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Research Article

Assessment of Antioxidant and Antimicrobial Activities of Silver Nanoparticles Biosynthesized by *Haplophyllum Obtusifolium*

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Abstract

Background: Plants comprise great antioxidant sources as a result of their redox and biochemical components, which are rich in secondary metabolites such as phenolic acids, flavonoids, and other constituents. *Haplophyllum obtusifolium* from polygonaceae is widely used for preventing and managing diabetes. This study investigated the antibacterial and antioxidant activities of silver nanoparticles (AgNPs) biosynthesized by *H. obtusifolium*.

Methods: The aerial parts of *H. obtusifolium* were gathered from the north of Khorasan Razavi province, Iran and desiccated at the chamber temperature. The shoots were powdered by grinding, 5 g of the powder was mixed with 250 mL of deionized water, and the resultant blend was then filtered. Bactericidal properties and antioxidant activity of the nanoparticles were assessed using disk diffusion and DPPH (2, 2-diphenyl-1-picrylhydrazyl) tests, respectively.

Results: The results of this study showed that the biosynthesized nanoparticles exhibited antibacterial activity against a gram-negative (*Klebsiella pneumoniae*) bacterium, but they had no effects on gram-positive *Staphylococcus epidermidis*. Antioxidant test results showed that these nanoparticles were capable of eliminating DPPH radicals in a concentration-dependent manner so that a more potent antioxidant activity was seen in higher concentrations of the nanoparticles.

Conclusion: Our results suggested that *H. obtusifolium* can be used as a key source of antioxidants/antimicrobial agents in food and pharmaceutical industries.

Keyword: Haplophyllum, Antimicrobial, Antioxidant, Silver nanoparticle.

Background

Recent studies have shown the substantial roles of nano-based materials in the development of nano-sciences and related technologies (1-6). In contrast to either small or bulk materials, nano-scale constructions show several physicochemical properties (7-9). Nanoparticles have specific intrinsic reactivity because of their excellent surface areas, providing suitable choices for manufacturing nanoparticle-based therapeutics (10,11). The surface functionalities of nanoparticles largely depend on their dimension, formation, and mass. The interaction between nano-materials and bio-systems occurs in different ways depending on cell type, employed uptake route, and target organelles (12).

Reactive oxygen species (ROS) consisting of superoxide and hydroxyl radicals can destroy living cells in bio-systems by causing oxidative stress. Antioxidant particles usually scavenge free radicals, and thus retain the balance between oxidative and antioxidative mechanisms (13). However, exposition to pollution or toxins can trigger the excessive production of ROS and upset the equilibrium between oxidant/antioxidant schemes, resulting in advanced infections (14). Antioxidant particles are found in great amounts in herbal medicines (15-17). Natural herbal antioxidants typically consist of anthocyanins, phenolic compounds, flavonoids, and carbohydrates (18-20) and present an extensive range of biomedical properties such as anti-inflammatory, anti-microbial, and anti-cancer effects (21-23).

Secondary metabolites are used in various research fields because of their numerous biomedical properties (24-26). *Haplophyllum* species have diverse secondary metabolites, and their biological functions have been less considered so far. The current study aimed to investigate the antimicrobial and antioxidant features of the silver nanoparticles (AgNPs) synthesized using *H. obtusifolium* extract, as well as the biological features of the plant extract itself.

Materials and Methods

Extraction and Synthesis of Nanoparticles

In this study, 5 g of leaf powder was used to obtain aqueous extract. Next, 10 mL of the aqueous extract was mixed with silver nitrate and stirred for 24 hours (27). A change of color into dark brown verified the synthesis of nanoparticles and the conversion of Ag^{+} to Ag^{0} (28).

Antibacterial Activity of the Plant Extract and Biosynthesized AgNPs

Bacterial samples were obtained from the Ghaem hospital of Mashhad, Iran. Antibacterial tests were performed on *Klebsiella pneumoniae* and *Staphylococcus epidermidis* by the agar diffusion manner (5). Initially, cultures were spread on agar plates to cultivate bacteria. Sterilized discs with 5 mm thickness were drenched with the extract, the biosynthesized nanoparticles, and deionized water, and kept at 37°C. Streptomycin and gentamicin were used as positive controls in this experiment (29). Antibacterial activity was clarified based on the inhibition zone nearby the disc.

Antioxidant Activity

The antioxidant activity of the AgNPs was assessed by DPPH (2, 2-diphenyl-1-picrylhydrazyl) test (30). In brief, DPPH solution (23 mg ml^{-1}) was prepared, and its absorbance was measured at 517 nm. BHA (butylhydroxyanisole) was used as a positive control. The experiment for all samples was performed in triplicate.

Results and Discussion

Antibacterial Activity

The shape of the synthesized nanoparticles was spherical, and their mean diameter was 13 nm (Figure 1) (28). Polydispersity index values for nanoparticles were 0.28. Zeta potential analysis indicated that the biosynthesized nanoparticles had a net charge of -20.67 ± 5.62 mV. The zeta-potential of NPs greater than +30 mV or less than -30 mV indicated the stability of nanoparticles (31).

The antibacterial properties of the AgNPs were studied by the agar diffusion method. The inhibition zones observed around the disks containing AgNPs indicated that the antibacterial properties of AgNPs were prominent (Figure 2). While AgNPs displayed anti-bactericidal activity against gram-negative *K. pneumoniae*, this was not observed against gram-positive *S. epidermidis*. Hamidi et al used *Tribulus* extract to synthesize AgNPs, and showed that the biosynthesized nanoparticles could kill bacterial cells via interacting with their membranes and releasing silver ions into the cytoplasm, a phenomenon which was attributed to the small size of approximately 25 nm of the nanoparticles (32). In another study by Taghavizadeh Yazdi et al, AgNPs were fabricated with an approximate size of 11 nm using *Helichrysum* extract, which were effective against various species of pathogenic bacteria (5). The capability of AgNPs in preventing bacterial cell growth can be in part related to their small size and large surface area, providing adequate contact interface with bacteria (27,32-34). It has been stated that bacteria death could be due to the great leakage of their macromolecules (35). The inhibition zones observed in this study have been shown in Table 1.

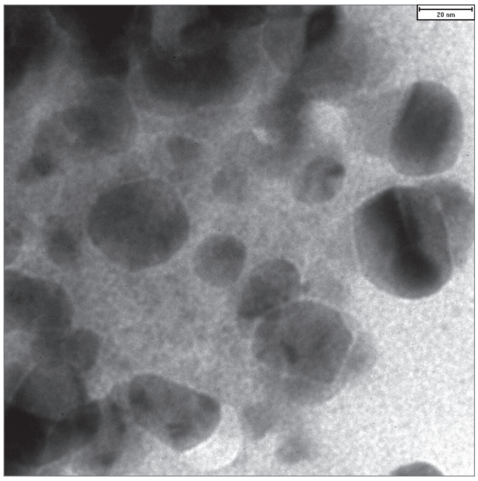

Figure 1. TEM Image of Biosynthesized Silver Nanoparticles (Mean Diameter: 13 nm).

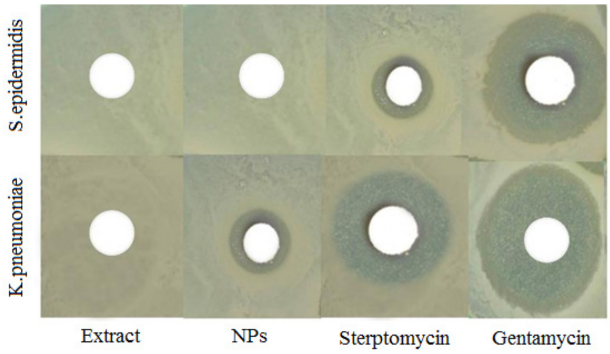

Figure 2. Antibacterial activity of the AgNPs Biosynthesized Using the Aqueous Extract of *Haplophyllum obtusifolium* Against Pathogenic Bacteria. Plant extract had no effect on pathogenic bacteria but biosynthesized nanoparticles had a specific inhibitory effect on *K. pneumoniae*.

Antioxidant Activity

Because of their usages in medicine, nutrition, and as

Table 1. Inhibition Zones of *Haplophyllum obtusifolium* Extract and Biosynthesized AgNPs Against Pathogenic Bacteria

Bacteria	Inhibition Zone (mm)			
	Extract	Nanoparticles	Streptomycin	Gentamycin
S. epidermidis	0	0	4.1	11.3
K. pneumoniae	0	3.9	9.7	13.2

cosmetics, it is essential to determine the antioxidant activity of natural molecules. Plants are predisposed to diverse types of stresses, such as salinity, extreme temperatures, and radiation during their life cycle (36,37). Depending on the extent of environmental stresses, they can limit plant growth and development and result in the creation of ROS, such as OH•, O2•, and H_2O_2. These radical species extensively damage cells by promoting the peroxidation of lipids, proteins, and DNA (38,39). Antioxidant particles and molecules are responsible for scavenging ROS within cells. DPPH radical dot analysis is normally used for calculating free radical scavenging potential and considered as one of routine and easy ways to assess the antioxidant activity of various compounds (40). In one study, the NPs synthesized by *Coriander oleoresin* extract exhibited significant dose-dependent antioxidant activity, as determined by the DPPH method (41). In another study, the AgNPs produced using *Chlorella* sp. and *Nannochloropsis oculata* extracts revealed significant antioxidant properties (42). Figure 3 compares the DPPH radical scavenging activity of AgNPs synthesized by green method via *H. obtusifolium* extract and that of BHA as a positive control. As indicated, these nanoparticles were able to eliminate DPPH radicals in a concentration-dependent manner, so that with growing in the concentration of the nanoparticles, their antioxidant activity became more potent.

Conclusion

We investigated the antibacterial and antioxidant properties of the AgNPs biosynthesized using the aqueous extract of *H. obtusifolium.* The prepared AgNPs displayed potent antibacterial activity against a pathogenic bacterium (i.e., *K. pneumoniae*) with an inhibition zone of 3.9 mm. As bacterial contamination can happen upon the proliferation of these organisms in the body and environment, causing many forms of infections and subsequently serious health threats and even death, AgNPs can be used as appropriate materials to manage infectious diseases and maintain humans' health. Considering our results, the AgNPs biosynthesized in this study can be employed for removing microbial, and particularly bacterial, contamination.

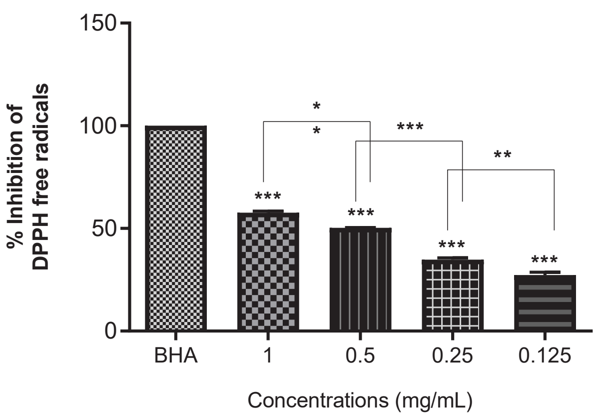

Figure 3. The Antioxidant Activity of Biosynthesized Nanoparticles Using DPPH Test. As the concentration of nanoparticles increases, the antioxidant properties of nanoparticles also increase. BHA used as a positive control. Data are expressed as mean ± SEM. *** $P<0.001$ indicated significant difference as compared to the control group.

Authors' Contributions

MRR performed the experiments. Both PY and MG authors contributed to the final version of the manuscript. AE supervised the project.

Conflict of Interest Disclosures

None declared.

Ethical Issues

Not applicable.

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