Antimicrobial, antioxidant, anti-glycation and toxicity studies on silver nanoparticles synthesized using *Rosa damascena* flower extract

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**ABSTRACT**

The perspective of this research was to investigate the antioxidant, antibacterial, cytotoxic activity and anti-glycation of the SNPs synthesized by aqueous flower extract of pink *Rosa damascena* plant and compare with the biological effect of plant extract. The antioxidant activity of plant extract, and SNPs, their inhibitory effect on albumin glycosylation and also the cytotoxic effect on the human fibroblast cells were assessed by DPPH, spectrophotometric assay and MTT test, respectively. The antibacterial activity of SNPs against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Klebsiella pneumoniae* was examined by the disk diffusion method. The percentage of free radicals inhibited by the extract was increased compared to the SNPs. The flower extract and the SNPs both showed anti-glycation effects under in vitro conditions, but SNPs showed the efficient inhibitory effect on glycation of BSA in the order; 1000 extract < 500 SNP < 250 SNP < 1000 SNP. The toxicity effects of the extract and SNPs were also found to depend on their concentration and time of incubation, still, SNPs showed 8% higher toxicity than the extract. Although SNPs showed antibacterial activity, their effect on *S. aureus* was significant. It is concluded that the SNPs exhibited multifunctional properties for many medicinal applications.

**Introduction**

Nanomaterials have attracted considerable attention due to their unique properties and a wide spectrum of applications. The most applied noble metal NPs include gold (Au), silver (Ag), palladium (Pd) and, platinum (Pt) (1–3). Ag NPs have been investigated in the medicinal field for their antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, anti-angiogenesis, antiplatelet and, antitumor anticoagulant, thrombolytic, antidiabetic, anticancer activities (4–10). The properties of NPs can be easily altered according to their morphological features such as size and shape, and chemical bonding features at the surface of the particle (11). For the synthesis of Ag NPs, an array of physical and chemical methods have been utilized in the past, which requires high energy and several hazardous compounds resulting in the environmental toxicity (12,13); Several biological resources that include plants, agro wastes, microbes, metabolites of animals among others have been used for the green synthesis of NPs under ambient and eco-friendly conditions (6,14–16).

Numerous studies in the recent past have revealed the application of varieties of extracts of plants such as...
Mangifera indica (17), Plectranthus amboinicus (18), Mangifera indica (17), Ziziphus clinopodioides (19), Morinda citrifolia (20), Saraca asoca (21), Verbena officinalis (22), Chrysanthemum morifolium (23), Tamarindus indica (10), Aloe vera (24), Chenopodium murale (25), Piper longum (26), Eupatorium odoratum (27), Nepeta deflersiana (28), Eupatorium odoratum (27), Salvia officinalis (29), Allium cepa (30), Emblica officinalis (31), Coffea Arabica (32), Jatropha gossypifolia (33), Ferulago macrocarpa (34), Mentha pulegium (35) medicinal plants Vernonia amygdalina Del. (36), Syzygium guineense (Willld.) DC (37), Hagenia abyssinica (Brace) JF. Gmel. (38), towards the successful biosynthesis of nano silver-particles. Researchers used different parts of plants such as stem, flower, leaf, fruit, bark, seed, shoot, peel and, root for the biosynthesis of Ag NPs (28). The Rosa damascena mill L., plant locally known as Gole Mohammadi in Iran, is an ornamental plant that belongs to Rosaceae family (39). This plant find application as anti-HIV (40), antibacterial (41, 42), antioxidant (43), antitussive (44), hypnotic (45) agent besides its use in perfume industry. The methanolic extract of this plant was found to exhibit an inhibitory effect on α-glucosidase and decrease blood glucose in normal and, diabetic mice (46). Although in numerous studies the sound effects of natural antioxidants from the herbal plant in the diabetic models have been proved (47), but to the best of our knowledge, the inhibitory activities of this plant extract and SNPs in the formation of advanced glycation end products (AGEs) in a diabetic model under experimental conditions has not yet been reported. Therefore, we aimed to synthesize SNPs using fresh aqueous extract of flowers of R. damascena plant and evaluate their antibacterial efficacy against some selected Gram-positive and negative bacterial strains, in vitro inhibitory effect on AGE production, and their toxicity on the human fibroblastoma cell line.

Materials and methods
Sample collection and preparation of extract
The fresh pink flowers of R. damascena were collected from the local region at Neyriz, Fars, Iran. The authentication of plant has been conducted at Lorestan University Herbarium (Voucher No. LUKH12111399). The flowers were thoroughly rinsed with tap water, followed by distilled water to remove dirt and impurities. Then the flowers were dried in the shade at room temperature for a week and were powdered with a grinder. The powdered flower (5 g) was mixed into 100 mL de-ionized water and, heated for 10 min in water bath at 100 °C. The mixture was cooled and centrifuged at 10000 rpm for 10 min and filtered to get the extract. The filtrate was stored at 4°C for further use.

Biosynthesis of SNPs
For the synthesis of SNPs, 5 mL of the flower extract was mixed with 5 mL of AgNO₃ solution in various concentrations (1, 2, 3, 4 and 5 mM), at different pH (7, 9 and 12) and at different temperature (40, 60 and 80°C). The reaction mixture was kept in a brown colored bottle to prevent auto-oxidation of silver. The formation of SNPs in the solution was evaluated by visual observation of the solutions turning brown. The reduction of positive Ag⁺ ions into Ag and formation of SNPs was assessed periodically. The precipitated particles were isolated by centrifuging at 10,000 rpm for 20 min and, were washed with deionized water. After repeated centrifugation at 10000 rpm for 10 min, pellets were dried at 60°C for eight hours and stored in proper containers. The purified SNPs were then subjected to characterization.

Characterization of SNPs
The as-synthesized SNPs were characterized by ultraviolet–visible (UV–Vis) JENWAY 6405 Spectrophotometer for the surface plasmon resonance (SPR) peak in a wavelength range from 300 to 700 nm. In addition, Fourier transform infrared spectroscopy (FTIR Shimadzu, Japan 8400S) and scanning electron microscopy (SEM, Tescan Mira 3 LMU), determine the possible biomolecules responsible for the reduction of silver ions to SNPs, morphological and surface features of SNPs, respectively. Morphological analysis of the sample was conducted using TEM instrument JEOL, JEM-2100 (accelerating voltage up to 200 kV, LaB6 filament) EDS-1.5 Å TEM resolution. The FTIR spectra were collected on Shimadzu, Japan 8400S spectrophotometer using the potassium bromide (KBr) disk technique over the wavelength regions between of 400 and 4000 cm⁻¹ with 4 cm⁻¹ resolutions. The external morphology and the size of SNPs were characterized by using Scanning electron microscope (Tescan Mira 3 LMU) and elemental composition was determined using energy-dispersive X-ray (EDX) spectroscopy maintained at 15 kV with 1 nm resolution. The X-ray diffractometer (Philips XPert Pro MPD X-ray) in a 2-theta range of 20–80° with Cu Kα radiation was used to analyze the crystallite structure of the obtained SNPs.

Antioxidant assay
Antioxidant activities of the plant flower extract and the synthesized SNPs were estimate by DPPH (1, 1-diphenyl-
2-picrylhydrazyl) radical scavenging assay (RSA) described by Blois method. DPPH exhibits purple color to solution. In order to affirm the stability of the solution composed of DPPH radical, the solution was stored uninterrupted for 3 h. There was no change in the λ max of the solution at 517 nm approving the stability of the solution all through the experiment. The molecule of DPPH is comprised of π system in addition to the unpaired electron residing over the nitrogen atom. The molar absorptivity increases upon the substitution of aromatic ring, however this effect is pronounced if the substituent escalates the conjugation length. Further this prolonged conjugation generally motivates the shift in the absorption band of benzene from shorter to longer wavelength. The absorption maxima at 517 nm is liable for n → π * energy transitions. Vitamin C was used as a standard compound for measuring the antioxidant activity (48) and calculated using Equation (1);

\[ \text{RSA(\%)} = \left( \frac{A_C - A_S}{A_C} \right) \times 100 \]  

\( A_C \) = Absorbance of the control (only DPPH solution) and, \( A_S \) = Absorbance of the samples when mixed in DPPH solution.

The IC_{50} is the extract concentration required to neutralize 50% of the initial DPPH radicals.

**Cell culture and cytotoxicity test**

The fibroblast cells were purchased from the Royan Institute (cell banks, Iran) and cultured in 96 welled plates at a concentration of 2 × 10^4 cells per well. After 24 h, the cells were exposed to different concentrations of extract and SNPs suspension (0, 25, 50 and 100 mg/mL) and kept in a humidified atmosphere with 5% CO2 at 37 °C for 24 h. At the end of the treatment period, the cell viability was measured by methyl thiazolyl diphenyl tetrazolium bromide (MTT) assay (50). The percentage of cell viability and growth inhibition were calculated using Equations (2) and (3), respectively;

\[ \text{%Cell Viability} = \frac{\text{Treated cell OD}}{\text{Control OD}} \times 100 \]  
\[ \text{%Inhibition of growth} = 100 - \text{Viability} \]

**Measurement (in vitro) of total advanced glycated end products (AGEs) in the presence of extract and SNP**

To investigate the effect of flower extract and SNPs on the non-enzymatic glycosylation process, AGE albumin was prepared by incubating 50 mg/mL of BSA with 30 mg/mL of glucose and various concentrations of extract and SNPs (250, 500, 1000 μg/ml) in 0.2 mM phosphate buffer pH 7.4. Gentamicin was used to prevent the development of bacterial infection in the medium. The incubation was done for 21 days at room temperature and kept away from light. At the end of each week of incubation, the total AGE level was estimated by measuring the fluorescence intensity of the samples through excitation and maximum emission wavelengths at 370 and 440 nm, respectively (51). To measure the fluorescence intensity, Varian spectrofluorometer, model Cary Eclipse was used.

**Statistical analysis**

The experiments were conducted in triplicate, and the value were expressed as means ± SD. The statistical analyses were performed using one-way ANOVA by SPPS version 15.0 and Excel 2016. Also, IC_{50} was calculated in the software ed50v10. The significance level considered is less than 0.05.

**Result and discussion**

**Biosynthesis of SNPs**

In this study, SNPs were synthesized using pink flowers of *R. damascene* and their antioxidant and, antimicrobial,
cytotoxicity capacity and inhibitory effect on the formation of AGE compounds were investigated. In this paper, the aqueous extract of R. damascena flowers were used for the efficient synthesis of SNPs. This plant contains various pharmacological compounds, possibly responsible for the reduction of silver ions to silver (45) and capping of SNPs to stabilize against agglomeration (52,53). A large number of functional molecules such as flavonoids, phenol, terpenoids, ketones, carboxylic acids, aldehydes, enzymes, amides, are present in plants and derived plant products, which serve as the source for bio reduction of metal ions to metal NPs (53).

To investigate the optimum environmental and physicochemical conditions for SNP synthesis, the R. damascena flower extract and silver nitrate solutions were mixed at different ratios. The color of the solution was observed to change slowly from faint yellow to colloidal brown, indicating the formation of SNPs, (Figure 1) which is believed to be due to the excitation of surface plasmon vibrations in the inoculated NPs. Surface plasmon resonance (SPR) is a phenomenon where the electrons in the metal surface layer are excited by photons of incident light with a certain angle of incidence, and then propagate parallel to the metal surface. It was concluded from the past studies, that the SPR wavelength depends on the morphological features of the nanoparticles. To examine the formation of NPs, a small amount of the mixture was diluted with deionized water (1:1) and monitored by measuring absorbance at different time intervals. The nitrate solution and plant extract did not show any specific absorption peak (Figure 2(a)) but, the same mixture maintained at pH = 4 and incubated at room temperature, exhibited the absorbance peaks in the wavelength range of 390–410 nm (Figure 2(b)). As shown in Figure 2(c), mixtures of different ratios of concentration of AgNO₃ and the extract showed that with an increase in the concentration of nitrate, the absorption peak shifted towards higher wavelength, indicating an increase in the size of synthesized Ag NPs. Thus, the concentration of 5 mM would be ideal for the further optimization of pH factor. For this purpose, the extract and 5 mM silver nitrate solution were adjusted to different pH (7,9,12); with increasing the reaction pH, the absorbance band exhibited a peak at the area of 400 nm (Figure 2(d)). It seems that electrical charges of biomolecules were changed by reaction pH, and possible to influence their capping and stabilizing capacities and subsequently the synthesis and growth rate of NPs (54). To obtain the best temperature for the synthesis of SNPs, the extract solution was added to nitrate at optimum pH and concentration and placed at three different temperatures 40, 60 and, 80 °C. As the reaction temperature rises, the absorption peak intensity was found to increase (Figure 2(e)). Increasing the reaction temperature is believed to the enhance the kinetic energy of the molecules and faster consumption of silver ions, and leaving less possibility for particle size growth (55). These results indicated that the color of the solution turned brown faster in higher pH and temperature. Hence, both alkaline pH and high temperature are more favorable for the synthesis of SNPs using the R. damascena aqueous flower extract. The UV absorption spectrometric analysis showed that the SNPs formed rapidly within 30 min and remained stable even after 24 h, but the maximum absorption occurred at 90 min (Figure 2(f)). It is concluded that R. damascena flowers extract acted efficiently as reducing and stabilizing agents.

The FTIR spectroscopy was used to determine the compounds according to the behavior and vibration of their functional groups. These compounds are expected to act as a reducing agent for Ag⁺ ions or as capping agent for SNPs. Even covalent bonds of metal–organic groups can be identified. Figure 3 showed the FTIR spectra of pure plant extract, and SNPs. The peak in the range of 500–1200 cm⁻¹ arises from the O–H group stretching of the phenol and alcohol in the extract that revives silver ions and are believed to have involved in the synthesis of NPs.

The intense peaks shown in Figure 3 (also Figure 12), respectively at 3375 and 1250 cm⁻¹ corresponds to phenolic –OH stretching and bending vibrational frequencies (56). The small peak at 2921 cm⁻¹ was correlated to the alkane C–H stretching mode (57). The peaks at 1645 and 1616 cm⁻¹ arises due to C=O vibration of ketonic groups (58). The C–O–C vibration displays at 1068 cm⁻¹. The presence of major IR peaks of plant extract in the IR spectrum of SNPs clearly indicate the

![Figure 1](https://via.placeholder.com/250)

**Figure 1.** Visual Observation of R. damascena Extract synthesized SNPs. Silver Nitrate, flower extract and SNPs (from left to right, respectively).
effective role played by the plant extract in reducing and stabilizing SNPs. The scanning electron microscopy is considered to be one of the best analytical methods to analyze the particle morphology and surface area of the internal structure in micron and nanometer dimension. Figure 4 shows SEM images of SNPs synthesized using R. damascena extract at low and high resolution. The particles were found to be nearly spherical, which corroborates the results obtained by UV–vis spectral studies. The crystallite size was found to be in the range of 18.92–40.55 nm. It should be considered that the properties and biological activity of these SNPs are related to their size. Nadagouda et al. (59) reported that the size of Ag and Au NPs synthesized using the antioxidants from blackberry, blueberry, pomegranate, and turmeric extracts depends on the nature of extract and method of preparation.

Similar to our study, the SNPs synthesized using Rosa damascena Hips Extract exhibited spherical shape with different sizes ranging from 11 to 30 nm (60). Azarbani and Shiravand (34) also showed that the NPs had a spherical shape with diameter ranging between 14 and 25 nm.

EDX analysis of SNPs showed an intense optical absorption peak at 3 keV energy indicating the revival of silver nitrate and formation of silver particles (61). The minor elements present are carbon, oxygen and, nitrogen that may originate from the biomolecules that are bound to the surface of the SNPs. In addition, small peaks of metals potassium, sodium, magnesium

**Figure 2.** Ultraviolet–visible (UV–vis) spectra of SNPs related to (a) nitrate and extract individually. (b) after mixing nitrate solution and extract with pH 4. (C) five concentrations of silver nitrate (1–5 mM). (d) Three reaction pH (7, 9 and, 12). (e) Three reaction temperatures (30, 60 and, 80 °C). (f) Seven times at intervals of 0, 30, 60, 90, 120, 150 and 180 min.
were present due to trace amount of impurities by the synthesis of samples (Figure 5).

The XRD analysis was performed to confirm the crystalline structure and the estimated crystallite size of the synthesized SNPs. As shown in Figure 6, the XRD spectrum of SNPs exhibited four sharp and distinct peaks at 2θ values of 38.11°, 44.30°, 64.44° and 77.40°, which are due to 111, 200, 220 and 311 crystallographic planes of silver (62,54). The XRD spectrum confirms the crystal structure of SNPs (55). The average crystalline size of SNPs was calculated from the XRD data using the Debye–Scherrer’s formula (Equation),

$$d = \frac{k \cdot \lambda}{\beta \cdot \cos \theta}$$  \( (4) \)

Where \( d \) is the particle size of the crystal, \( k \) is Scherrer’s constant (0.9), \( \lambda \) is the wavelength of X-ray source used in XRD (0.15406 nm), \( \beta \) is the breadth of the observed diffraction peak at its half maximum and \( \theta \) is the Bragg angle. From Scherrer’s formula, the average crystalline size of synthesized SNPs was deduced to be 12 nm, which is in compliance with the particle size measured from using the SEM micrographs. The impurity peaks were not observed in XRD patterns.

The TEM images of SNPs are depicted in Figure 7. The almost spherically structures with various the size ranging from 8.6 to 49.7 nm with a median particle size of 31.8 nm are as shown in Figure 7(a,b). The six spots appeared on the SAED pattern, were correlated to specific crystal planes of SNPs as shown in Figure 7. The most prominent 4 spots were represented in colored concentric circles which represents (111), (200), (220) and (311) planes. The interplanar spacing (IPS) value of 0.242 nm for Ag (111) plane (36).

### Antioxidant activity

The flower extract and SNPs showed strong antioxidant DPPH radical scavenging activity in a dose dependent manner with an IC50 value of 278.9 ± 0.1, 237.9 ± 0.1 and, 207.1 ± 0.1 µg/mL for ascorbic acid, extract and SNPs respectively. Many researchers have investigated the antioxidant activities of SNPs and reported that NPs exhibited superior antioxidant activity compared to plant extract or silver nitrate (25,26,55). Biogenically synthesized SNPs using the extract of R. damascena petals were reported to exhibit less scavenging activity in comparison with the flower extract (63). The result of the antioxidant assay for 250, 500 and 100 µg/mL of R. damascena extract obtained 65.2, 83.6 and, 89.1 percent respectively (Figure 8), while for these concentration of SNPs less antioxidant activity of 61.2, 72.5 and 73.1 percent as observed. It seems that during the reduction of silver, the antioxidant molecules in the extract were oxidized and, could not quench electrons to the free radicals (63).

### Antimicrobial activity

The SNPs showed antimicrobial activity against all the tested bacterial strains. The maximum non-growth halo diameter was observed in the case of S. aureus and increased with the concentration of SNPs. The observed antibacterial activity followed against B. cereus, then for K. pneumoniae and E. coli.

The results of the broth micro dilution method correspond to the disk diffusion test show that the antibacterial activity of the SNPs was higher against the tested Gram-positive bacteria than the Gram-negative ones. The synthesized SNPs had both MIC and MBC values of 62.5 µg/mL toward B. cereus bacterial strain (Table 1).
silver nanoparticles synthesized by hips extracts of Rosa damascene showed the highest antibacterial activity against Enterococcus faecium (+) than Pseudomonas aeruginosa (−) and Escherichia coli (−). Bacillus cereus (+), and Salmonella aureus (+) (60). SNPs, as a safe and promising antibacterial agent have been used alone or in combination with antibiotics. However, similar to antibiotics, the development of bacterial resistance towards SNPs occurred (64–66). Recently Ipe reported that when SNPs combines with some antibiotics, they not only increase the antibacterial activities of antibiotics but also, inhibit the growth of bacterial species that were
Figure 6. XRD of nanoparticle.

Figure 7. TEM micrographs of Ag NPs at (a) weak magnification (100 nm) and (b) strong magnification (50 nm) (c) SAED pattern (4 spots) and (d) HRTEM micrographs of lattice fringes of Ag NPs with IPS value of 0.242 nm.
resistant to either the antibiotics or SNPs alone (67). The antibacterial activity of SNPs depends on some physiochemical features, including size, form, dose, colloidal state and charge distribution (68). The specific control over size, shape, and, charge surface of SNPs can be achieved by optimizing some parameters such as the nature of plant extract, pH, and reaction time (69). Although the precise mechanism underlying antibacterial activity of SNPs is not fully recognized (70), the adhesion of these particles onto the surface of bacteria, penetration inside the cell and destruction of bacterial biomolecules and intracellular structures, production of ROS and free radical that result in cellular toxicity and oxidative stress and, modulation of bacterial signal transduction pathway are the most problematic mechanism for their action (71,72). Many studies and researchers examined and reported the antibacterial activity of SNPs against over 650 bacteria, fungi, and viruses. They proved the effectiveness of SNPs against some drug resistant pathogenic bacteria such as E. coli, Bacillus subtilis, E. faecalis, K. pneumoniae, P. aeruginosa, and S. aureus (73). A few reports also showed that these NPs have more potent antibacterial activity against Gram-positive bacteria than negative, but some researchers reported conflicting results (74–76).

The effect of extract and SNPs on the inhibition of the glycation reaction of albumin

The total amount of AGE compounds formed during the experiment was evaluated by the fluorescence measurement for the incubation period of 3 weeks. According to Figure 9, the fluorescence intensities of flower extract and SNPs were found to be different during the period of 21 days. The inhibitory effect was initiated after 72 h and continued until the third week. The amount of AGE compounds formed from the extract with a concentration of 250 μg/mL and SNP with concentration of 1000 μg/mL were found to be the lowest. Hence, these two concentrations exhibited the highest inhibitory effect. At first, the intensity peak of these two concentrations did not differ significantly, but over time, the peak corresponding to SNP showed lower concentration than the extract. Each value represents the mean ± SD (n = 3). Statistically significantly different from Control+ (P < 0.05). The inhibitory effect of the extract is not dose-dependent and follows the order; 1000 < 500 < 250 μg/mL. However, in the presence of SNPs, while the concentration increased, the fluorescence intensity and formation of AGE compounds were found to decline and the inhibitory effect was observed to follow the order; as 250 < 500 < 1000 μg/mL. In general, order of inhibitory effect of different concentration of extract and NPs is as follows; 1000 extract < 500 Extract < 500 SNP < 250 SNP < 250 extract < 1000SNP (Figure 10). It seems that hyperglycemia in diabetes results in the production of AGEs through nonenzymatic glycosylation in plasma proteins such as albumin, fibrinogen and, globulins. It leads to some various deleterious effects, including loss of natural function of the protein, generation of free radicals, alteration in drug binding in the plasma, platelet activation, impaired fibrinolysis and impairment in immune system regulation. The

Table 1. Non-growth hole diameter of SNP against all tested bacteria.

| B. sature | S. aureus | K. pneumonia | E. coli | SNP (μg/ml) |
|-----------|-----------|--------------|---------|-------------|
| 250       | 10        | 9            | 10      | 12          |
| 500       | 12        | 11           | 15      | 15          |
| 1000      | 15        | 16           | 19      | 18          |
| Amikacin  | 19        | 20           | 19      | 20          |
| MIC       | 500       | 500          | 125     | 62.5        |
| MBC       | 500       | 250          | 125     | 125         |
fluorescence spectroscopy is a type of electromagnetic spectroscopy that investigates the fluorescence properties of the samples studied. The technique uses light beam of specific intensity to excite the electron. As a result, electrons are transported to higher energy levels by measuring the intensity of the fluorescence light to study the concentration, properties, or interactions of the molecules. The diabetic model was developed by glycation of bovine serum albumen under laboratory conditions. the extent of AGE generation was detected via fluorescence absorption at excitation and emission wavelengths of 335 and 385 nm, respectively. Therefore, in this study, we examined the inhibitory effect of SNPs and flower extract against the formation of AGE compounds in a diabetic model under experimental conditions. In the present study, both plant extract and, SNPs reduced the effects of blood glucose and prevented the formation of AGE compounds. However, the anti-diabetic activity of the synthesized NPs was higher than that of the extract mostly. This activity lasts up to three weeks, which can say to be a long-lasting drug effect. The inhibitory effect of plant extract and SNPs can be attributed to the presence of various antioxidant compounds. The FTIR spectra were applied to identify distinct functional and organic groups in control containing BSA (Bovine Serum Albumin)-GLU (Glucose). Then two concentrations including 250 µg/mL of extract and 1000 µg/mL of SNPs with the highest inhibitory effect on albumin glycation among all tested concentrations.

Figure 9. The Effect of control, plant extract and SNP (250, 500 and 1000 µg/mL) on fluorescent AGES formation in the BSA/glucose system. Each value represents the mean ± SD (n = 3). * Statistically significantly different from Control+ (P < 0.05).

Figure 10. Percentage of inhibition of different concentrations of plant extract and SNP (250, 500 and 1000 µg/mL) on glycosylation in laboratory conditions.

Figure 11. Effects of aqueous extract of plant extract (A) and SNP (B) on the viability of fibroblastoma cells. Cells were incubated with increasing concentrations of extract and SNP (0, 25, 50, 100, and 200 mg/ml) in culture medium for 24, 48, and 72 h. Data presented are the mean ± SEM of three independent experiments. P < 0.05.
were added to control and subjected to FTIR analysis. A wide and strong band in the range of 3300–3400 cm$^{-1}$ for OH stretching vibration, a weak peak at 2500 cm$^{-1}$ of C–H bending vibrations, peaked at 1609 cm$^{-1}$ of C=O asymmetric vibrations and, a peak at 1405 cm$^{-1}$ of –COOH stretching vibrations are displayed in Figure 11. In the spectra at 500 cm$^{-1}$, furthermore, a strong extensive absorption at 900–1200 cm$^{-1}$ for coupled C–O–C glycoside band vibration and C–O–H bending vibrations of side groups indicated the characteristic absorption of polysaccharides (77). The diagnostic absorption peaks at 991 and 675 cm$^{-1}$ may suggest the presence of β-d-pyranoid glucose and α-isomers of pyranose (78). The peaks in the 1600–1700 cm$^{-1}$ region are due to C=O stretch. The band located between the region 1500–1600 cm$^{-1}$ corresponds to C–N stretch coupled with N–H bending mode, representing the amount of carbonyl and amino bonds in side chains of amino acid residues of the BSA-GLU (78). The native BSA showed spectra at 1603 and 1548/72 cm$^{-1}$, respectively. The shift in peak positions in FTIR spectra demonstrated a change in the secondary structure of BSA after modification with GLU. However, the effects of GLU on BSA secondary structure were found to gradually decrease with increasing concentration of SNPs (79). FTIR analysis of the GLU-BSA mixture with and without SNPs and extract corroborate that the SNPs and the flower extract played a significant role in maintaining

![Figure 12. FTIR of Control (A), 250 μg/ml concentration of extract (B) and 1000 μg/ml concentration of SNP (C).](image-url)
the secondary structure of BSA protein. Amino acids containing free amino groups (Lys, Arg) are potential sites for glycation in addition to the N-terminal amino acid. It can be concluded that SNPs competitively binds to these free amino groups. These observations demonstrate the decrease in the glycation upon masking of the free amino groups or sequestration of reacting group of glycation agents by SNPs (those residing on the lysine residues) (80,81). However, the exact mechanism behind the inhibitory effect of SNPs and extract is not yet fully known. In summary, the results of our study indicate that both the SNPs and the flower extract can reduce the rate of non-enzymastic modification of BSA by GLU. However, the anti-AGE production activity of the synthesized SNPs was found to be higher than that of the extract. Mostly, this activity lasts up to three weeks, which can be meant to be a long-lasting drug effect.

**MTT assay**

The cytotoxic properties of the SNPs and flower extract were investigated using the various concentrations (25, 50 and 100 mg/mL) and time durations (0, 24, 48 and 72 h). For concentration of 25 mg/mL, cytotoxicity was not observed for the flower extract and SNPs, even cells were proliferated significantly, specially, after 24 h of treatment. For 50 mg/mL, a little of toxicity was observed specially, after 72 h, still, for 100 mg/mL the longer duration and maintenance time, a higher inhibitory effect was observed, suggesting that both the SNPs and the extract reduce the viability of cell lines in a dose and time-dependent manner (Figure 12). The fact the lower inhibition concentration (IC50) for SNPs (Table 2) than the extract revealed that the SNPs toxicity is more than that of the extract. Over time, IC50 for SNPs was reduced, meaning that with increasing treatment time, a lower concentration of the SNPs was required to inhibit 50% of cells.

**Conclusion**

In conclusion, this investigation provides an economical, rapid, reproducible and environment-friendly procedure, for the synthesis of SNPs (Ag NPs) by the application of flower extract of *R. damascena* plant. The phyto-synthesized SNPs were characterized thoroughly by UV-Vis spectroscopy, SEM, FTIR and, XRD techniques. The average size of synthesized SNPs was found to be 12 nm, which could be affected by the extract concentration, reaction temperature, and, pH. The FTIR results found several phyto-constituents possibly involved in the silver ions reduction, leading to the formation of SNPs. Especially, hydroxyl groups in phenolic compounds. The flower extract showed less enhanced antioxidant properties compared to the SNPs. The SNPs showed antibacterial activity against both tested Gram-positive and Gram-negative bacterial strains which cause hospital-acquired infections. Both the flower extract and the SNPs have reduced the effects of blood glucose and preventing the formation of AGE compounds. The reason for their inhibitory effect on AGE formation can be attributed to the presence of various antioxidant compounds. In addition, they also keep the secondary structure of BSA in a dose-independent manner. However, they did not exhibit significant toxicity on human normal fibroblastoma cells at concentrations several times more than the ones used in anti-glycation and antibacterial assay. Even the increase of cell proliferation during treatment with some concentration of the extract and the SNPs was observed. But at higher concentration and incubation time, the cell viability was observed to decrease specially for SNPs. Thus, the significance of this study demonstrate a wide range of multifunctional applications of green synthesized SNP.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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**Research involving humans and animals statement**

Not applicable.

**Informed consent**

Not applicable.

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| Table 2. Inhibition concentration (IC50) for SNP and plant extract at different times. |
|-----------------|-------|-------|-------|
| Time (h)       | SNP   | Extract |       |
| 72             | 111.98| 137.8  |
| 48             | 158.51| 212.7  |
| 24             | 168.47| 177.41 |
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