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Green Synthesis of Silver Nanoparticles Using Saffron (*Crocus sativus*) Purple flower and Their Antimicrobial activity

Mehmet Fırat Baran[1(ID)], Hatice Becerekli[1(ID)], Ülkü Karaman[2(ID)]

[1]Department of TBMYO Food Technology, Batman University, Batman, Turkey.
[2]Department of Parasitology, Faculty of Medicine, Ordu University, Ordu, Turkey.

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Abstract

Objective: In this research, CS-AgNPs were created, described, and their antibacterial activity assessed utilizing an inexpensive, simple, and ecologically friendly extraction approach from the waste saffron flower's purple flower parts.

Methods: : In this study, silver nanoparticles (AgNPs) were synthesized quickly, cheaply, and environmentally friendly utilizing purple flower extract from saffron (*Crocus sativus* (CS)). For the explanation of the structure of silver nanoparticles synthesized with saffron flower extract (CS-AgNPs); UV-visible (UV-vis.) Spectrophotometer, Fourier Scanning Electron Microscope (FE-SEM), Scanning Electron Microscope (SEM), Electron Distributed X-rays (EDX), Fourier Transform Infrared Spectroscopy (FT-IR), X-Ray Characterized using Diffraction Diffractrometer (XRD), Transmission Electron Microscope (TEM), Zeta potential data. It was discovered that the UV-visible spectrum of the biologically produced silver nanoparticles generated at a maximal wavelength of 405.68 nm in the spectrum collected after 15 minutes. Furthermore, it was noted that the synthesized nanoparticles' UV spectrum, taken a month later, revealed the same wavelength and that the resulting nanoparticles were stable. CS-AgNPs' antimicrobial effects against gram-positive, gram-negative, and fungal pathogens were assessed employing the minimum inhibition concentration approach.

Results: The generated CS-AgNPs were found to be active against both fungi and bacteria as a consequence.

Conclusion: It turned out that even at very low concentrations, the antimicrobial activity of silver nanoparticles enhanced with decreasing size and had high antibacterial and anticandidal implementation.

Key Words: *Crocus sativus* , CS-AgNPs, MIC, Antibacterial effect and Antifungal effect.

Safran (*Crocus sativus*) Mor Çiçekleri Kullanılarak Gümüş Nanopartiküllerin Yeşil Sentezi ve Antimikrobiyal Aktiviteleri

Özet

Amaç: Bu araştırmada, safran çiçeğinin mor çiçekleri parçalarından ucuz, basit ve çevre dostu bir ekstraksiyon yaklaşımı kullanılarak CS-AgNP'ler oluşturulmuş, tanımlanmış ve antibakteriyel aktiviteleri değerlendirilmiştir.

Metod: Bu çalışmada saffrondan (*Crocus sativus* (CS)) elde edilen mor çiçek özü kullanılarak gümüş nanoparçacıklar (AgNP'ler) hızlı, ucuz ve çevre dostu olarak sentezlendi. Safran çiçeği özü (CS-AgNPs) ile sentezlenen gümüş nanopartiküllerin yapısının açıklanması için; UV-görünür (UV-vis.) Spektrofotometre, Fourier Taramalı Elektron Mikroskobu (FE-SEM), Taramalı Elektron Mikroskobu (SEM), Elektron Dağıtılmış X-ışınları (EDX), Fourier Dönüşümlü Kızılötesi Spektroskopi (FT-IR), X-Ray Kırınım Kırınım Ölçer (XRD), Geçirgen Elektron Mikroskobu (TEM), Zeta potansiyel verileri kullanılarak karakterize edilmiştir. Biyolojik olarak üretilen gümüş nanopartiküllerin UV-görünür spektrumunun 15 dakika sonra toplanan spektrumda maksimum 405.68 nm dalga boyunda üretildiği keşfedildi. Ayrıca sentezlenen nanoparçacıkların bir ay sonra alınan UV spektrumunun aynı dalga boyunu ortaya çıkardığı ve ortaya çıkan nanoparçacıkların kararlı olduğu kaydedildi. CS-AgNP'lerin gram-pozitif, gram-negatif ve mantar patojenlerine karşı antimikrobiyal etkileri, minimum inhibisyon konsantrasyonu yaklaşımı kullanılarak değerlendirildi.

Bulgular: Çok düşük konsantrasyonlarda bile gümüş nanoparçacıkların antimikrobiyal aktivitesinin küçülen boyutla arttığı ve yüksek antibakteriyel ve antikandidal uygulamaya sahip olduğu ortaya çıktı.

Anahtar Kelimeler: *Crocus sativus*, CS-AgNP'ler, MİK, Antibakteriyel etki ve Antifungal etki.

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Address for correspondence/reprints:

Ülkü Karaman

Telephone number: +90 553 618 52 45

E-mail: ulkukaraman44@hotmail.com

INTRODUCTION

Silver nanoparticles show large surface areas and good conductivity (1). Biosensor is one of the most frequently studied subjects by scientists in recent years due to its chemical stability, drug transport, catalytic properties, electrical conductivity. The creation of multi-purpose nanostructures for application in many industrial industries is an important area for researchers today due to their unique properties. Food safety and food protection, Engineering, biomedicine, waste and environment (environmental remediation) and bio-energy, Drug design, drug active ingredients and alternative structures to antibiotics are considered as the main areas for nanomaterial applications. Additionally, when making NPs, environmentally friendly methods were preferred over chemical and physical methods, particularly when used to reduce environmental waste and stop the growth of pathogenic microbes. (2). Saffron flowers-Silver nanoparticles (AgNPs) are employed in a wide range of industries, including medicine, bioremediation research, catalysis, the food and cosmetics sector, agriculture, electronics, and medication antibiotics. Antibiotics have become indispensable in the world of medicine since the day they were discovered. It can be used in almost every disease. However, humans' unconscious use of antibiotic-resistant microorganisms increases antibiotic resistance. Biological sources such as bacteria, fungi and plants are used in the synthesis of AgNPs by the biological method, which is a very common method in the synthesis process (3). The use of plant sources, the ability of plants to generate more nanoparticles than other species, the stability of the particles produced, and the simpler, more cost-effective, ecologically friendly, and easier ways to apply them are a few of these factors that contribute to the popularity of this sector (4). Due to this, "green synthesis" techniques that are quick, inexpensive, and do not harm the environment are preferred over currently used conventional methods for producing nanoparticles. Plant leaves (6), berries, roots, tubers, seeds, flowers (7), plant leaves (6), The plant's aerial components are most frequently used in the manufacture of AgNPs. The biological process, which is simple, non-toxic, and less expensive, is the ideal way for creating nanoparticles. The green method is the best method of synthesizing nanoparticles, the biological method, as it is easy, non-toxic and cheaper. It can also be used for the synthesis of large quantities of nanoparticles and dimensional (nm). Another benefit is that biotechnology is easier to use, more environmentally benign, and more repeatable than waste materials. Additionally, the nanoparticles created using this technique are typically more stable (6-8).

Traditional medicine uses the saffron flower plant, which was used in this study, to treat liver conditions, constipation, typhoid fever, among other ailments. Our goal was to create bioactive Ag-NPs (antibacterial and antifungal) using bioactive components from plant sources, such as terpenes, alkaloids, flavonoids, phenols, and terpenoids, which convert Ag+ ions to Ago form in aqueous structure and create CS-AgNPs (8).

The saffron plant has a pungent taste and iodoform or straw-like odor. The reason for this is the chemicals picrocrocin and saffronal found in the composition. It also contains a carotenoid dye called crocin, which adds a golden yellow color to the environment when used for food consumption. This unique property makes saffron (*Crocus sativus*) a highly sought after product around the world. It is also known to be used in the medical field.

The Mediterranean region is where saffron, or *Crocus sativus* (CS), is typically grown. It is an herbaceous plant that is growing in the Black Sea region of Turkey. Since it is a bulbous culture plant, it is now widely grown throughout the world.

In this study, CS-AgNPs were created, described, and their antibacterial activity assessed utilizing an inexpensive, simple, and ecologically friendly extraction approach from the waste saffron flower's purple flower parts.

METHODS

In the study, purple saffron (*Crocus sativus*) flowers (CS) from the Safranbolu area of Karabük Province were employed. Vancomycin, fluconazole, colistin, and AgNO3 (99.8% pure) were purchased from Sigma Aldrich. The anti-microbials properties of CS-AgNPs were tested on grampozitive and gram negative bacterials.

Extraction of purple flowers of saffron (*Crocus sativus* (CS))

The CS purple flowers were dried after being thoroughly cleaned with distilled water. 100 cc of distilled water and three grams of dried purple blossoms were combined and cooked in a beaker. Following this procedure, it underwent filtration. The resultant filtrate was used to create silver nanoparticles (CS-AgNPs) and kept chilled at +4 °C.

Synthesis of Crocus sativus Supported-Silver nanoparticles (CS-AgNPs)

The solid silver nitrate salt was converted into a thirty mM AgNO3 aqueous solution for the environmentally friendly synthesis of Cs-AgNPs. In a glass container, the previously produced CS Extract and AgNO3 were added in a 2:3 ratio to the reaction medium at 50 oC. By using wavelength scanning using UV-vis spectroscopy, it was discovered that the reaction was complete depending on the synthesis of CS-AgNPs and color change (9,10). The dark solution was centrifuged for 15 minutes at 8500 rpm after the reaction. After multiple washings, the solid fraction that had been recovered after centrifugation was dried for 24 hours at 90°C in an oven. The produced nanoparticles were then kept sealed at room temperature for the evaluation of their antibacterial activity.

Characterization of Saffron Flower Supported-Silver nanoparticles

By using a spectroscopic approach, the UV-vis spectra of CS-AgNPs produced with biological support were identified in the wavelength range of 250-700 nm. CS-AgNPs were examined using the following techniques: FTIR, SEM, FE-SEM, TEM, XRD, EDX and Zetasizer. The analyses of size, morphology, crystal structure, surface distribution, and zeta potential were highlighted. The Debye-Scherrer equation (11, 12)) was applied to calculate the crystal size of CS-AgNPs.

Evaluation of antimicrobial activities of saffron flower supported-silver nanoparticles (CS-AgNPs)

The MICs of Cs-AgNPs towards gram-negative bacteria (*P. aeruginosa* ATCC27833, *E. coli* ATCC25922), gram-positive bacteria (*S. aureus* ATCC29213, *B. subtilis* ATCC 11774), and a gram- bacteria (*C. albicans*) were calculated using a microtiter plate containing 96 wells. Murphy Hinton The wells were filled with RPMI, which (Growth Medium Used in Cell Growth) for yeast and broth for bacteria. The microplates containing the medium and microorganisms received an addition of AgNPs solution. Each time, 100 L was drawn and transferred to the following well. The microplates were then filled with a certain volume of microbe solutions that had been created and adjusted in accordance with 0.5 McFarland. At 37°C, it had been incubated for one full day. The MIC value was established as the lowest concentration at which growth did not occur following incubation (13-15). Additionally, the antibacterial properties of CS-AgNPs on *C. albicans, S. aureus, P. aeruginosa, B. subtilis* and *E. coli* were evaluated in comparison to those of the commercial antibiotics vancomycin, colistin, and fluconazole as well as 30 mM $AgNO_3$ solution.

RESULTS

Antibiotics used, the antimicrobial capabilities of CS-AgNPs grows into more significant. Foodborne infections tend to be caused by the pathogenic microorganisms *E. coli, B. subtillis, P. aeruginosa, S. aureus, and C. albicans* (16, 17). The results of the investigation demonstrated that bio-derived CS-AgNPs strongly suppressed the spread of these bacteria even at low doses. (Table 1). It was found that

Staphylococcus and Bacillus subtilis were more effectively repressed by CS-AgNPs than other bacteria. Due to their propensity to interact with the phosphorous and sulfur atoms in bacterial cell walls, plant-derived silver nanoparticles compromise with the thiol molecules and phosphate groups in bacterial cell membranes, which prevents bacteria from respirating. Bacteria perish as a result of this (16).

Table 1 MIC values of CS-AgNPs, AgNO3 and antibiotics (mg/mL)

Microorganisms	CS-AgNPs	$AgNO_3$	Antibiyotics
Staphylococcus aureus ATCC 29213	0. 125	3.75	2
Bacillus subtilis ATCC11774	0.0625	1.87	1
Escherichia coli ATCC25922	1.0	0.93	2
Pseudomonas aeruginosa ATCC27853	1.0	7.50	4
Candida albicans	0.5	0.47	2

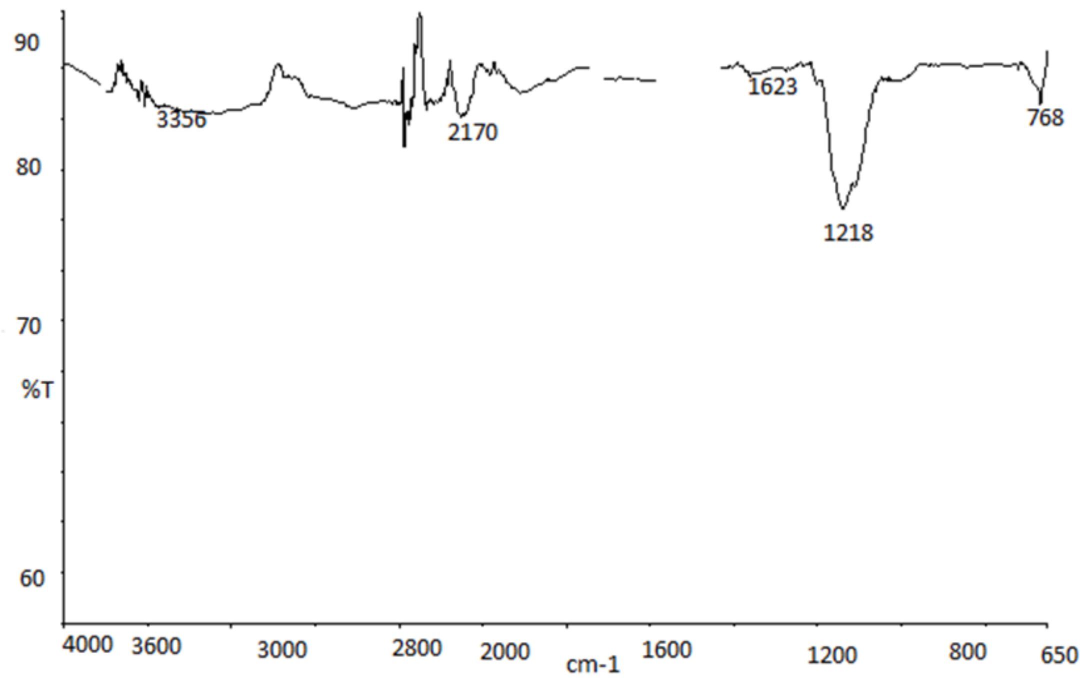

Figure 1. Purple parts of saffron flower FTIR spectrum.

DISCUSSION

The main objective of this study was to first synthesize and characterize reproducible, highly monodisperse CS-AgNPs. Next, we determined the in vitro antibacterial and antifungal activity of CS-AgNPs of saffron flower origin. antimicrobial inhibitory actions of CS-AgNPs, AgNO3 solution, and conventional antibiotics on human infection growth were assessed. The concentrations ranged from 0.0625-0.125 g mL-1. There were several significant MICs for growth inhibition of Gram-negative bacteria. On the other hand, an experimental test was used to identify the effective growth suppression concentrations, which were 1.0 mL-1 for a Gram- strains and 0.5 mL-1 for C. albicans yeast. At a lower level than the two antibiotics, CS-AgNPs were active. On the other hand, gram-positive bacteria appear to be more sensitive to the AgNO3 solution. It has been verified that CS-AgNPs minimize the growth of several pathogens tested at extremely low concentrations when compared to standard antibiotics and AgNO3 solution. On the other hand, gram-positive microbes have a hard polysaccharide layer that gram-negative bacteria do not, making the transition to the a gram- bacterium wall more difficult. AgNPs have a larger regulatory impact on gram-positive bacteria than gram-negative bacteria as a result (5,18). In other comparable experiments, they discovered that gram positive S. aureus had resistance to AgNPs that was roughly 2-3 times higher than that of gram-negative P. aeruginosa and E. coli. In the studies of silver nanoparticles with a green synthesis origin conducted by many researchers, it is observed that AgNPs have inhibitory effects on both gram-positive and - bacteria as well as yeasts (13,19,20).

CONCLUSION

The studied strains were resistant to the biogenic AgNPs' antibacterial and anticandidal effects. The deformation and distortion in bacteria and candidal cells stimulated with the AgNPs that are biogenic were confirmed by ultrastructural analysis. The ability of biogenic AgNPs to penetrate cells due to their small particle size as well as the capping effect of biomolecules adsorbed on the outer layer may both contribute to their high efficacy. One of the fastest-growing technological fields is bionanotechnology, which consists of biologically inspired nanostructures and metallic nanoparticles made from the substances that make up their fundamental building blocks. This study used CS flower extract to quickly and easily create green CS-AgNPs without the use of any harmful chemicals. UV-vis intake, XRD and EDX, FESEM, TEM examination of the bioassisted produced CS-AgNPs revealed that they were generally cylindrical with a mean length of roughly 67.9 nm. It was discovered that silver nanoparticles showed excellent antibacterial and anticandidal activity even at extremely low concentrations and that their ability to fight bacteria increased with decreasing size. It may be utilised to produce commodities obtained from the life sciences on a tight budget by using easy, basic, and practical methods. Synthesized nanoparticles derived from biological sources are available in a variety of sizes and exhibit a wide range of properties, including anticancer, anti-diabetic, stability, surface functionality, biocompatibility, and antibacterial activity. The science of nanotechnology needs more research and a greater range of species (21, 26-28). But it is obvious that more investigation is required to reveal the medical uses of metallic nanoparticles.

Ethics Committee Approval: There is no need for an ethics committee.

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Author Contributions: Concept: MFB. Design: MFB, HB. Literature search: MFB, HB. Data Collection and Processing: MFB, HB, UK Analysis or Interpretation: MFB, HB, UK. Written by: MFB, HB, UK

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