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Original Research

Antimicrobial, Antioxidant and DNA Damage Prevention Effect of Nano-Copper Particles Obtained from *Diplotaenia turcica* Plant by Green Synthesis

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Abstract

Metal nanoparticles have been intensely researched and developed in recent years due to their superior properties. There is a growing interest in economical and environmentally friendly techniques. Cu metal is preferred in research studies due to its cheapness and effects on human health (such as production of blood cells, oxidation and reduction).

In recent years, the use of plant extracts in nanoparticle synthesis has become quite popular. In this study, we aimed to investigate the antioxidant capacity and protective properties of Cu nanoparticles obtained by using *Diplotaenia turcica* plant against damage to pBR322 plasmid DNA. In addition, we investigated the antimicrobial effect of Cu nanoparticles against *Staphylococcus aureus* ATTC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 25952, *Candida albicans* ATCC 25952. *Diplotaenia turcica* plant characterization with Cu metal (Cu NPs/Dt) ultraviolet and visible light absorption spectroscopy (UV-vis), fourier-transformed infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), X-ray diffraction (XRD) and energy dispersive X-ray (EDX) analysis. When looking at the antioxidant activity analysis, it is understood that it is a powerful antioxidant. It was determined that Cu NPs/Dt have significant antimicrobial activity. Its effect against some pathogens was found to be stronger than the positive control antibiotic. It was determined by the obtained DNA images that there is a potential to prevent breaks that may occur in DNA depending on the concentration.

Keywords: antimicrobial, antioxidant, *Diplotaenia turcica*; DNA damage, nanoparticle

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Introduction

Nanotechnology is a growing field of study in the scientific world to obtain new nanoscale particles [1-5]. Nanoparticles can be prepared using various chemical, electrochemical, radiation, photochemical and biological methods developed in recent years. However, due to the toxicity of materials used in chemical synthesis, it is imperative to use environmentally friendly and non-toxic stabilizing agents in the synthesis of nanoparticles. In this context, biological methods provide a significant advantage in the production of non-toxic nanoparticles compared to physical and chemical methods. In recent years, the use of plant extracts in nanoparticle synthesis has gained popularity due to its simple, non-toxic, cheap and applicability in various fields [6-8]. Metal nanoparticles are being studied extensively due to their unique chemical properties such as catalytic activity, optical properties, electronic properties, antimicrobial activity, antioxidant, anticancer and magnetic properties [9-13]. Studies have proven that Cu nanoparticles show strong antibacterial properties and at the same time show effective antimicrobial properties against some pathogens [14, 15]. Cu, Zn and Ag nanoparticles have the ability to inhibit the growth of these microorganisms due to the damage they cause on the membrane of bacterial cells [16]. Synthesized Cu NPs by using *Artemisia haussknechtii* leaf extract have been found to have inhibitory effects against *Escherichia coli*, *Staphylococcus aureus* and *Serratia marcescens* bacteria [17, 18]. It was stated in the study that Cu nanoparticles show activity against many pathogens and have the ability to reduce bacterial populations to zero [19]. *Diplotaenia turcica* is an endemic plant that has existed for many years with a woody root structure of about 1.5-2 m long. *Diplotaenia turcica*, which blooms in white in August, is popularly known as "siyabo" [20]. *Diplotaenia turcica* plant is used by the people of the region in meals and treatments, as well as in the structure of herby cheese. In addition, *Diplotaenia turcica* plant is popularly used for diabetes, blood pressure and rheumatic conditions [21].

In this study, CuNPs were synthesized using the extracts of *Diplotaenia turcica* plant (Cu NPs/Dt), an endemic species that grows in rural areas of Van and using biological synthesis method. Particles with Cu NPs, Ultraviolet / visible light absorption spectroscopy (UV-vis), X-ray diffraction (XRD), fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) analyzes has been characterized. The antimicrobial effects of synthesized Cu NPs against some pathogens that cause disease in the human body, the antioxidant level that has an important effect on health, and the activities to prevent damage to DNA have been investigated.

Material and Methods

Synthesis of Cu NPs/Dt

Diplotaenia turcica plant, which grows in the higher parts of Hakkari province, was collected properly. Later, he was diagnosed at Van Yüzüncü Yıl University, Faculty of Science, Department of Biology, Herbarium Techniques Laboratory (Herbarium no: Vanf 32858). Leaf parts were removed, washed and left to dry. After drying at room temperature for 15 minutes, the grinding process was performed [22]. For the synthesis of nanoparticles, Gurunathan et al. [23] method was used in a modified form. For the synthesis of Cu NPs/Dt, 1 mM 500 ml $CuSO_4$ aqueous solution was prepared. 100 ml of *Diplotaenia turcica* plant leaf extract was allowed to react under constant conditions at room temperature in a 1000 ml flask. Color change occurred in the solution after 40-45 minutes. The resulting solution was centrifuged at 10.000 rpm for 5 minutes and the liquid phase was removed. The remaining solid part in the tubes was subjected to washing with distilled water. The resulting solid part was left to dry in the oven at 48-50ºC for 2 days. And as a result of this process, nanoparticles were obtained.

Characterization of Cu NPs/Dt

UV measurements were made using the PEL 750 instrument in the wavelength range of 250-800 nm. FT-IR analysis was carried out with a spectrometer device in the wavelength range of 500-4000 cm^{-1} to identify the functional structures of various molecules in the plant extract. XRD analysis was performed using an X-Ray diffraction diffractometer. The size and morphology of Cu NPs/Dt were obtained using scanning electron microscopy (SEM, Zeiss SmartEDX).

Antioxidant Activity of Cu NPs/Dt

Which is the extract of the study subject. The DPPH quenching activity of Cu NPs/Dt was calculated using the previously found method [24]. BHA was used as a positive control in this procedure.

Table 1. Tested microorganisms used in the study.

Tested microorganisms
Staphylococcus aureus ATTC 29213
Pseudomonas aeruginosa ATCC 27853
Enterococcus faecalis ATCC 29212
Bacillus subtilis ATCC 6633
Bacillus cereus ATCC 10876
Escherichia coli ATCC 25952
Candida albicans ATTC 90028

Table 2. Factors added to pits in electrophoresis process.

Pit	Factors added to pits
1. Pit	pBR322+ Loading dye
2. Pit	pBR322+ Loading dye+H_2O_2+UV
3. Pit	pBR322+ Loading dye+H_2O_2+UV+ 50 mg/L Cu NPs/Dt
4. Pit	pBR322+ Loading dye+H_2O_2+UV+100 mg/L Cu NPs/Dt
5. Pit	pBR322+ Loading dye+H_2O_2+UV+250 mg/L Cu NPs/Dt

The experiment was carried out using methanol solutions of 0.1 mg/ml DPPH. DPPH and Cu NPs/Dt extract solutions were prepared in 7 different concentrations of 5, 10, 15, 20, 25, 50 and 100 µg/ml. Cu NPs/Dt extract and positive control 3 ml were taken and DPPH solution was added on them. The mixtures formed in the tubes were incubated for 30 minutes in the dark and at room temperature. At the end of this period, absorbance values were read at 517 nm.

$$\% I = [(A_{control}-A_{sample}) / A_{control}] \times 100$$

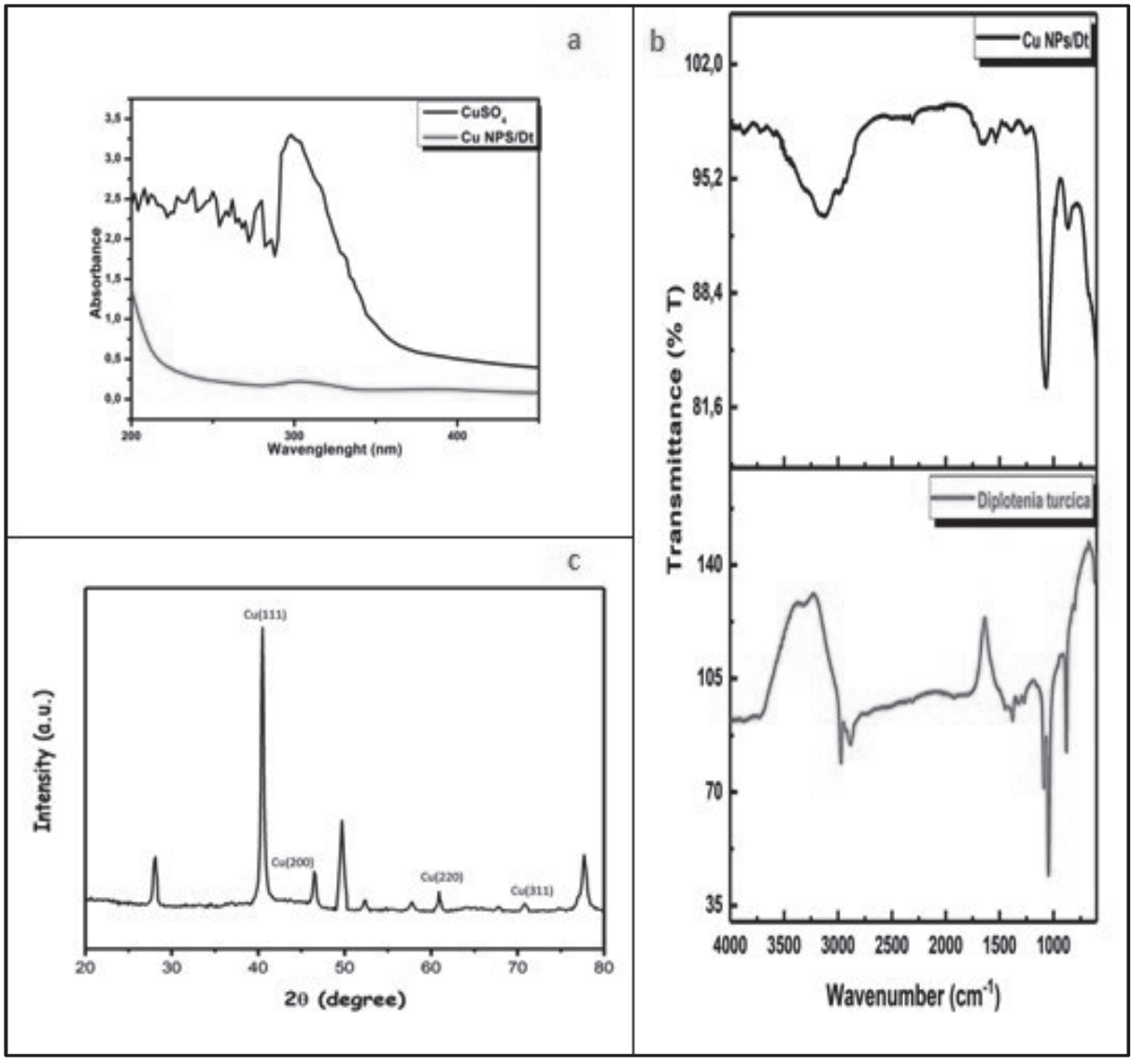

Fig. 1. a) UV-vis spectra of CuSO4 and Cu NPs/Dt samples, b) FT-IR spectra of *Diplotaenia turcica* plant extract and Cu NPs/Dt samples and c) XRD pattern of Cu NPs/Dt.

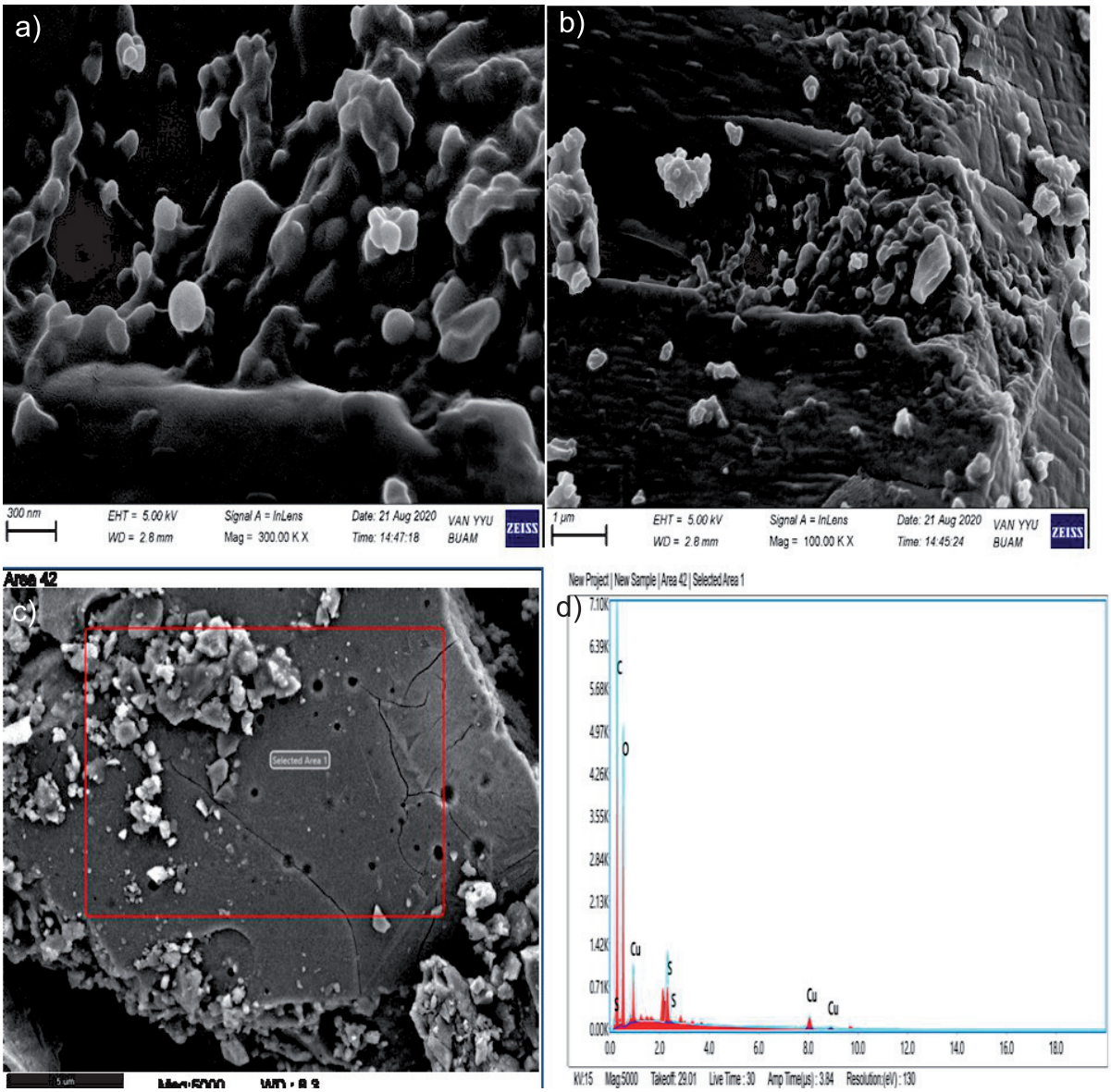

Fig. 2. (a-c) SEM images of Cu NPs/Dt sample taken at different scales. It shows the homogeneous distribution of Cu nanoparticles from SEM image at 300 nm scale. (d) EDX spectrum obtained from one of these images. EDX spectrum, on the other hand, clearly reveals the existence of Cu, C, S and O elements that form the structure of the Cu NPs/Dt sample.

As a result of these processes, the graph of Cu NPs/Dt concentration versus increasing DPPH ethanol concentration was obtained. This graph is obtained using the above equation.

Antimicrobial Activity of Cu NPs/Dt

Copper nanoparticles were obtained using *Diplotaenia turcica* extract and $CuSO_4$. Antimicrobial activity of Cu NPs/Dt against some pathogenic microorganisms was investigated. The disk diffusion method was used to examine the antimicrobial effect [25]. Test microorganisms used in the study are given in Table 1 Microorganisms were obtained from Van Yüzüncü Yıl University, Faculty of Health Sciences.

30 µL of extract (1) and 30 µL of nanoparticle (2) solution were absorbed into the blank discs and allowed to dry at room temperature. Pathogens previously activated in Nutrient Broth medium were transferred to Müller Hinton Agar medium and smear was performed. Neomycin (3) antibiotic was used for positive control. After the discs were placed, they were kept in the oven at 36.5-37ºC for 48 hours for incubation. The zones formed at the end of the incubation period were measured and images were obtained.

DNA Damage Preventive Effect of Cu NPs/Dt

The damaging effect of Cu NPs/Dt on DNA was investigated. Electrophoresis was performed by preparing a 1% agarose gel [26, 27]. pBR322 plasmid

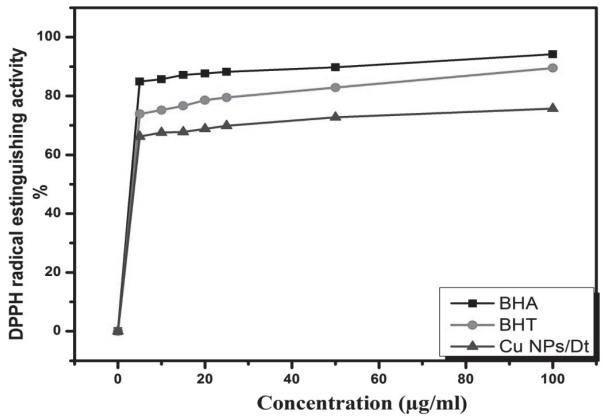

Fig. 3. DPPH scavenging activity values BHA (Butyl Hydroxy Anisole) 94.1558%, BHT (Butyl Hydroxy Toluene) 89.4805% and Cu NPs/Dt 75.7142%.

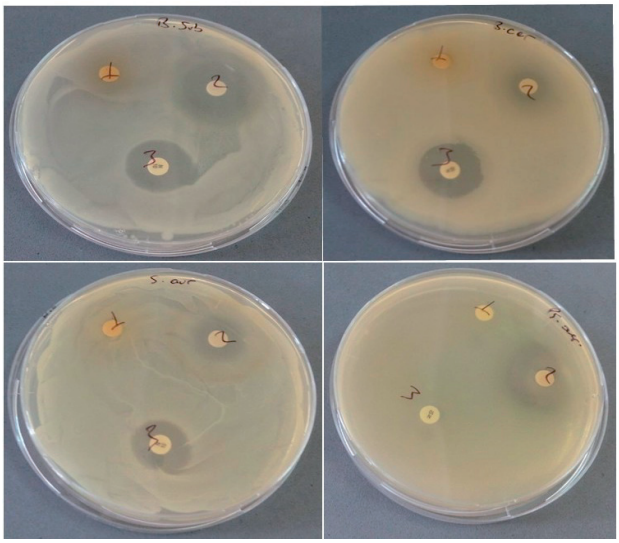

Fig. 4. Some images of antimicrobial activity (Extract →1, Cu NPs/Dt →2, Neomycin →3)

DNA was used as nucleic acid in the study. Loading was made into five pits. Factors added to pits in electrophoresis process are given in Table 2 After the loading process was completed, 45 minutes of execution was done at 110 Volts. From the third well, copper nanoparticles were added at the rate of 50-100-250 mg/L Cu NPs/Dt, respectively. The results obtained were recorded.

Results and Discussion

Characterization of Synthesized of Cu NPs/Dt

SEM/SEM-EDX, FT-IR, XRD and UV-vis techniques were used for the structural and morphological characterization of Cu nanoparticles prepared by green synthesis using *Diplotaenia turcica* plant, respectively. Fig. 1a) shows the UV-vis spectra of $CuSO_4$ and Cu NPs/Dt samples. Signals observed at 305 nm wavelength in the UV-vis spectrum of the $CuSO_4$ (Cu^{+2}) solution can be attributed to d-d transitions. On the other hand, it was observed that signals around 305 nm in the UV-vis spectrum of the Cu NPs/Dt sample completely disappeared. This situation reveals that the Cu^{+2} cation is largely reduced to metallic copper [28]. Fig. 1b) shows FT-IR spectra of a Cu NPs/Dt sample with *Diplotaenia turcica* plant extract. The signals observed at 1500 and 1550 cm^{-1} in the IR spectrum of *Diplotaenia turcica* plant extract originate from the functional groups of organic compounds (Gallic acid, Vanillin, Quercetin, Malic acid etc.) in the structure of *Diplotaenia turcica* sample. The peaks observed in the 3100 and 3300 cm^{-1} regions correspond to aromatic C-H vibrations. In the FT-IR spectrum of the Cu NPs/Dt sample, it shows that there is a decrease in the peak intensities and shifts in some peaks. When the XRD pattern of the Cu NPs/Dt sample (Fig. 1c) is examined, the signals of Cu (111), Cu (200), Cu (220) and Cu (311) surfaces are seen at 36.54°, 42.44°, 61.57° and 73.58°, respectively. It is understood that these

Table 3. Antimicrobial effect results.

Tested Microorganisms	Zone of Inhibition (mm)		
Bacteria	Extract (1)	Cu NPs/Dt (2)	Neomycin (3)
Bacillus cereus ATCC 10876	8.10	13.20	20.25
Bacillus subtilis ATCC 6633	21.15	28.10	21.05
Escherichia coli ATCC 25952		8.15	14.20
Enterococcus faecalis ATCC 29212		7.20	
Pseudomonas aeruginosa ATCC 27853		17.25	
Staphylococcus aureus ATTC 29213		15.10	16.05
Fungus			
Candida albicans ATTC 90028		8.20	20.20

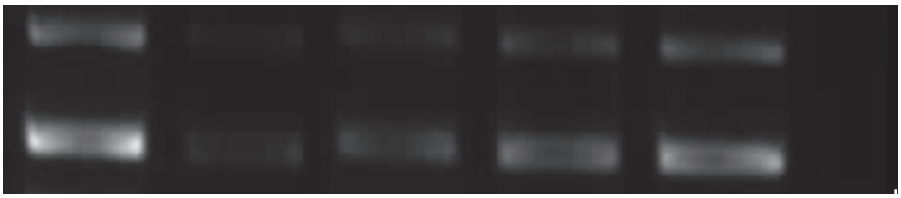

Fig. 5. Gel electrophoresis image.

values are quite compatible with the literature data [29]. Fig. 2(a-d) shows SEM images of Cu NPs/Dt sample taken at different scales and EDX spectrum obtained from one of these images. It shows the homogeneous distribution of Cu nanoparticles from SEM image at 300 nm scale. EDX spectrum, on the other hand, clearly reveals the existence of Cu, C, S and O elements that form the structure of the Cu NPs/Dt sample.

Antioxidant Activity of Cu NPs/Dt (DPPH)

DPPH radical quenching method is one of the most used cheap, fast and safe spectrophotometric methods in antioxidant activity measurement [30]. It was determined that Cu nanoparticles obtained by using cellulosic walnut shell material showed a significant antioxidant activity (97.2%) [31]. The antioxidant effect of copper nanoparticles synthesized by using *Dioscorea bulbifera* a medicinal plant, was investigated and it was observed that it showed 40.81±1.44% scavenging activity against DPPH radicals. [32]. In our current study, considering the values in the graphic, it is seen when looking at the radical quenching activity that Cu NPs/Dt has antioxidant properties. DPPH radical quenching activity is seen to be 75.7142% at 100 µg/ml, giving better results as the concentration increases (Fig. 3). That is, the higher the concentration, the higher the DPPH radical quenching activity accordingly. This value was found as 94.1558-89.4805 for the positive controls BHA and BHT, respectively. As a result, it is seen that Cu NPs/Dt nanoclusters give a result close to these values when compared to BHA and BHT used as positive control and have a good radical quenching effect.

Antimicrobial Activity of Cu NPs/Dt

Cu nanoparticles, like other metallic nanoparticles, show their antibacterial effects by disrupting the membrane structure [33]. Pathogenic bacteria such as *Bacillus subtilis* are more sensitive to copper nanoparticles as they bind more strongly to Cu, which has cell walls rich in amine and carboxyl groups [34]. Aygün et al., [35] found that nanoclusters have antimcrobial effects against seven different pathogens. In this study, it was determined that *Diplotaenia turcica* extract showed antibacterial effect against *Bacillus cereus* and *Bacillus subtilis* species. However, it was observed that Copper nanoparticles obtained by using plant extract and $CuSO_4$ affected all test microorganisms and formed zones varying between 8.15 mm and 28.10 mm. It was determined that Cu NPs/Dt have significant antimicrobial activity. Copper nanoparticles showed the best zone against Bacillus subtilis ATCC 6633 bacteria, while the lowest zone showed against Enterococcus faecalis ATCC 29212 bacteria. In addition, Copper nano clusters were determined to be more effective against *Bacillus subtilis* ATCC 6633 *Pseudomonas aeruginosa* ATCC 27853 *Enterococcus faecalis* ATCC 29212 pathogens than the antibiotic used as positive control. Although Cu NPs/Dt clusters showed a low level of antifungal effect against *Candida albicans* fungus, it was observed that they showed a strong antibacterial effect against the pathogenic bacteria used. Antimicrobial activity results are shown in Table 3 and some images related to antimicrobial activity are shown in Fig. 4.

DNA Damage Preventive Effect

It was determined that the nanostructures obtained using chamomile flower extract and CuO disrupted the DNA helix structure [36]. On the other hand, in our electrophoresis study, it was seen that Cu NPs/Dt had a protective effect on pBR322 plasmid DNA. The image obtained is shown in Fig. 5. When the banding formed is evaluated, it is clearly seen that there are differences between the strip with the control DNA and the strips to which the nano clusters are added. Accordingly, it was seen that 100-250 mg/L concentrations of Cu NPs/Dt have the potential to prevent breaks in DNA.

Conclusions

It was determined that copper nanoparticles have an inhibitory effect against some pathogenic microorganisms. Moreover, it was determined that it formed zones more effective against some pathogen bacteria than the antibiotic used. The number of bacteria that cause antibiotic resistance is constantly increasing. Due to the antimicrobial effect of Cu NPs/Dt, it is thought that it can be developed as a bio agent for the treatment of some diseases. The antioxidant capacity of copper nanoclusters has shown very important results. In the study, it was observed that as the concentration of extraction increased, the radical quenching activity increased. Various breaks and

damages in DNA can cause mutation. Some diseases, especially cancer, occur as a result of these situations. The agents used in the treatment of these diseases damage healthy cells and disrupt the DNA chain. In this study, it was observed that Cu NPs/Dt had an anti-damage effect on pBR322 plasmid DNA and its protective effect increased with the increase of nanoparticle concentration.

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Conflict of Interest

The authors declare no conflict of interest.

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