



Research article

Synthesis and characterization of *Rosa canina*-mediated biogenic silver nanoparticles for anti-oxidant, antibacterial, antifungal, and DNA cleavage activities

Fulya Gulbagca ^a, Sadin Ozdemir ^b, Mehmet Gulcan ^{c, **}, Fatih Sen ^{a,*}^a *Sen Research Group, Biochemistry Department, Dumlupinar University, Evliya Celebi Campus, 43100, Kütahya, Turkey*^b *Food Processing Programme, Technical Science Vocational School, Mersin University, TR-33343 Yenisehir, Mersin, Turkey*^c *Chemistry Department, Van Yuzuncu Yıl University, Zeve Campus, 65080, Van, Turkey*

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ABSTRACT

In biomedical applications, silver nanoparticles (Ag NPs) are of great interest due to their cost-effective and environmentally friendly properties. Green synthesis of nanoparticles for biological research is a preferred choice since it does not require additional reducing agent. For this purpose, in this study, we aimed to synthesize the biogenic silver nanoparticles with the help of *Rosa canina* plant (Rc-Ag NPs) and then they have been tried for their antioxidant and antibacterial properties. UV-Vis spectrophotometer, transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS) and X-ray diffraction (XRD) analyses were performed for characterization of Rc-Ag NPs. Antioxidant properties of silver nanoparticles synthesized with *Rosa canina* plant were investigated against 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH). DNA dissociation activity of synthesized Rc-Ag NPs was studied, and DNA dissociation activity was shown. The antimicrobial activity of Rc-Ag NPs was also tested using micro-dilution. According to the results, Rc-Ag NPs synthesized were found to be highly effective for anti-oxidant, antibacterial, antifungal, and DNA cleavage activities.

1. Introduction

Nanoparticles (NPs) are very popular in the scientific world due to their properties [1, 2, 3, 4, 5]. The advantageous features of NPs can be listed as follows: NPs gain different properties and functions due to their volumetric ratio. The main reason for this situation is the fact that the rules of quantum physics are affected instead of normal physics rules. The surface/volume ratios of NPs are extremely high. This has become the main reason for choosing NPs in both in vitro and in vivo studies. NPs have extremely small dimensions. Therefore, they can easily pass through the capillaries and pores [6, 7, 8, 9, 10, 11]. One of the methods used to synthesize nanoparticles with all these properties is the green synthesis method. This method is a process that aims to obtain more complex structures from materials that are simple in structure. This process is used to express the chemical events in the metabolic processes of living things. However, this phenomenon is expressed as the process of obtaining NPs using living organisms or chemical materials in nanotechnology. Living organisms such as algae, bacteria, plants, fungi are

involved in this process [12, 13, 14]. This method, which has been used more frequently in recent years, is generally preferred due to its simple application, economy, and structure suitable for large quantities of commercial production. It is also applicable in biomedical processes and does not have to work with a toxic substance. Silver nanoparticles, which have industrial and biomedical applications, are highly preferred in terms of low cost and reliability. Plants used in the green synthesis are the most suitable reducing and stabilizing options for use in biological applications without the use of additional reducing chemicals. For the selection of materials in the green synthesis process, the materials must be safe, contain a single reaction step, not cause a waste release, be environmentally friendly, renewable and have a high yield capacity [14, 15, 16]. In addition, there should be a material that can be easily separated from the environment at the end of the reaction. Although it is difficult to combine all these properties, the selection of a material containing the majority is crucial to increase the success of the synthesis [16]. The extracts of the *Rosa canina* species of the Rosaceae family are potential phytochemicals for the reduction reaction due to the OH groups they

* Corresponding author.

** Corresponding author.

E-mail addresses: mehmetgulcan65@gmail.com (M. Gulcan), fatihsen1980@gmail.com (F. Sen).

contain [17, 18]. Saware et al. (2014) reported that silver nanoparticles obtained from *Ficus benghalensis* (F.B.) leaf extract are environmentally friendly, fast, economical and renewable and that proteins with amino groups play an important role in the stability of silver nanoparticles in solution. They have emphasized that silver nanoparticles help stabilize, probably due to phytochemicals such as protein [19].

The strong antimicrobial effects in plant-mediated products are very important because the resistance of many pathogens to antibiotics is one of the major problems of medical science. Antibiotic resistance is considered to be one of the most important concerns in public health, due to the overuse or misuse of antibiotics. Increasing antibiotic resistance, increasing the cost of health care due to long-term treatment, including admission and recovery, results in the introduction of new antibiotic agents and the implementation of effective and widespread infection control methods to prevent the spread of antibiotic-resistant pathogens. In all stages of the infection process, free radicals are produced by stimulating the various defense mechanisms of the organism. However, these free radicals cause various damage to the organism. In recent years, studies on neutralizing the harmful effects of free radicals by using antioxidants are of great importance [20].

In this study using the green synthesis method, we aimed to prove the antioxidant (DPPH) and antibacterial properties of silver nanoparticles synthesized by *Rosa canina* plant. UV-Vis spectrophotometer, transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS) and X-ray diffraction (XRD) analyses were performed for characterization. The ability of DNA cleavage of silver nanoparticles mediated by *Rosa canina* has been demonstrated for plasmid DNA (pBR322) using agarose gel electrophoresis.

2. Materials and methods

2.1. Synthesis of Rc-Ag NPs

Rosa canina was purchased from Turkey. The fruit parts of the obtained plant were dried and pulverized. *Rosa canina* plant extract was assisted by microwave method in a 5 min [21]. 1 mM 50 ml silver nitrate solution was prepared, and added to *Rosa canina* plant extract to a 10 ml extract section at 85 °C on a magnetic stirrer. After color change, washing with alcohol was carried out by centrifugation at 7000 rpm. The supernatant was discarded to remove unreacted particles, and the pellet consisting only of the reacted particles was dried and stored [22, 23].

2.2. Characterization of synthesized Rc-Ag NPs

Rc-Ag NPs were characterized by UV, TEM, XRD and XPS analyses. JEOL 200 kV was used for TEM. The prepared Rc-Ag NPs solution was placed on a carbon-coated copper grid, and images were obtained. The crystal structure of Rc-Ag NPs was obtained by using XRD at high resolution (Panalytical Empyrean diffractometer, 40 mA, 40 kV, $k = 1.54056$ Turkey). The oxidation state of the metals was examined by X-ray photoelectron spectroscopy (XPS, Specs Spectrometer) with $K\alpha$, 1253.6 eV, 10 mA lines. XPS analysis was performed concerning the carbon line at 284.6 eV as a reference point. Characteristic absorption bands for Rc-Ag NPs were investigated using UV-Vis absorption photometers. The spectral result showed an absorption band around 400–450 nm for Rc-Ag NPs [24].

2.2.1. Antioxidant activity (DPPH)

The DPPH radical scavenging activities of Rc-Ag NPs were performed according to the Agirtas et al. (2015) method [25]. For this test, 2 mL of 0.004% DPPH solution was taken into each tube and added to 0.5 mL of Rc-Ag NPs at different concentrations (10, 25, 50, 100, 200 and 500 mg/L). The tubes were shaken rapidly and then incubated at room temperature for 30 min in the dark. DPPH solution was used for control. DPPH was measured at 517 nm with trolox and ascorbic acid used as

standard. The percentage of free radical scavenging was calculated using the following equation (Eq. (1)).

$$\% \text{ Inhibition} = (A_C - A_1) / A_C \times 100; (A_C: \text{Control Absorbance}, A_1: \text{Sample Absorbance}) \quad (1)$$

2.2.2. DNA cleavage ability

DNA cleavage ability of Rc-Ag NPs was investigated using agarose gel electrophoresis [26, 27]. The plasmid DNA (pBR322) was used as target DNA molecules. 7 μ L Rc-Ag NPs at 100 mg/L and 200 mg/L with 3 μ L plasmid DNA were reacted at 37 °C for 60 min and 90 min. After incubation, the reaction solution was treated with loading dye (0.25% bromphenol blue, 50% glycerol). The mixture was then run on electrophoresis gel at 50 V using a 0.8% agarose gel in a buffer in TAE buffer (50 mM Tris base, 50 mM acetic acid, 2 mM EDTA, pH: 7.8). The gel was monitored and photographed under UV light after electrophoresis studies.

2.2.3. Antimicrobial activity

The MIC is described as the lowest concentration of a substance that inhibits a microbial growth [28]. The antimicrobial activity of Rc-Ag NPs was performed by using microdilution methods in 96 wells of the microplate. The Rc-Ag NPs were prepared at a concentration of 2048 μ g/mL. Following dilutions (1024, 512, 258, 64, 32, 16, 8, 4, 2 and 1 μ g/mL) of the Rc-Ag NPs were prepared in the microplate. *Bacillus cereus*, *Enterococcus hirae* (ATCC 10541), *Escherichia coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 6538), *Legionella pneumophila* subsp. *pneumophila* (ATCC 33152), *Pseudomonas aeruginosa* (ATCC 9027), and *Candida albicans* were used as test microorganisms. The microorganisms' cultures were inoculated with 2.4×10^7 CFU/mL. After inoculation, microplates were kept in an incubator for 24 h at 37 °C for bacteria and 48 h at 25 °C for fungi. The MIC was determined as the lowest concentration of Rc-Ag NPs that inhibited of microbial growth.

3. Results and discussion

3.1. Characterization of the synthesized Rc-Ag NPs

According to the TEM analysis results, it is clear that the synthesized nanoparticles are spherical, isotropic in structure (Figure 1). TEM images showed that the particle size of Rc-Ag NPs was 13–21 nm (with the help of the elimination of outliers) consistent with the literature [29, 30].

XRD pattern shows the peaks at 20 values of 37.57°, 46.54°, 63.66°, 78.63°, 82.79° corresponding to Ag (111), (200), (220), (311), and (222) lines [21, 31, 32]. Findings from the XRD analysis show that the silver particles have a surface-centered cubic structure and a crystallite size of approximately 19.75 nm, and the results are similar when compared to TEM. The oxidation state of Ag metals present on the obtained nanoparticles was investigated using X-ray photoelectron spectroscopy; Specs Spectrometer, and both XRD, and XPS analysis result is given in Figure 2. The peaks of Ag (0) $3d_{3/2}$ – $3d_{5/2}$ are divided into doublets corresponding to 366.0–372.0 and 368.0–374.0 eV. The peaks observed in the energy values at 366.0–372.0, and 368.0–374.0 eV shows the existence of Ag (0) mostly and Ag (I) which is in a very small amount. This indicates that most of the silver was reduced to metallic silver with the help of plant extract as an efficient way. The results are consistent with the literature [33, 34].

UV-Vis spectrophotometer was also used to confirm the formation of Rc-Ag NPs in the aqueous colloidal dispersion. Samples showed a sharp SPR band at 422 nm, which is characteristic peak of NPs as shown in Figure 3.

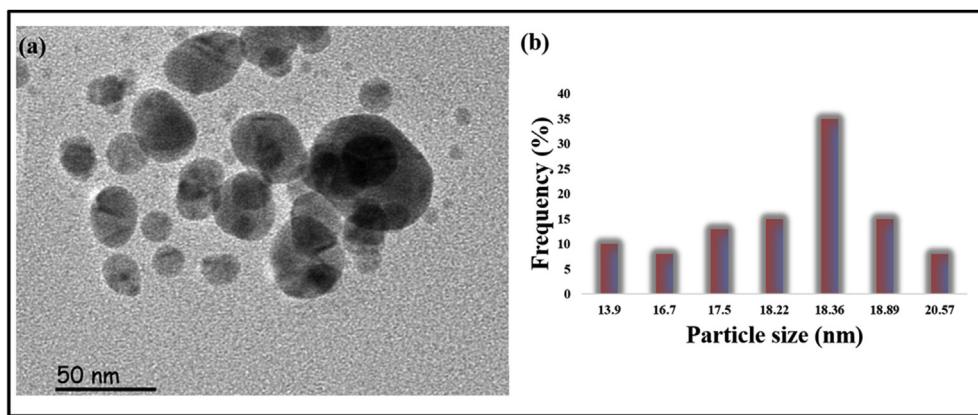


Figure 1. (a) TEM patterns and (b) particle size histogram of Rc-Ag NPs.

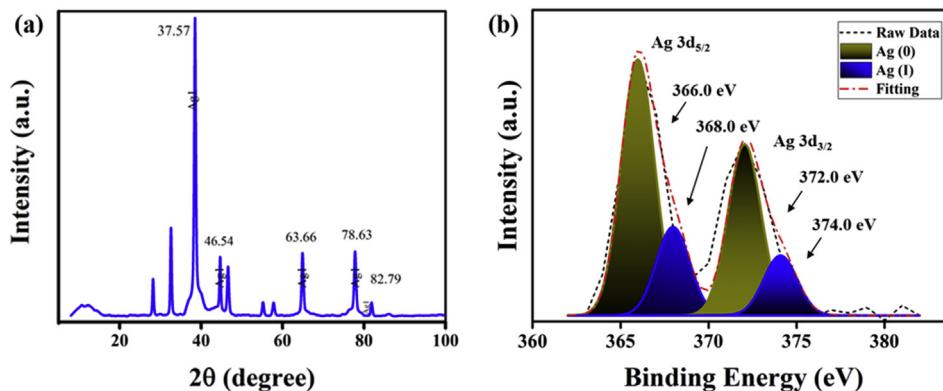


Figure 2. (a) XRD and (b) XPS graphs of Rc-Ag NPs.

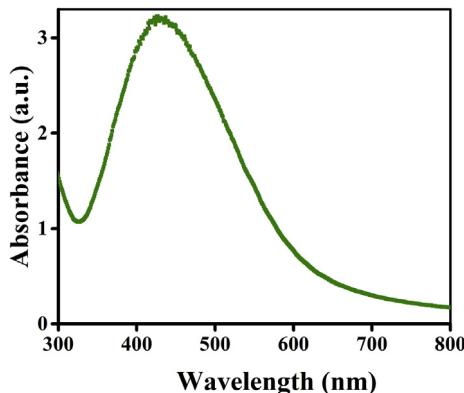


Figure 3. UV spectrum of Rc-Ag NPs.

3.1.1. DPPH radical scavenging ability

After fully characterization of Rc-Ag NPs, they have been tried for their antioxidant and antibacterial applications. For this purpose, free radicals are the first therapeutic targets because they are markers of inflammatory diseases. Potential products are antioxidants in inhibiting free radicals. The development of antioxidant compounds is the potential route to effective drugs. Antioxidants, which can inhibit free radicals, have anti-cancer, anti-aging, and antifungal properties in addition to their anti-inflammatory properties. Antioxidant compounds can also be used to prevent rheumatoid arthritis and inflammation. The antioxidant activities are also considered to be through oxygen-mediated hydrogenation [35, 36, 37]. For these reasons, DPPH scavenging abilities of Rc-Ag NPs were examined. Trolox and ascorbic acid were used as

standards. The DPPH scavenging ability of plant-mediated green synthesized of Rc-Ag NPs is represented in Figure 4. Both Rc-Ag NPs and standart antioxidants established an important scavenging ability against the DPPH radical and thereby expressed a source for antioxidants. The DPPH scavenging ability of Rc-Ag NPs tended to rise with rising its concentration. The DPPH scavenging abilities were 8.3%, 18.4%, 32.6%, 58.7% and 79.5% at 10 mg/L, 25 mg/L, 50 mg/L, 100 mg/L and 200 mg/L, respectively. The strongest antioxidant ability was recorded as 86.4% at 500 mg/L. In the previous study, green synthesis of silver nanoparticles using *Lippia nodiflora* was found the antioxidant activity of 67% at 500 µg/mL [38], which is lower with our findings. The DPPH scavenging ability of Rc-Ag NPs indicated previously also showed higher activities with their findings [39, 40].

3.1.2. DNA cleavage activity

The cleavage activity was performed by gel electrophoresis. The cleavage performance of the green synthesized of Rc-Ag NPs compared to that of the control is because of its efficient DNA cleavage ability [41, 42]. The electrophoresis clearly revealed that Rc-Ag NPs acted on plasmid DNA molecules. Results are shown in Figure 5. As seen in Figure 5, there are differences in the bands of Lanes 2–5 when compared with control DNA. Plasmid pBR322 was changed from Form I into Form II in Lanes 2–4. In addition to these, the results showed that plant-mediated green synthesized of Rc-Ag NPs acted as chemical nucleases by cleaving the DNA Form I into Form III, at the concentration of 200 mg/L for 90 min. As the plant-mediated green synthesized of Rc-Ag NPs was monitored to cleave the DNA, it can be indicated that new green Rc-Ag NPs inhibits the growth of the cancer cell and pathogenic organism by cleaving the genome. Further, in-vivo and in-vitro studies are necessary for new plant-mediated green synthesized of Rc-Ag NPs toxic effects.

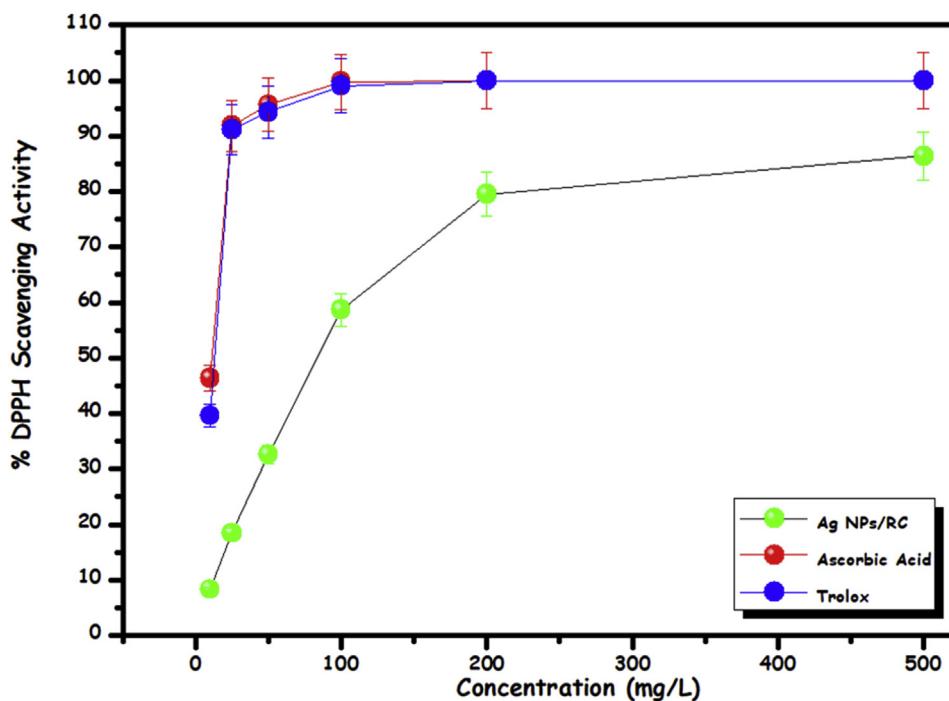


Figure 4. DPPH scavenging activity of Rc-Ag NPs.

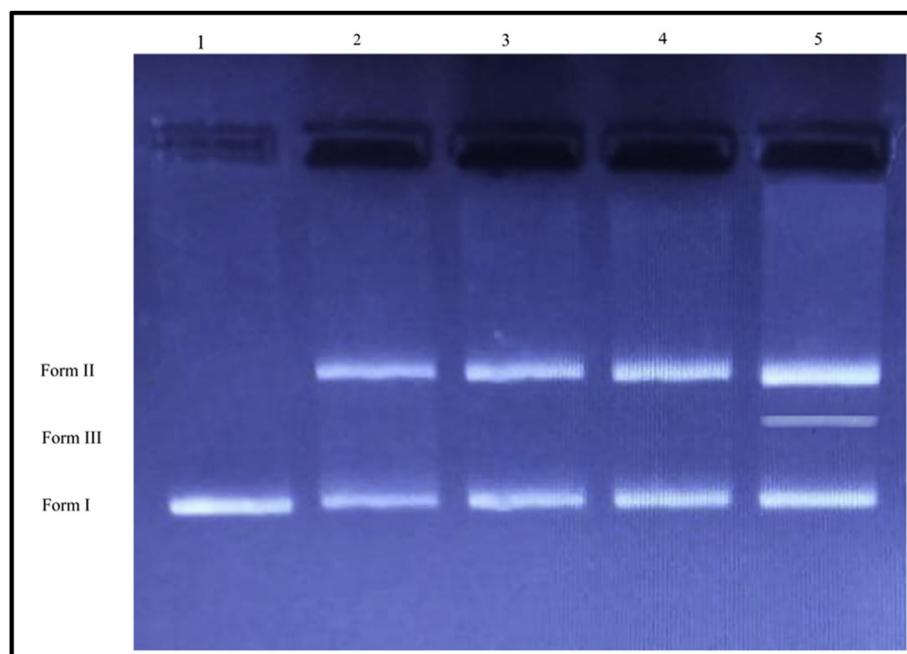


Figure 5. DNA cleavage activity of Rc-Ag NPs. Lane 1, pBR 322 DNA; Lane 2, pBR 322 DNA +100 mg/L of Rc-Ag NPs (60 min incubation); Lane 3, pBR 322 DNA +200 mg/L of Rc-Ag NPs (90 min incubation); Lane 4, pBR 322 DNA +100 mg/L of Rc-Ag NPs (60 min incubation); Lane 5, pBR 322 DNA +200 mg/L of Rc-Ag NPs (90 min incubation).

3.1.3. Antimicrobial activity

Along with the developing pharmaceutical industry, the antibiotic resistance mechanism in microorganisms has increased linearly and become the agenda in the medical industry. As a result of drug researches, antimicrobial activities of Ag NPs have been proved nanotechnologically [13, 14, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52]. In the present investigation, the Rc-Ag NPs were tested for their antimicrobial activity against test microorganisms. Results show that green synthesized

of Rc-Ag NPs had antimicrobial activity. The MIC results are shown in Table 1. The MIC values were found as 32 µg/mL, 256 µg/mL, 256 µg/mL, 256 µg/mL, 16 µg/mL, 128 µg/mL, and 128 µg/mL for *B. cereus*, *E. hirae*, *S. aureus*, *E. coli*, *L. pneumophila*, *Candida albicans*, and *P. aeruginosa*, respectively. According to these results, silver nanoparticles showed the most effective antimicrobial activity against *L. pneumophila*. The results are consistent with similar studies in the literature [53].

Table 1. MIC values of *Rosa canina* plant-mediated green synthesized of silver nanoparticles.

Microorganisms	MIC values (µg/L)
<i>E. coli</i>	256
<i>E. hirae</i>	256
<i>S. aureus</i>	256
<i>P. aeruginosa</i>	128
<i>B. cereus</i>	32
<i>L. pneumophila</i> subsp. <i>pneumophila</i>	16
<i>C. albicans</i>	128

4. Conclusions

As a conclusion, in recent years, many researchers have been looking for a technology that cannot develop resistance to new and effective antimicrobial agents in order to develop single or multiple antibiotic resistances of microorganisms and to provide sustainable health conditions economically. Silver; as a broad spectrum antimicrobial agent with antibacterial, antifungal and antiviral properties, it has also been used safely in many fields for centuries. The mechanism by which silver kills microorganisms remains unclear. The morphological and structural changes of metallic silver, silver ions and silver nanoparticles in bacterial cells are investigated and the mechanism is tried to be understood more clearly. In our study, the green synthesis method was used and silver nanoparticles were obtained in this direction. Antioxidant, antibacterial and DNA cleavage activity experiments were performed and the results were highly positive. The results show that DPPH sweep, DNA cleavage and antimicrobial activities of Rc-Ag NPs are very effective. For instance, The MIC values were found as 32 µg/mL, 256 µg/mL, 256 µg/mL, 256 µg/mL, 16 µg/mL, 128 µg/mL, and 128 µg/mL for *B. cereus*, *E. hirae*, *S. aureus*, *E. coli*, *L. pneumophila*, *Candida albicans*, and *P. aeruginosa*, respectively. Besides, The DPPH scavenging ability of Rc-Ag NPs tended to rise with raising its concentration. The DPPH scavenging abilities were 8.3%, 18.4%, 32.6%, 58.7% and 79.5% at 10 mg/L, 25 mg/L, 50 mg/L, 100 mg/L and 200 mg/L, respectively. In addition to these, the results showed that plant-mediated green synthesized of Rc-Ag NPs acted as chemical nucleases by cleaving the DNA Form I into Form III, at the concentration of 200 mg/L for 90 min. In light of these results, such research may be useful in the synthesis and preparation of nano-drugs and targeting drug delivery in the near future. This study has shown an innovative way to synthesize antimicrobial Ag NPs using natural products that can be used in a variety of biomedical applications. In addition, the environmentally friendly method would be a competitive alternative to existing methods for producing nanoscale inorganic materials.

Declarations

Author contribution statement

Fulya Gulbagca: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sadin Ozdemir: Performed the experiments.

Mehmet Gulcan: Analyzed and interpreted the data.

Fatih Sen: Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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