Evaluation of plant-mediated Silver nanoparticles synthesis and its application in postharvest Physiology of cut Flowers

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Abstract Today the use of silver nanoparticles is becoming increasingly widespread due to their wide applications as antimicrobial agent. Green synthesis of silver nanoparticles (SNPs) using the petal extract of saffron (*Crocus sativus*) as a reducing agent from 5 mM AgNO₃ has been investigated in this work. Diverse petal extracts quantities and reaction times were used for the synthesis of SNPs. The resulting SNPs were characterized by means of UV–Vis, XRD and FTIR techniques. SNPs were synthesized rapidly within 30 min of incubation period and synthesized SNPs showed an absorption peak at 380–400 nm in the UV–Vis spectrum. XRD spectrum confirmed the formation of metallic silver, too. Green synthesized SNPs were used as antimicrobial agent against three bacterial genera of *Bacillus*, *Pseudomonas* and *Acinetobacter* which contaminate preservative solution of cut-flowers, too. According to the results biosynthesized SNPs using saffron petals successfully controlled these bacteria and have made them promising candidates as new generation of antimicrobials. This route is rapid, simple without any hazardous chemicals and economical to synthesized SNPs.

Keywords Saffron · Antibacterial · Vase life · FTIR · Green synthesis · SNPs

Introduction

Ion and silver-based compounds is well known as one of the most universal antimicrobial substances. Silver nanoparticles are attractive especially for antimicrobial sterilization among

the nanoparticles (Nabikhan et al. 2010). It is well known, that silver is an effective antibacterial agent and possesses a strong antibacterial activity against bacteria, viruses and fungi (Kaviya et al. 2011).

Today, the synthesis of metals nanoparticles is a more important research area in nanotechnology. Silver nanoparticles (SNPs) are receiving great attention due to their applications in different areas such as biotechnology, packaging, electronics, medicine and coatings. Chemical procedures are the most widely used methods for synthesis of SNPs. Hazardous materials such as sodium borohydride, hydrazine and polyvinyl pyrrolidone used in reduction and stabilization of nanoparticles were found to cause adverse effects in this approach. In contrast, nanoparticles synthesized using plant extracts are cost effective, ecofriendly, easily scaled up for large scale synthesis, non-toxic, and there is no need to use high pressure, energy, temperature and toxic chemicals (Dubey et al. 2010a; Kaushik et al. 2010; Solgi and Taghizadeh 2012). Recently, the potential of various plants for the synthesis of SNPs has been studied. Like pomegranate and Damask rose (Solgi and Taghizadeh 2012), rose (Dubey et al. 2010a), banana (Bankar et al. 2010), hibiscus (Philip 2010), geranium leaves (Shankar et al. 2003), cinnamomum (Sathishkumar et al. 2009), aloe (Chandran et al. 2006), basil (Ahmad et al. 2010) and tansy fruits (Dubey et al. 2010b).

Saffron (*Crocus sativus*) which is known as the world's most expensive spice is a bulbous perennial belonging to the iris family (*Iridaceae*) (Hosseinzadeh et al. 2007). It seems that saffron originally comes from Mediterranean region and Greece. There are some researchers who believe that origin of this plant comes from Iran (Moghaddasi 2010). Saffron is valued for its golden-colored, pungent stigmas, which are dried and used to flavor and color foods as well as a dye. Crocin is the major coloring pigment which is easily soluble in water and picrocrocin is the principal component giving

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saffron its special "bitter" flavor. Safranal is the main aroma factor in saffron, too (Hosseinizadeh et al. 2007).

Iran, Greece, Morocco, Kashmir (India), Spain and Italy are the main saffron producer's countries. Iran as the origin of saffron has the largest production and the amount of saffron production in Iran is 230 tons which constitutes 93.7 % of the world saffron production (Ghorbani 2008). Saffron petals are by-products of fields and are thrown out after harvesting. The amount of petals is more than 10000 tons each year. Nowadays, the only usage of saffron petals is dye extraction, which is not increased yet (Abbasi-Alikamar et al. 2007).

On the other hand, several studies have sought to attribute vascular blockage and subsequent senescence of cut-flowers to bacterial plugging of stem elements (Zagory and Reid 1986). Microbial contaminations of cut-flower's vase solution are thought to be the most common cause of stem blockage and decrease longevity. The presences of microorganisms in cut-flower's vase solution are able to cause physical plugging of the cut stem, release toxic metabolites and enzymes or produce ethylene (Rodney and Hill 1993). Thus, many antimicrobial agents such as 8-hydroxyquinoline sulphate, silver nitrate and aluminium sulphate have been used in vase solution for cut-flowers to increase vase life (longevity) by improving their water uptake. Many researchers have demonstrated the importance of silver nanoparticles as an antibacterial agent (Lu et al. 2010).

Many studies reported that three genera of *Bacillus*, *Pseudomonas* and *Acinetobacter* are the main bacteria which were identified in a few cut-flowers vase solution (Balestra et al. 2005; Van Doorn and De Witte 1994; Jowkar et al. 2012; Zagory and Reid 1986).

The aim of this experiment was to investigate the saffron petal extract-mediated biosynthesis of SNPs and their effects on some bacteria genera which are involving in decreasing flowers longevity in order to find the possibility of using the huge amount of petal residues as green biosynthesis. The SNPs have been characterized by UV-visible spectroscopy, XRD and FTIR analysis.

Material and Methods

Preparation of saffron petals extract

Saffron flowers have been obtained from Khorasan province (Iran). These materials were washed several times, air-dried, cut into fine pieces, ground into fine powder for further experiments. 2 g of above ground powder were placed in a 250 ml beaker containing 100 ml distilled water and placed on boiling steam bath for 15 min at 65–70 °C till color of the solvent changed. They were cooled at room temperature, gently pressed and filtered through Whatman No. 40 filter

paper. These solutions were treated as source extracts and were utilized in subsequent procedures.

Synthesis of silver nanoparticles (SNPs)

Silver nitrate was obtained from Sigma Aldrich chemicals. Silver nitrate solution (5 mM) was prepared and was reduced using saffron petals extract at room temperature. The effects of reaction conditions such as the saffron petals extract/silver nitrate ratios and reaction mixture contact time were also studied. The 5 mM silver nitrate solution without any addition of extract was used as control.

Characterization of SNPs

Visual observations and UV-Vis spectra analysis

SNPs gave sharp peak in the range of visible region (200–700 nm) of the electromagnetic spectrum. The synthesis and their development of SNPs at different saffron petals extract/silver nitrate ratios (1:20, 1:10, 1:5, 1:1, and 2:1 v/v) and reaction mixture contact time (zero time, 30 min and 2 h after reaction) were observed by visual observations (yellowish-brown color) and UV-Vis spectrophotometer (T80+ UV-VIS Spectrophotometer PG Instruments Ltd).

FTIR spectroscopy

FTIR spectroscopy was used to recognize the function groups that bound on the silver surface and involved in the formation of SNPs. Samples for the FTIR were prepared by drying saffron petals, resulting saffron petals extract with silver nitrate (1:20). Hand-ground samples were measured by Galaxy Series FTIR 5000 FTIR spectrometer and using spectral range of 500 – 4000 cm⁻¹ and resolution of 4 cm⁻¹.

Powder X-ray diffraction

Resulting solutions of the synthesized SNPs (1:20) and saffron petals powders were dried at 80 °C for X-ray powder diffraction measurements. XRD measurements were carried out on a Philips-X'Pert MPD X-ray diffractometer. The pattern was recorded by Cu-Kα1 radiation with λ of 1.5406 Å and nickel monochromator filtering the wave at tube voltage of 40 kV and tube current of 30 mA. The scanning was done in the region of 2θ from 20° to 80° at 0.02°/min and the time constant was 2 s.

Antibacterial activity of SNPs

The synthesized SNPs using saffron petals extract was tested for antimicrobial activity by agar disk diffusion method. The bacterial test cultures included *Pseudomonas aeruginosa*,

Pseudomonas fluorescens (gram negatives), *Bacillus subtilis*, *Bacillus cereus* and *Acinetobacter sp.* (gram positives). Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. The discs with 6 mm diameter were placed on Muller Hinton Agar. Using a micropipette, 50 μ l of SNPs prepared from saffron petals extracts (1:20, 1:5 and 2:1) were poured onto each disc on all plates. Control samples lacking the silver nitrate were used to assess the antimicrobial activity of the pure petals extract. The samples were initially incubated for 15 min at 4 °C (to allow diffusion) and later on at 37 °C for 24 hours for the bacterial cultures. Positive test results were scored when a zone of inhibition was observed around the disc after the incubation period.

Statistical analysis

SNPs characterization experiments were carried out in triplicate. For antibacterial experiment, each treatment had four replications with 4 sample in each replication. A completely randomized design (CRD) with factorial arrangements was used for experiment. Data were analyzed using the ANOVA procedure of SAS statistical software. Duncan's multiple range test (DMRT) were performed to determine the statistical significance of differences between means of treatments at the 5 % probability level.

Results and Discussions

Visual observations and UV–Vis spectra analysis

When the saffron petals extracts were mixed in the aqueous solution of the silver ion complex, they started to change the color from watery or green-colored to brown due to reduction of silver ions. It is well known that SNPs exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles, which indicated formation of silver nanoparticles [Jain et al. 2009; Solgi and Taghizadeh 2012]. Visual observation of the solutions revealed color changes from watery or green-colored to brown on addition of silver solutions with decrease of saffron petals extract quantity (from 2:1 to 1:20) in each reaction solution. Control silver nitrate solutions did not develop the brown colors (Fig 1). Also, with increase in saffron petals extract quantity from 2:1 to 1:20, consistently an increase in peak absorbance was found in UV–Vis spectrum (Fig 2). Even though, SNPs were synthesized rapidly within 30 min of incubation period, results showed that reaction mixture contact time also affected the SNPs development and with an increase in time the color became deep brown and the peak become shaper (Fig 1 and 2). Intensity of brown color increased in direct proportion to the reaction time which was found in earlier researches. It may be

Fig. 1 Visual observations of reaction mixtures containing different saffron petals extract/5 mM silver nitrate (left to right; control, 2:1, 1:1, 1:5, 1:10 and 1:20) incubated at zero time (a), 30 minutes (b) and 2 hours after contact (c)

due to the excitation of surface Plasmon resonance (SPR) effect and reduction of AgNO_3 (Dubey et al. 2010a; Krishnaraj et al. 2010; Solgi and Taghizadeh 2012). Fig 2 shows the UV–Vis spectra recorded from the reaction medium. Absorption spectra of silver nanoparticles formed in the reaction media by saffron petals extract had absorbance peak at 380–400 nm. Broadening of peaks indicated that the particles are polydispersed (Jain et al. 2009; Solgi and Taghizadeh 2012).

FTIR spectroscopy

The FTIR spectra of untreated and treated SNPs samples are indicated that untreated saffron petals sample showed absorption bands at 3342, 2922, 1764, 1051 and treated samples showed absorption bands at 3367, 2931, 1770, 1074, respectively. These absorbance bands are known to be associated with the –OH, aliphatic –CH stretching or $\text{C}\equiv\text{C}$, $\text{C}=\text{O}$ stretching frequency and C–O stretching, respectively (Dubey et al. 2010b; Bankar et al. 2010; Jain et al. 2009; Solgi and Taghizadeh 2012). Saffron petals extract contains phenolic compound such as Flavanoids (e.g. kaempferol) and anthocyanins (e.g. anthocyanidin, delphinidin and plargonidin) (Isao and Ikuyo 1999; Hadizadeh et al. 2003) The hydroxyl groups of

Fig. 2 UV-visible absorption spectra of different saffron petals extract/5 mM silver nitrate; 2:1 (a), 1:5 (b), 1:10 (c) and 1:20 (d) after different contact time (0 min, 30 min and 2 hour)

these compound have a stronger ability to bind silver ions and involve in the biosynthesis of SNPs and act as reducing agent for the reduction of silver ions (Ag^+) to silver nanoparticles (Ag^0) (Dubey et al. 2010b; Bankar et al. 2010; Jain et al. 2009; Solgi and Taghizadeh 2012).

X-ray diffraction

Biosynthesized SNPs by saffron petals extract were further confirmed by the characteristic peaks observed in the XRD

image. The XRD patterns of air-dried control saffron petals sample are shown in Fig 4 (a). The control did not show the characteristic peaks. After the reaction of saffron petals with the silver nitrate (1:20), the XRD spectra that were obtained are shown in Fig 3 (b). After the reaction, the diffraction peaks at $2\theta=38.06^\circ$, 44.19° , 69.44° and 77.35° [assigned to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes of a faced center cubic (fcc) lattice of silver] were obtained. Similar results were reported in silve nanoparticles synthesized using banana (Bankar et al. 2010) Hibiscus rosa sinensis (Philip 2010),

Fig. 3 XRD patterns of saffron petals powder (a) and SNPs synthesized by 1:20 saffron petals extract with silver nitrate (b)

Fig. 4 Antibacterial activity of different concentrations of synthesized SNPs by saffron petals extract in comparison to pure saffron petals

papaya (Jain et al. 2009) and pomegranate and Damask rose [Solgi and Taghizadeh 2012].

Antibacterial activity of SNPs

The antibacterial effects of different concentration of synthesized SNPs were significant in comparison to pure saffron petal extracts (Fig 4). Synthesized SNPs, particularly 1:20 and 1:5 concentrations, showed antibacterial activity against both gram negative and gram positive bacteria which tested. However, pure petals extract didn't display any antibacterial activity (Fig 5).

Fig. 5 Antibacterial activity of synthesized SNPs by saffron petals extract (from left to right; saffron extracts, 2:1, 1:5 and 1:20) on different gram negative and gram positive bacteria (from top row to bottom;

Bacillus cereus, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Acinetobacter* and *Bacillus subtilis*)

SNPs show more antibacterial activity compared to other silver containing compounds due to their extremely large surface area. They get attached to the cell membrane and also penetrate inside the bacteria. The membrane of bacteria includes sulfur-containing proteins and the SNPs interact with these as well as with the phosphorus containing compounds similar to DNA (Rai et al. 2009; Nabikhan et al. 2010). The mechanism of the inhibitory effects of SNPs on microorganisms is partially known. The antimicrobial effect of SNPs is due to structural changes in bacterial cell membrane, loss of DNA replication, dissipation of the proton motive force and finally cell death (Solgi et al. 2009; Kaviya et al. 2011). Antibacterial activities of plant-mediated SNPs have been reported earlier. Antibacterial activity of synthesized SNPs using banana peel extracts was observed towards *E. coli*, *E. aerogenes*, *Klebsiella sp.* and *Shigella sp.* by Bankar et al. (2010)). Kaviya et al. (2011) showed higher antibacterial activity of synthesized SNPs by *citrus sinensis* peel extracts against *E. coli* and *P. aeruginosa* (Gram negative) than *S. aureus* (Gram positive), too.

The synthesized SNPs exhibited good antibacterial activity against both gram negatives and gram positives significantly. However their effects on gram positive genera (*Bacillus* and *Acinetobacter*) were higher than gram negative ones (Fig 6).

This result is possible due to the difference in the structure of the cell wall between gram positive and gram negative bacteria. The Gram negative bacteria are usually surrounded by two membranous structures. The inner one is a trilamellar structure that bounds the bacterial protoplasm and is composed of a phospholipids bilayer. The outer membrane also presents a trilamellar structure and consists of proteins, including porins, receptors, and an asymmetric distribution of lipids. The outer leaflet is composed primarily of lipopolysaccharide (LPS) projecting outside and the inner leaflet containing phospholipids and lipoproteins. The LPS of a Gram-negative

bacterium consists of three different sectors: (i) lipid-A, (ii) the core polysaccharide comprising the inner and the outer cores, and (iii) the O-specific polysaccharide chains (Chatterjee and Chaudhuri 2012). Thus, such structure forming more rigid structure leading to difficult penetration of the SNPs compared to the gram positive bacteria where the cell wall do not possesses outer membrane (Goldfine 1984). The effects of different concentrations of produced SNPs were evaluated on vase life of rose cut flowers. Their results showed that, low applied silver nanoparticles concentrations particularly 1:20 significantly increased approximately two times of vase life in comparison to control, too (data not shown).

Conclusion

In summary, visual observations, UV–Vis, XRD and FTIR spectroscopic techniques confirmed the formation of SNPs by saffron petals extracts. This work indicates that saffron petals extract had a good valuable potential in the future for production of SNPs. This route is rapid, simple without any hazardous chemicals as reducing or stabilizing agents and economical route to synthesized SNPs. These synthesized SNPs displayed efficient antibacterial activity against most important bacteria which are involving in decreasing the cut flowers longevity. To our knowledge, this is the first study on the green synthesis of SNPs by saffron petals extract and its application against these bacteria genera.

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Fig 6 Antibacterial activity of synthesized SNPs by saffron petals extract on different gram negative and gram positive bacteria

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