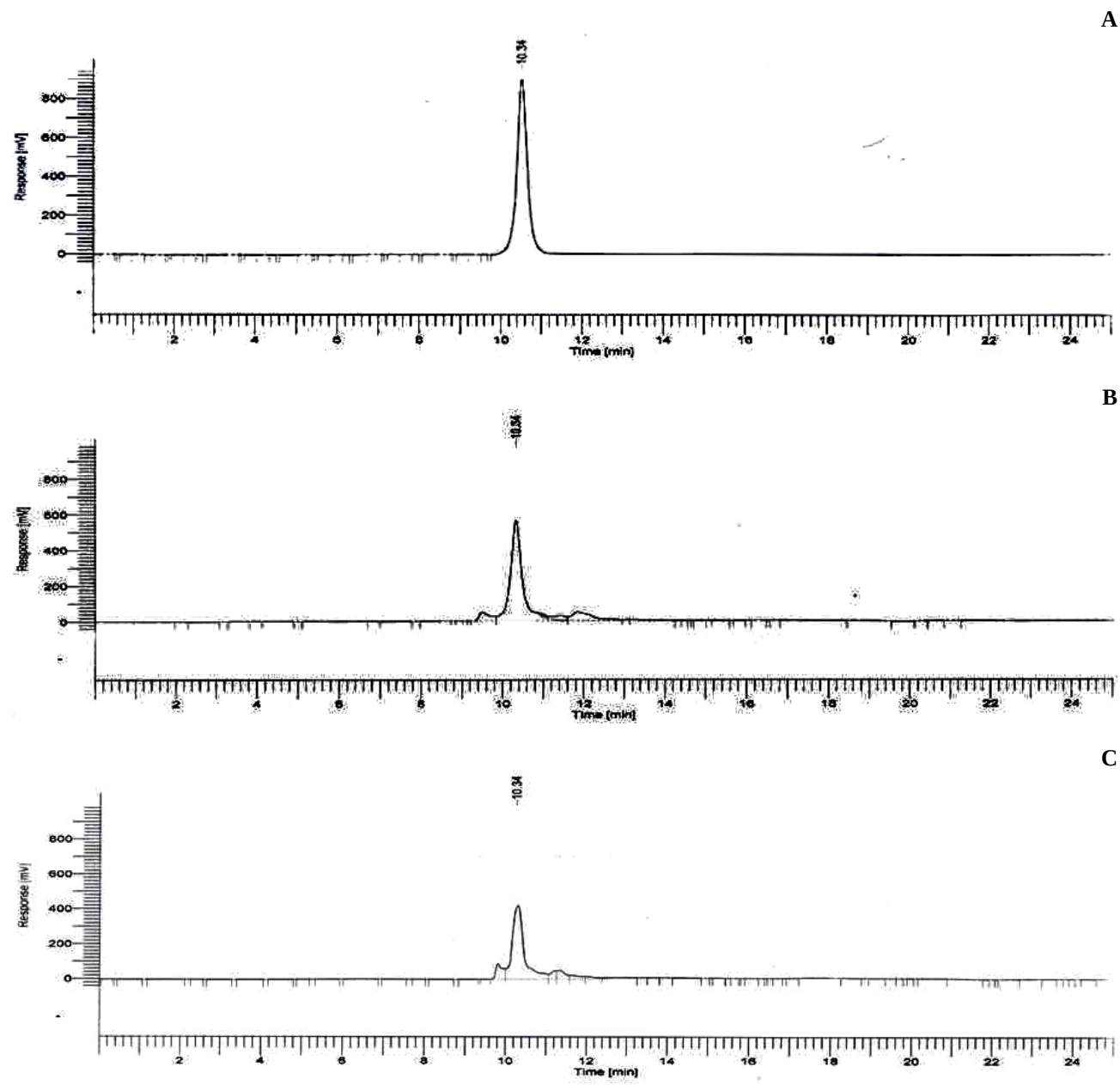

Fig. 9 HPLC analysis of aflatoxin B1: A) standard curve plotted concentrations of AFB1 standard (100 ppb) with a retention time of 10.34 min B) chromatogram of AFB1 sample without Ag nanoparticles (FB5) C) chromatogram of AFB1 sample after treated with Ag nanoparticles 50 ppm (FB5).

Discussion

In this study synthesized AgNPs exhibit yellowish brown color in aqueous solution was noted by UV-visible spectroscopy cause formation of silver nanoparticles, which leads to excitement of surface plasmon vibrations in silver nanoparticle solution (Roy *et al.,* 2013, Solgi & Taghizadeh 2012, Jain *et al*., 2011). The intensity of the color increased over the incubation period, indicating an increase in the number of synthesized AgNPs (Roy *et al.*, 2013, Abd El-Aziz 2014, Solgi & Taghizadeh, 2012). Reflecting the crystalline nature of the AgNPs were observed by X-ray analysis corresponding to the (111), (200), (220) and (311) planes at 2θ angles of 38.25, 44.31°, 68.31°, and 77.35°, respectively. These results can be assigned to face-centered cubic (fcc) metallic silver (JCPDS File No 03-0921), as reported previously (Li, 2012, Shanmugavadivu *et al.,* 2014). FTIR analysis was appeared four bands at 3281.21 cm-1, possibly indicating the presence of O–H functional group in the synthesis of nanoparticles. The peak at 1,635.22 cm-1 may be assigned to C = N bending in the amide group or C = O stretching in carboxyl. The broad peaks around 431.95 and 421.28 cm-1 are related to AgNPs bonding with oxygen from hydroxyl groups (Solgi &Taghizadeh, 2012, Jain *et al.*, 2011, Kumar et al., 2015). Amino acid and peptides prevent agglomeration by coating the silver nanoparticles and may possibly be responsible for their stabilization (Ovais *et al.*, 2016, Roy *et al.*, 2013, Abd El-Aziz, 2014). Morphological characterization of AgNPs by SEM and TEM image were shown spherical shaped, highly dense silver nanoparticles and without agglomeration, possibly because of coating of silver nanoparticles with amino acid and peptides consistent with previous results (Abd El-Aziz 2014, Jain *et al.,* 2011). The particle size of silver nanoparticles ranged from 5 to 50 nm, comparable to results from previous studies (Roy *et al.* 2013, Shanmugavadivu *et al.*, 2014). There is large variability in the size and shape of AgNPs produced by different plants, depending on the plant type and other factors such as temperature and pH of the medium (Shanmugavadivu *et al.,* 2014, Kumar *et al.,* 2015, Ovais *et al.,* 2016, Khatami *et al.,* 2015). Maybe cofactors can reduce and play effective role in salt reduction to produce nanoparticles (Roy *et al.*, 2013). In our study, we report an inhibition of aflatoxin B1 production dependent on the concentration of AgNPs. All concentrations of AgNPs led to a decrease in aflatoxin B1 production, as compared to the control. Nanoparticles have a large surface area due to their small size, allowing more contact with microorganisms (Rai *et al*., 2009, Dar *et al.,* 2013). Many studies have reported the antifungal activity of AgNPs on different fungus species (Kim *et al.*, 2009, Rathnayake *et al.*, 2012).while few studies reported their effect on aflatoxin production (Abd El-Aziz 2014, Mousavi & Pourtalebi 2015).

Conclusion

In the current work, we noted that pomegranate peel is a good, environment friendly source for synthesis of silver nanoparticles. Analytical techniques such as UV-Vis spectroscopy, FTIR, XRD, SEM, and TEM confirmed the synthesis of AgNPs. TEM revealed an average nanoparticle size range of 5-50 nm. FTIR was used to reveal the active functional groups in the synthesized silver nanoparticles, confirming the role of pomegranate peel as a reducing agent. The synthesized AgNPs showed inhibitory effects on aflatoxin B1 production in *A. flavus*.

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