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ORIGINAL PAPER

Biofabrication of copper oxide nanoparticles using *Solanum tuberosum* L. var. Vitelotte: characterization, antioxidant and antimicrobial activity

Gamze Topal Canbaz[1] · Zehra Seba Keskin[2] · Ahmet Yokuş[3] · Rukiye Aslan[2]

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Abstract
In this study, *Solanum tuberosum* L. var. Vitelotte extract and copper oxide nanoparticles (CuONPs) were synthesized for the first time by the green synthesis method, which is the cheapest and most effective method. The synthesized nanoparticles were analyzed by SEM, EDX, XRD, and FTIR. In the SEM analysis, the synthesized particles had a spherical morphology in the size range of 190–220 nm. In the EDX analysis, the amounts of Cu and O atoms forming the structure of the nanoparticles were determined as 66.77% and 31.75%, respectively. In XRD analysis, the crystal size of nanoparticles was calculated as 7.22 nm. The bonds that are effective in nanoparticle synthesis were identified by FTIR spectroscopy. The antibacterial activities of CuONPs obtained by green synthesis against gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* were examined and determined that they had antibacterial effects against both bacteria. The antioxidant activity of CuONPs was also investigated on the basis of free radical scavenging activity by the DPPH method. The antioxidant capacities of *Solanum tuberosum* L. var. Vitelotte extract and CuONPs were determined in the range of 28–31% at 50–250 µg/mL concentrations, and over 80% activity was determined at 500 µg/mL concentration.

Keywords CuONPs · Green synthesis · Antimicrobial · DPPH scavenging · *Solanum tuberosum* L. var. Vitelotte

Introduction

Nanotechnology allows the controlled application of materials to science at the nanoscale (1–100 nm) and provides advanced technologies for biological applications. Nanomaterials have physicochemical properties, such as ultra-small size, large surface area/volume ratio, and high reactivity. Since they have similar dimensions and chemical stability with biological molecules, they show high biomedical efficiency in areas where large molecules are poorly accessible (Reyes-Torres et al. 2019; Dhandapani et al. 2020).

Recently, the antibacterial and antioxidant values of metallic and metal oxide nanoparticles such as Ag, Au, TiO, ZnO, CuO, FeO, and Al_2O_3 have been investigated.

CuONPs are preferred in many application areas among metal oxides because they are economical and versatile, abundant starting materials, non-toxic, different acid–base properties, and reduction properties.

CuONPs are of interest in areas such as high Tc superconductors (Khene 2021), sensors (Bang et al. 2021), optics (Velliyan and Rajendran 2021), electricity (Pramothkumar et al. 2021), giant magnet resistor materials, gas sensors (Zhang et al. 2021), heat transfer (Khooshechin et al. 2020), solar cells (Siddiqui et al. 2020) and batteries (Li et al. 2021), photocatalysis (Sharma et al. 2021), antimicrobial (Nithiyavathi et al. 2021) and biomedical industry (Ruddaraju et al. 2021).

CuONPs, which is more stable, robust, and have a longer shelf life than organic antimicrobials, do not harm human tissues but show activity on microorganisms even at low concentrations. In addition, CuONPs and hybrid nanocomposites are increasingly preferred in wastewater treatment

✉ Gamze Topal Canbaz
gamzetopal@hotmail.com

[1] Department of Chemical Engineering, Engineering Faculty, Sivas Cumhuriyet University, 58140 Sivas, Turkey

[2] Department of Pharmacy, Health Services Vocational School, Sivas Cumhuriyet University, 58140 Sivas, Turkey

[3] Suşehri Timur Karabal Vocational School, Sivas Cumhuriyet University, 58600 Suşehri, Sivas, Turkey

and degradation of organic pollutants (Iqbal et al. 2020a, b, 2022; Kuang et al. 2022).

Physical and chemical methods are used in the synthesis of CuONPs. However, alternative methods have been investigated due to disadvantages such as high energy density, use of toxic chemicals, and high-temperature requirement in these methods (Zaman et al. 2020).

Therefore, in recent years, researchers have been trying to develop non-toxic, cost-effective, easily synthesized, environmentally friendly green methods as an alternative to these methods. Green methods are a method that makes use of biochemicals found in macro- and microorganisms, such as fungi, algae, bacteria, plants and plant parts. However, the use of plants and plant extracts as biological material is more advantageous in terms of being a one-step method, non-pathogenic, inexpensive and producing large amounts of metabolites. (Salem and Fouda 2021; Derakhshani et al. 2023). In addition, plant extracts used in the synthesis of nanoparticles act as reducing, stabilizing and capping agents due to the phytochemicals they contain, such as polyphenols, flavonoids, amino acids, terpenoids, alkaloids (Atri et al. 2023).

In recent years, plant extracts such as, *Eichhornia Crassipes* leaf extract, (Saligedo et al. 2022), *Wedelia trilobata* flower extract (Sowmya et al. 2023), *Punica granatum* L. peel extract (ben Mosbah et al. 2022), *Abutilon indicum* leaf extract, apple peel extract (Rajamohan and Lee 2023), *Calotropis procera* leaf extract (Shah et al. 2022) have been used for the synthesis of CuONPs.

Solanum tuberosum L. is a high-carbohydrate product preferred as an energy source in the diet of a significant part of the world's population after rice and wheat (Bvenura et al. 2022). Potato tubers contain phytochemicals, such as phenylpropanoids, carotenoids, polyamines and glycoalkaloids.

In recent years, the *Solanum tuberosum* L var Vitelotte species has attracted the attention of consumers due to its attractive colors, superior flavors and crushability. The pigments responsible for the dark blue skin and violet flesh of this potato variety were determined as 5-glucoside-3-rhamnosylglucoside derivatives of anthocyanidins, which are known to have anti-atherosclerotic, anti-inflammatory, antioxidant and anti-cancer properties, monoacylated with p-coumaric or ferulic acids. It has also been reported that colored potatoes contain polyphenols such as chlorogenic acid as phenolic acid, caffeic, cinnamic and sinapinic acid as flavonoids, catechin and epicatechin (Cebulak et al. 2023).

These colored genotypes contain three to four times higher phenolic compounds than the white and yellow varieties. These phytochemicals have been reported to have antiproliferative, antifungal and antimicrobial activities in vitro (De Masi et al. 2020).

As far as we know, there is no study in the literature in which CuONPs were synthesized using *Solanum tuberosum* L. var. Vitelotte peel. Therefore, the aim of the study is to synthesize CuONPs by the green synthesis method using *Solanum tuberosum* L. var. Vitelotte peel as a reducing and stabilizing agent and to examine the antioxidant and antibacterial activities of biosynthesized nanoparticles.

The characterization of CuONPs synthesized in the study was performed by UV–Vis spectroscopy, SEM, EDX, XRD, and FTIR analyses. Then, their antibacterial activities against *Escherichia coli* and *Staphylococcus aureus* were analyzed. The antioxidant activity of synthesized nanoparticles was determined by the DPPH method on the basis of free radical scavenging activity.

Experimental

Materials

The *Solanum tuberosum* L. var. Vitelotte used in the study was purchased from the Sivas Cumhuriyet University sales office. $CuSO_4.5H_2O$ (Sigma-Aldrich) was used as the metal solution. pH adjustments were made with concentrated and diluted NaOH and H_2SO_4.

Solanum tuberosum extract preperation

The *Solanum tuberosum* L. var. Vitelotte was washed with water and distilled water to remove dust and dirt, and their peels were removed for use in the study. The peels were dried at ambient temperature until the moisture was completely removed. 6 g of the dried peel was extracted by boiling with 100 ml of distilled water for 30 min. Extract and peels were separated with Whatman no 1 filter paper. The extract was stored in the refrigerator at + 4 °C to be used in the experiments.

CuONPs synthesis

$CuSO_4.5H_2O$ was used as the starting metal solution to synthesize CuONPs produced by green synthesis. 10 ml of purple potato extract was added dropwise onto 50 ml of 0.2 M $CuSO_4.5H_2O$ solution, the pH was adjusted to 10 and mixed in a magnetic stirrer for two hours. The precipitate formed after mixing was centrifuged at 10,000 rpm for 15 min (Hitachi, CR22N) and separated. The synthesized CuONPs were dried in an oven at 50 °C (Dashtizadeh et al. 2021).

Characterization

The characterization of CuONPs produced by the green synthesis method using *Solanum tuberosum* L. var. Vitelotte peel extract was analyzed by UV–Vis spectrophotometer, XRD, FTIR, SEM, and EDX.

CuONPs synthesis were confirmed by absorbance scanning in the 200–900 nm range with a UV–Vis spectrophotometer (UV-2600, Shimadzu). The size and morphology of CuONPs were examined by SEM (TESCAN MIRA3 XMU). Qualitative chemical analysis of CuONPs were determined by EDX. FTIR (Bruker Mode: Tensor II) analysis was performed to identify CuO NPs and possible functional groups in the structure of the extract. The crystal structure of CuONPs was determined by XRD (RIGAKU-MINI FLEX 600).

CuONPs antibacterial assay

Disk diffusion assay

The disk diffusion test was studied to investigate the antimicrobial activities of nanoparticles solutions (Biemer 1973). For this purpose, gram-negative *Escherichia coli* ATCC 25,922 standard strain; gram-positive *Staphylococcus aureus* ATCC25923 standard strain was used. Bacterial strains were inoculated on blood agar and incubated at 37ºC for 24 h, and overnight fresh cultures of bacteria were used. The next day, bacterial concentrations were adjusted to the standard McFarland 0.5 density and inoculated onto the entire agar surface with a sterile cotton swab on Mueller Hinton agar (MHA). Different concentrations of CuONPs solutions were inoculated on the empty sterile antibiotic disks in an empty petri dish with 15 µl of CuONPs solution in each disk. The disks were allowed to absorb the solutions for 20 min at room temperature. Then, the disks were placed on MHA medium at a homogeneous distance. For comparison, plates of the same diameter with 20 µl Gentamicin (10 µg/disk) for gram-negative bacteria, Ciprofloxacin (5 µg/disk) for gram-positive bacteria were used for positive control. For negative control, 15 µl of distilled water was impregnated on the disks an MHA medium was incubated at 37 °C for 24 h. The next day, the inhibition zone diameters formed in the media were measured. All assays were carried out in triplicate.

Antioxidant assay

In vitro DPPH free radical scavenging assay

CuONPs, extract of *Solanum tuberosum* L. var. Vitelotte were evaluated for their radical scavenging capacity following the procedure described by Sathiskumar vd. (Sathishkumar et al. 2016). Briefly, different concentrations (50–500 µg/ml) of CuONPs, *Solanum tuberosum* L. var. Vitelotte extract and ascorbic acid (as a positive control) were mixed separately with 1 mL of DPPH% solution in ethanol (0.1 mM). The mixtures were kept for 30 min in the dark, then the absorbance at 517 nm was acquired. The DPPH % inhibition percentage was estimated using the following formula 1:

$$\%\text{Inhibition} = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] * 100 \quad (1)$$

where A_{sample} is the absorbance of DPPH/sample solution and A_{control} is the absorbance of DPPH % solution without including the sample.

Results and discussion

Characterization of CuONPs

SEM and EDX analysis

Structural morphologies of CuONPs synthesized using *Solanum tuberosum* L. var. Vitelotte peel extract were investigated using SEM analysis. It is seen in Fig. 1 that the synthesized nanoparticles have a spherical morphology in the size range of 190–220 nm. Agglomeration is observed between nanoparticles in the SEM image. The reason for this agglomeration is thought to be the large surface area and high surface energy of the synthesized nanoparticles (Pakzad et al. 2019).

EDX analysis results of CuONPs are given in Fig. 2. The strong signals of Cu and O atoms in the EDX spectrum confirm that the synthesized structure is CuO.

In addition, weak signals of the C atom due to the organic structure of the extract used in biosynthesis and the S atom due to the structure of the metal salt are observed (Ali et al. 2021).

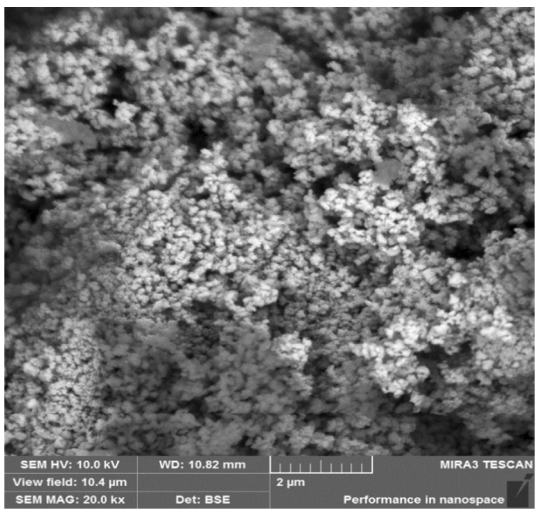

Fig. 1 SEM image of CuONPs

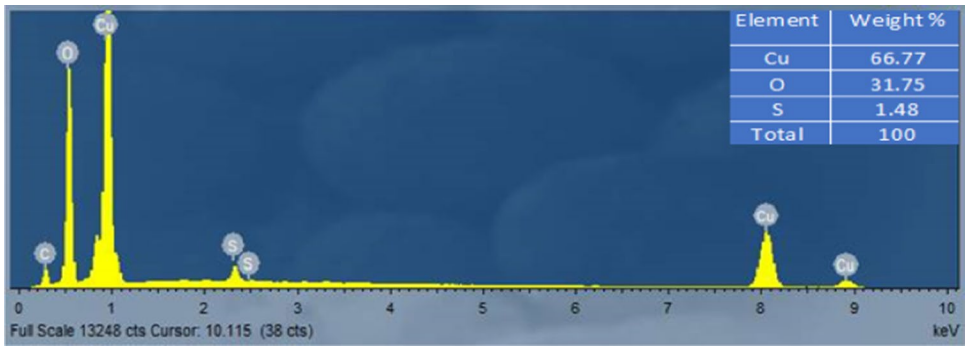

Fig. 2 EDX spectrum of the synthesized CuONPs

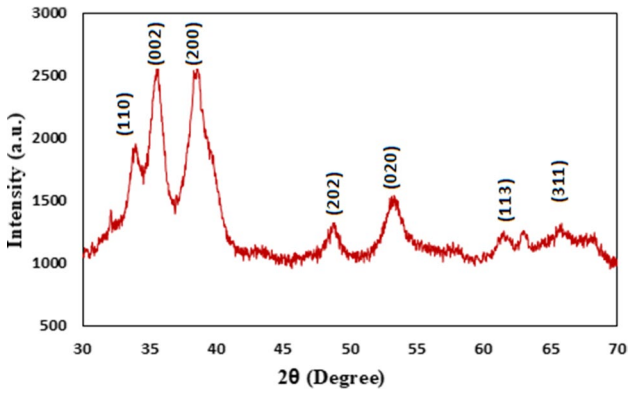

Fig. 3 XRD pattern of synthesized CuONPs

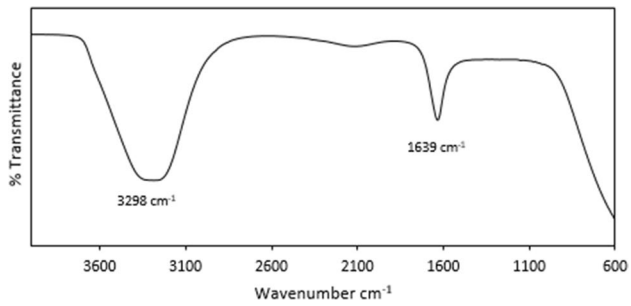

Fig. 4 FTIR spectra of *Solanum tuberosum* L. var. Vitelotte extract

XRD analysis

XRD analysis of biosynthesized CuONPs is shown in Fig. 3.

The characteristic peaks observed at $2\theta = 33.82°$, 35.55°, 38.8°, 48.75°, 53.34°, 61.89, 68.17 values belonging to CuONPs in the XRD spectrum, corresponds to the (110), (002), (200), (202), (020), (113) and (311) planes, respectively, and it was determined to be monoclinic. The fact that all the diffraction peaks determined in the spectrum are compatible with CuO (JCPDS no. 80-1917) and no other peaks are observed indicates that the CuONPs are püre. The accuracy of this result is supported by the results of previous studies (Iqbal et al. 2020b; Lv et al. 2022; Atri et al. 2023). In addition, the presence of narrow and sharp peaks observed in the XRD spectrum is attributed to the crystal structure of CuONPs. (Sardar et al. 2022).

The crystal size measurement for the synthesized CuONPs was calculated using the following Debye–Scherrer equation (Aladpoosh and Montazer 2015).

$$D = \frac{K\lambda}{\beta \cos\theta} \quad (2)$$

D: crystal size, K: Debye Scherrer constant (0.94), λ: Cu-Kα radiation (1,54 A), β: half-length width of maximum peak (FWHM), θ: Bragg angle value obtained from the 2θ value of the maximum peak in the XRD diffraction pattern.

The average crystal size of the synthesized CuONPs was determined as approximately 7.22 nm according to the Debye–Scherrer formula.

The XRD analysis results obtained show parallelism with the studies in the literature. (Das et al. 2013; Sharma et al. 2021).

FTIR analysis

The determination of the functional groups in the material structure is carried out by FTIR analysis. FTIR analysis of the *Solanum tuberosum* L. var. Vitelotte extract is given in Fig. 4. The peaks corresponding to 3298 cm^{-1} define the alcohol group (-OH), and the peaks observed at 1639 cm^{-1} define the C=C group as phenyl (Yasser et al. 2021).

FTIR analysis of biosynthesized CuONPs is given in Fig. 5. The peak observed at 3567 cm^{-1} corresponds to the –OH bond, while the peaks observed at 1456 cm^{-1} and 1115 cm^{-1} correspond to the C–O bond. The peak observed at 689 cm^{-1} corresponds to the Cu–O vibration (Rajeshkumar et al. 2021).

Antibacterial activity results

Inhibition zones formed by CuONPs solutions were measured (Table 1). For each microorganism, it was expressed in

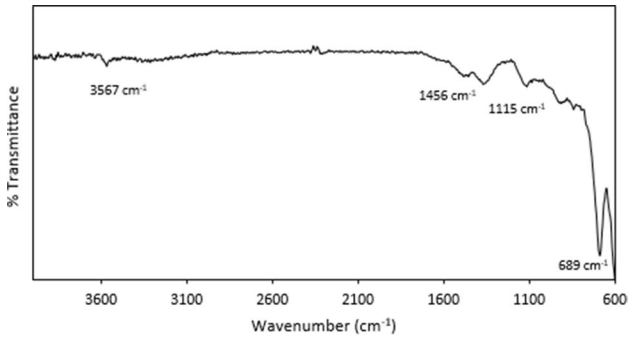

Fig. 5 FTIR spectra of CuONPs

Table 1 The inhibition zones of CuONPs solutions

Concentrations	Bacterial strains	
	Staphylococcus aureus	*Escherichia coli*
100 mg/ml	21 mm	12 mm
50 mg/ml	19 mm	9 mm
40 mg/ml	12 mm	7 mm
30 mg/ml	12 mm	–
20 mg/ml	12 mm	–
10 mg/ml	–	–
Positive control	50 mm	25 mm

"mm" units. It was observed that the inhibition zones created by the CuONPs solutions were higher for *S. aureus* bacteria. It was determined that CuONPs solutions have antibacterial activity for *E. coli*, and as the concentration value decreases, the antibacterial activities also decrease. *S. aureus* and *E. coli* are bacteria that have different cell structure. Since the cell wall, metabolic and physiological activities that surround the cell membrane of these bacteria and ensure the survival of the bacteria will be different, their interactions with CuONPs solutions, their resistance or sensitivity status are also different. Due to the presence of amine and carboxyl groups on the cell surface of Gram-positive bacteria S. aureus, its affinity with CuONPs solutions also increases. *E.coli*, which is a Gram-negative bacterium, has a different cell wall structure, making it resistant or less sensitive to CuONPs solutions (Azam et al. 2012; Liang et al. 2012).

Generally, it was determined that CuONPs solutions have an effective antimicrobial activity against both gram-positive and gram-negative bacteria.

Antioxidant activity

It is known that antioxidants protect cells against harmful effects caused by reactive oxygen species and consequently reduce the incidence of some degenerative diseases, such as cancer, neurodegenerative, and heart diseases (Zayed et al. 2020).

The principle of % DPPH analysis is that the intense purple color of a freshly made DPPH solution tends to fade or disappear when an antioxidant sample is present in the medium. As a result, antioxidant molecules can reduce absorption at 517 nm by quenching DPPH free radicals and converting them to a colorless product.

This is a fast, easy and reliable method. This method is based on the principle that DPPH can easily take an electron from antioxidant molecules to become a stable diamagnetic molecule (Soares et al. 1997). In this study, the antioxidant activities of CuONPs synthesized by the green synthesis method were investigated at different concentrations (50–500 mg). Ascorbic acid was used as a positive control (Fig. 6).

The antioxidant capacities of the extract and CuONPs at 50–250 µg/mL concentrations range from 28 to 31%. When the concentration was 500 µg/mL, the antioxidant activities of the extract and CuONPs were determined as 83.5% and 84.75%, respectively. Obtained results showed that DPPH inhibition activity is concentration-dependent.

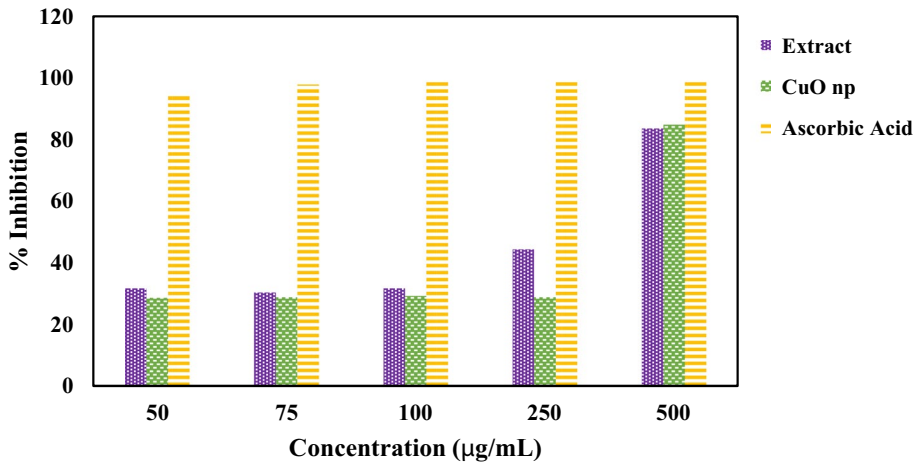

Fig. 6 DPPH % scavenging activity of *Solanum tuberosum* L. var. Vitelotte extract, CuONPs, and ascorbic acid

Table 2 The comparison of DPPH radical scavenging activity of synthesized CuONPs of the present study with other biosynthesized CuONPs

CuONPs	IC_{50} (µg/ml)	Reference
L-CuONPs-*Mussaenda frondosa*	1570	Manasa et al. (2021)
S-CuONPs-*Mussaenda frondosa*	1536	Manasa et al. (2021)
CuONPs-*Tinospora cordifolia*	566	Udayabhanu et al. (2015)
CuONPs-*Capsicum frutescens*	289.97	K. et al. (2021)
CuONPs-*Solanum tuberosum*	275	This study
CuONPs-*Cissus vitiginea*	45.29	Thakar et al. (2021)
CuONPs-*Plectranthus amboinicus*	40.10	Velsankar et al. (2020)

DPPH inhibition activity was above 90% in all ascorbic acid concentrations studied as a positive control.

The IC_{50} values of biosynthesis CuONPs using different plant extracts are given in Table 2. As shown in Table 2, the values of CuONPs obtained from the *Solanum tuberosum* Vitelotte peel extract are in the previous study range.

Conclusion

The synthesis of CuONPs produced by the green synthesis method using *Solanum tuberosum* L. var. Vitelotte extract was carried out for the first time in this study. SEM analysis of the synthesized nanoparticles shows that spherical-shaped particles are formed in the size range of 190–220 nm. In CuONPs EDX analysis, Cu and O ratios were determined as 66.77% and 31.75%, respectively. The average crystal size of the synthesized CuONPs was determined to be approximately 7.22 nm The presence of narrow and sharp peaks observed in the XRD spectrum is due to the crystal structure of CuONPs. The peak observed at 689 cm^{-1} in the CuONP spectrum obtained from the FTIR analysis corresponds to the Cu–O vibration. The synthesis of CuONPs was characterized by XRD, SEM, EDX analyzes and it was proved that the synthesis was successful with Solanum tuberosum L var Vitelotte extract.

The antibacterial and antioxidant capacities of the synthesized CuONPs were investigated. Due to the presence of amine and carboxyl groups on the cell surface of Gram-positive bacteria S. aureus, its affinity with CuONPs solutions also increases. E.coli, which is a Gram-negative bacterium, has a different cell wall structure, making it resistant or less sensitive to CuONPs solutions. In the study conducted with *S. aureus* and *E. coli,* it was determined that the inhibition zones were higher for *S. aureus* bacteria, and for *E. coli*, the antibacterial activity of the particles decreased as the concentration value of the particles decreased. It was determined that CuONPs had effective antimicrobial activity against gram-positive and gram-negative bacteria. In antioxidant activity studies, the free radical scavenging activity's results were at 28.75%, 29%, 29.37%, 29% and 84.75% with 50, 75, 100, 250 and 5000 µg/mL concentrations for CuONPs, whereas for ascorbic acid were at 94.12%, 97.62%, 98.5%, 98.75% and 99% with 50, 75, 100, 250 and 80 µg/mL concentrations, respectively.

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