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BIOSYNTHESIS OF SILVER NANOPARTICLES USING DIFFERENT GENOTYPES OF TALL FESCUE (*FESTUCA ARUNDINACEA* SCHREB.) EXTRACT

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Abstract

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In order to produce green synthesis of silver nanoparticles, the plant extract of 10 genotypes of tall fescue were used to reduce the silver ions present in the solution of 5M silver nitrate. This research was conducted in complete randomize design with 3 replications. The reaction was done at 70°C that the color of the extract changed from pale yellow to dark brown revealed the production of silver nanoparticles. The differences between genotypes extract were significant for evaluated traits such as percent of carbohydrate and potential of reduction based of UV spectrophotometry. The results showed that genotype number 4 had the highest means for the evaluated traits. In this study, the formation of silver nanoparticles was confirmed (by the presence of an absorption peak at 450 nm) using a spectrophotometer. The size and morphology of silver nanoparticles were determined using scanning electron microscopy. The shape of particles was spherical and their average size was about 90 nm. Comparison of different genotypes extracts showed that the plant extract with higher carbohydrate had the higher potential of reduction.

Key words: reducing agent; silver nanoparticle; tall fescue

Introduction

The genus Festuca is one of the largest families of grasses which are widely distributed in temperate regions and mountainous. Tall Fescue (*Festuca arundinacea* Schreb.= *Lolium arundinaceum* (Schreb) S. J. Darbysh) has been important in terms of forage. Due to having deep, wide and thick roots this plant can decreases the soil erosion (Sleper and West, 1996).

In Iran tall fescue naturally grows in the northern, central and western of Iran and plays an important role in forage production and protection of the soil (Tehrani et al., 2009). This plant has the best grow in cool conditions and only cool season grasses that can tolerate warm summers and cold winters for several years (Tehrani et al., 2009). Due to the high availability of tall fescue in all seasons of the year, use of this plant for the production of green silver nanoparticles is very economic.

Nanotechnology is a science that can be encompasses the understanding of the fundamental physics, chemistry, biology and technology of nanometer-scale objects (Ghorbani et al., 2011). Due to the different size, distribution and morphology of nanoparticles they exhibit completely new or improved behavior. Among different metal nanoparticles silver nanoparticles play an important role in the field of biology and medicine due to their attractive physiochemical properties. Silver nanoparticles are reported to possess anti-fungal, anti-viral and anti-bacterial such as *Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis* and *Klebsiellapneumo-*

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nia (Ahmed et al., 2016).

Up to know different methods used for the synthesis of silver nanoparticles, such as chemical approaches (Starowicz et al., 2006), thermal decomposition (Navaladian et al., 2007), the use of microwave (Sreeram et al., 2008) and green chemistry methods (Begum et al., 2009). Green chemistry is biosynthesis of nanoparticles using plant extract (Veerasamy et al., 2011). Green synthesis of nanoparticles is one of environmentally friendly green processes. Due to wide distribution of the plant and safety in handling this biosynthesis can be regarded as an economic method (Mittal et al., 2013). Processes employed for making nanoparticles using plant extracts are readily scalable and for the most part, less expensive (Iravani, 2011). In comparison to the relatively expensive methods based on microbial processes (Mittal et al., 2014), nanoparticles produced from plant extract, because of their medicinal properties, could be used in drugs, targeted drug delivery and cosmetic applications (Saranyaadevi et al., 2014). Furthermore, in comparison with chemical and physical methods, green synthesis has many benefits such as environmentally friendly, cost effective, and easily scaled up for large scale synthesis.

The present study was aimed to rapidly green synthesize of silver nano particles using aqueous leaves extract of 10 genotypes of tall fescue and evaluate antibacterial activity.

Materials and Methods

The fresh leaves of 10 genotypes of tall fescue (Table 1) were washed several times using running tap water, and then with distilled water to eliminate impurities. Twenty gram leaves of each genotype boiled for 15 min in 100 ml water. The prepared solution was initially filtered through normal filter paper mesh and the leafy materials removed then the extracts filtered through Whatman filter paper No. 1. The filtered extracts were stored in refrigerator at 4°C. This extracts were used as stabilizing agent to produce silver nanoparticles.

Synthesis of silver nanoparticles

Thirty ml of 5 M solution of silver nitrate was taken in Erlenmeyer; and 3 ml of each leaf extract was added to it separately at 70°C temperature. After 15 min the solution turned yellow to dark brown indicating the formation of silver nanoparticles.

The construction of silver nanoparticles was confirmed by UV spectroscopy using UNICO (Model SUV S2100, USA) spectrophotometer instrument. Here the sample was centrifuged at 9000 rpm for 20 min, dried using hot air oven and ground with KBr to form a pellet. The crystalline structure of the silver nanoparticles were determined by X-Ray diffraction analysis using an X-Ray diffractometer (XRD, Philips) instrument operating at 40 kV with 2 s time interval at the room temperature, 27°C. The morphology and the mean particle size of the silver were determined by SEM microscopy (HITACHI S-4160).

Table 1
Characteristic of 10 genotypes of *Festuca arundinacea* used in this research

Genotype Number	Code	Origin
1	L12	Iran, Isfahan, Semirom
2	6000/112	Iran, Isfahan, Isfahan
3	6000/112	Iran, Isfahan, Isfahan
4	06477	Iran, IsfahanDaran
5	RCAT041877	Iran, Isfahan, Mobarake
6	6000/A4	Iran, Charmahal, Shahrekord
7	6000/V3	Iran, Isfahan, Isfahan
8	1000.52	Iran, Shahrood, Semnan
9	1000.247	Iran, Isfahan
10	4000.44	Iran, Shahrood, Semnan

Antimicrobial assay

Synthesized compound are tested for inhibition against the human pathogenic bacteria. Pathogenic bacterial such as *Escherichia coli* (ATCC 35218) and *Staphylococcus aureus* (ATCC 29213) strains are inoculated in sterile nutrient broth and incubated at 37°C for 24 h. Pathogens are swabbed on the surface of the Muller Hinton Agar plates were infused with the 50 μl of plant leave extracts on the surface. Control petri dishes are placed with $AgNO_3$, DMSO and distilled water to assess the effect of $AgNO_3$ on pathogens. The plates are incubated at 37°C for 24 h and the antibacterial activity was measured.

Results and Discussion

UV–Vis spectral studies

The green synthesis of Silver nanoparticles with 10 genotypes extracts were carried out and confirmed by visual observation. The analysis of variance showed that the difference between genotypes extract were significant for percent of carbohydrate and potential of reduction (Table 2). Genotype number 4 had the highest amount for percent of carbohydrate and potential of reduction (Table 3). The correlation between percent of carbohydrate and potential of reduction was significant ($r = 0.58$). It means the genotypes with higher carbohydrate might have higher potential of reduction.

Due to reduction of silver ions the color was changed greenish brown color. Sivarman et al. (2014) and Sathyavathi et al. (2010) also observed leaf extracts were mixed

Table 2
Analysis of variance of tall fescue for Carbohydrate and reduction power based on UV spectrophotometer

Source of variation	df	Mean square	
		Carbohydrate (%)	Potential of Reduction
genotype	9	0.73*	0.075**
error	20	0.0625	0.01

*, **significant at $P< 0.05$ and $P< 0.01$, respectively

Table 3
Comparison of Mean value for percent of Carbohydrate and Potential of reduction based on UV Spectrophotometer in 10 genotypes of tall fescue

Genotype	Carbohydrate (%)	Potential of reduction
1	14.45^{b}	1.2^{cd}
2	13.83^{dc}	1.1^{d}
3	14.27^{bc}	1.3^{bc}
4	15.40^{a}	1.6^{a}
5	13.61^{d}	1.4^{b}
6	14.25^{bc}	1.1^{d}
7	13.93^{dc}	1.3^{bc}
8	14.12^{bc}	1.2^{dc}
9	13.88^{dc}	1.2^{dc}
10	13.98^{dc}	1.1^{d}

Values in column followed by the same letters are not statistically significant according to Duncan's multiple range test (P = 0.05)

with the aqueous solution of the silver ion complex, it was changed into reddish brown color due to excitation of surface plasmon vibrations, which indicated that the formation of Ag nanoparticles. It was well known that Silver nanoparticle exhibits greenish brown color in aqueous solution due to evitation of plasma on vibrations in silver nanoparticles.

The synthesized silver nanoparticle using 10 genotypes of tall fescue extracts were detected by UV-Vis spectrophotometer (Figure 1). The UV-Vis spectrum of colloidal solution of Silver nanoparticles has maximum absorbance peak at 400 nm, which is proved the synthesis of silver nanoparticles in the colloidal solution. Ashokkumar et al. (2015) studied the effect of varying concentration of leaf extract on the size of AgNPs. As the concentration of leaf extract is increased, more number of biomolecules is available in the leaf extract which will be helpful for the metal reductive process. However, in this study the plant extract of genotypes number 4 had the highest absorption peak so this genotype extract selected for SEM and test of antibacterial activity.

Scanning electron microscope (SEM)

Scanning electron microscope analysis was used to measure the size and shape of silver nanoparticle. In this analysis the mean size of silver nanoparticles was 90 nm and the shapes of them were spherical. Figure 2 shows the silver nano particles with 100nm magnifications.

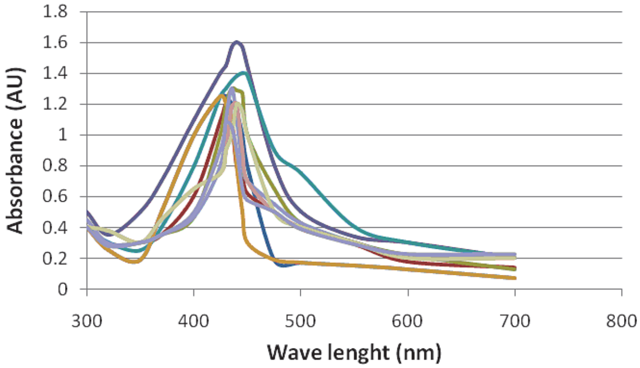

Fig. 1. UV spectophotometer of silver nanoparticles using leaf extract of 10 tall fescue different genotypes

Antibacterial activity of synthesized silver nanoparticle from Festuca auroundinacea

The antibacterial activity of the synthesized silver nanoparticles has been investigated against *Staphylococcus aureus* and *E. coli*. Green synthesis of silver nanoparticles of *tall fescue* showed very strong inhibitory actions against *S. aureus* and followed by *E. coli* (Figure 3). Priya et al. (2011) observed the antimicrobial activity of synthesized Ag nanoparticles against six different bacteria such as *E. coli, S. pyrogens, S. auereus, B. Subtilis, S. typhi and Citrobacter* sp. Benakashani et al. (2016) also reported that the nano- sized silver produced by *Capparis spinosa* showed excellent antibacterial property and high antimicrobial activity compared to the ionic silver.

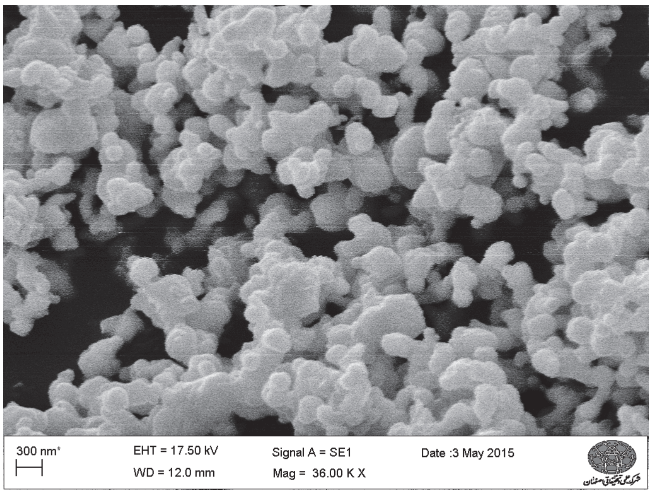

Fig. 2. SEM images of silver nanoparticles using tall fescue leaf extract at 100 nm magnificent

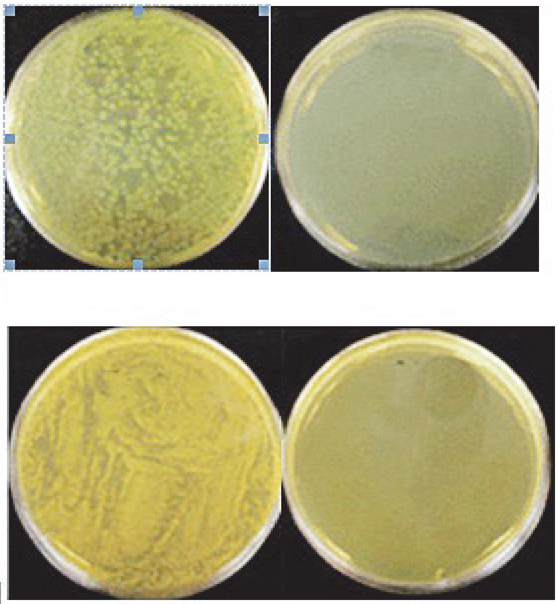

Fig. 3. Antibacterial activity of silver nanoparticles against *Escherichia coli* (top) and *Staphylococcus aureus* (down) in the left without tall fescue leaf extract and in the right with tall fescue leaf extract

Conclusion

In this study, tall fescue leaf extract used to produce Ag nanoparticles. The synthesized AgNPs were analyzed using UV spectrophotometer and SEM. Comparison of different genotypes extracts showed that the plant extract with higher carbohydrate had the higher potential of reduction. The biosynthesized silver nanoparticles were proved to have excellent antimicrobial antibacterial performance against *E. coli* and *S. aureus* using tall fescue leaves extract. Therefore, AgNPs producing *C. spinosa* may be potentially utilized for the economical production of AgNPs for many pharmaceutical applications.

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