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Utilization of mistletoe for the synthesis of copper nanoparticles and its clinical applications

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ABSTRACT

Nanoparticles are substances with dimensions smaller than 100 nm. Nowadays, nanoparticles can be synthesized by many methods. Copper nanoparticles (CuNPs) were made using the green synthesis approach, using aqueous extracts of the pine mistletoe plant (*Viscum album* ssp. *austriacum*) and $CuSO_4$ metal salt. The formation of CuNPs was determined both by observing the color change and by the UV-vis method. Especially peaks were observed around the leaf (567 nm), fruit (560 nm), and branch (565 nm). Specific functional groups involved in the formation of CuNPs and the reduction of Cu^0 were determined by FT-IR spectroscopy. In addition, SEM and EDS analyses of the synthesized CuNPs show that they are nanosized and their average size is less than 100 nm. In particular, it was determined that the size of fruit-derived CuNPs was the smallest (between 23.21 and 54.63 nm), and all synthesized CuNPs were spherical in shape. In addition, the antioxidant capacities of these plant extracts and CuNPs synthesized from them were investigated. For this purpose, DPPH$^\bullet$ and $ABTS^{\bullet+}$ radical scavenging activities of the samples were determined. The DPPH$^\bullet$ radical IC_{50} value of CuNPs obtained from the aqueous extract of fruit was determined as 151.41 µg/mL, and the $ABTS^{\bullet+}$ radical IC_{50} value was 160.43 µg/mL. The antioxidant results were compared with the standard ascorbic acid results. Furthermore, copper nanoparticles obtained from fruit extract were found to have the highest antioxidant activity. Furthermore, the antimicrobial activities of all samples were examined. In particular, the antiquorum activity of CuNPs synthesized from plant parts of *V. album* was determined for the first time. As a result, it was determined that the copper nanoparticles obtained from these plant parts had superior antioxidant and antimicrobial properties.

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1. Introduction

Substances with dimensions of 100 nm or less are called nanoparticles. Thanks to these features, they have an extraordinary structure and attract attention [1,2]. With these properties, nanomolecules have become widely used in the fight against microbes, diagnosis and treatment, prevention of diseases, purification of water and air, food production, cosmetics, and clothing sectors [3]. Many methods are used in the synthesis of nanoparticles. In particular, the green synthesis method, which is less costly and harmless to the environment, is frequently used. In this sense, green plant extracts are used [4]. In addition, the method of synthesis of nanoparticles from plant extracts is highly preferred due to its ability to produce large amounts of nanoparticles and control production processes [5,6].

Copper and silver have been used extensively in medicine for many years to heal burns and infections [7,8]. Because of their beneficial redox characteristics, copper(II) compounds have been proposed as antibacterial, antioxidant, and anticancer chemicals. They are regarded as one of the most promising anticancer drugs, second only to cisplatin [9,10]. The creation of metal-based medications from transition metal complexes is also heavily dependent on copper complexes. Numerous oxidative metabolism-related enzymes, including superoxide dismutase and ceruloplasmin, require copper as a cofactor in order to carry out fundamental biological processes that are essential for cell growth and development [11]. Copper compounds also function as antifungal, antibacterial, and therapeutic agents for leishmaniasis, diabetes, amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, inflammatory disorders, skin lesions, and cardiovascular problems [12]. In fact, in a study, the antibacterial properties of copper nanoparticles (CuNPs) were compared with triclosan and it was determined that both showed strong antibacterial effects [13].

Mistletoe (*V. album* ssp. *austriacum*) genus belongs to the Loranthaceae family and is an evergreen plant with pea-sized, bright, white, and slippery inside fruits. It does not shed its leaves; The fruits are pea- or chickpea-sized, shiny, white in color, slippery, and sticky inside. Although this sticky white substance is poisonous to humans, it is consumed by birds because its fruits are fleshy and soft, and does not harm birds [14]. The chlorophyllous plant that clings to the branches and trunks of trees as a semiparasite is called mistletoe. Mistletoe is rich in bioactive compounds that it obtains from plants because

Figure 1. Appearance of pine mistletoe.

it lives as a parasite on plants. It has been used especially for medical purposes since ancient times. Its medicinal properties vary depending on the plant species [15]. Research conducted on *V. album* ssp. *austriacum* species is a semiparasitic plant that lives on the branches and trunks of pine trees. It has been determined that this plant, in particular, has positive effects against cancer. It has also been reported to have anti-inflammatory, hypotensive, antipsychotic, and anti-diabetic properties. Studies have reported that *V. album* ssp. *austriacum* extracts prevent the mutagenic and harmful effects of radiation therapy and chemotherapy [16,17]. This study aims to investigate the synthesis, analysis, and *in vitro* clinical use (antioxidant capacity and antibacterial activity) of copper nanoparticles synthesized from aqueous extracts of the mistletoe plant.

2. Experimental

2.1. Material and methods

The pine mistletoe plant (*V. album* ssp. *austriacum*) (Figure 1) was collected from the gardens in Kırsehir center in October. Impurities were removed from the fresh plant collected by washing it with tap water. Then it was rinsed with distilled water and filtered. The samples were dried in the shade and under room conditions for two weeks. The leaves, branches, and fruits of the dried mistletoe were ground to powder using a grinder.

2.2. Preparation of plant extracts

Ten grams of powdered plant material were placed in beakers. After adding 100 mL of distilled water, the samples were boiled for 30 minutes at 80 °C. After being cooled to room temperature, the samples were filtered using filter paper [18].

2.3. Green synthesis of copper nanoparticles (CuNPs)

A 10 mM copper sulfate solution ($CuSO_4 \cdot 5H_2O$) (Merck) was prepared with distilled water. 10 mL of the obtained plant extracts were taken and slowly added to 10 mM 90 mL $CuSO_4 \cdot 5H_2O$ solution. The mixture was stirred on a magnetic stirrer for 45 minutes at room temperature. A color change was observed, indicating the formation of copper nanoparticles [19]. The samples were centrifuged at 4.500 rpm for half an hour. The nanoparticles in the samples were precipitated, and the supernatant was removed. After three rounds of washing with distilled water, the nanoparticles were placed in Eppendorf tubes. After the solid nanoparticles were dried in an oven at 50 °C, they were weighed on a precision scale.

2.4. Characterization of copper nanoparticles (CuNPs)

The characterization of copper nanoparticles (CuNPs) synthesized by green synthesis was performed by ultraviolet-visible spectroscopy (UV-vis). This method is an important and reliable method used to determine the properties of surface plasmon resonance (SPR) of nanoparticles [20]. CuNPs were synthesized from different parts of the *V. album* ssp. *austriacum* plant. The formation of CuNPs via green synthesis was monitored by absorbance measurements at 300-800 nm on a Shimadzu UV-1800 model spectrophotometer. The FTIR approach is often used to determine the average characteristics of molecular species on nanoparticle surfaces [21]. Using FT-IR spectroscopy, the functional groups of leaf, fruit, and branch extracts of *V. album* ssp. *austriacum* plant and CuNPs obtained from these extracts were determined. All materials were examined using an FT-IR instrument ranging from 4000-500 cm^{-1}. Scanning electron microscopy (SEM) is an imaging technique that directly visualizes the size, surface, and form morphologies of nanoparticles [22]. The most important method used, especially for the analysis of the elemental composition in solid samples, is energy-dispersive X-ray spectroscopy (EDS). It offers comprehensive details on the principles of nanoparticles [23]. The existence of a copper element in CuNPs was found using SEM-EDS (Jeol JSM 6390; scanning electron microscopy-energy dispersive X-ray spectroscopy).

2.5. Determination of the antioxidant capacity of plant extracts and copper nanoparticles

2.5.1. Radical scavenging activity with DPPH• (1,1-Diphenyl-2-picrylhydrazil)

This method is based on the detection of the DPPH• radical, which is a free radical, by the antioxidant substance and the determination of its purple color by measuring it on a spectrophotometer [24]. The lightening of the purple color of the radical indicates the presence of antioxidant activity. The samples were prepared from plant extracts, copper nanoparticles and a reference chemical (ascorbic acid) at three different concentrations (25, 50, and 100 µg/mL). 1 mL of these samples was combined with 4 mL of 0.1 mM DPPH solution.

After the vortexing process, all samples were left for a half hour at room temperature in a dark location. The absorbance values of the samples were recorded with a spectrophotometer at a wavelength of 517 nm. The DPPH• radical scavenging capabilities of the samples were determined using Equation 1:

$$\text{DPPH} \bullet \text{ Radical Scav. Act. (\% Inhib.)} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100 \quad (1)$$

The value of IC_{50} is the amount of antioxidant material needed to eliminate 50% of the concentration of DPPH radicals in samples [25,26]. Inhibition-concentration graphs of the mixtures prepared at three different concentrations for each sample were drawn. The IC_{50} values were calculated using the correct equation shown on the graphs.

2.5.2. ABTS•+ [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging activity

Another method by which the antioxidant activity of the samples is measured is the ABTS•+ radical scavenging activity [27]. Pure water was used to create a 2.45 mM $Na_2S_2O_8$ solution and a 7 mM ABTS solution. To produce the radical solution, the prepared solutions were mixed in a mixer for 16 hours in a 1:0.5 ratio. The prepared radical solution was wrapped in aluminum foil to protect it from light. Using the prepared ethanol-water (80%) mixture, the absorbance of the radical was adjusted to be 0.7 at the wavelength of 734 nm. Samples of plant extracts, copper nanoparticles, and standard substance were prepared at three different concentrations. 50 µL of each sample was collected and 2 mL of radical solution was added. The absorbance of the samples kept in the dark for 30 minutes was determined at 734 nm. Equation 2 was used to determine the inhibition values. The IC_{50} values were computed and contrasted with the reference values.

$$\text{ABTS} \bullet + \text{ Radical Scav. Act. (\% Inhib.)} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100 \quad (2)$$

2.6. Determination of the antimicrobial activity of plant extracts and copper nanoparticles

2.6.1. Antimicrobial activity

The agar-well diffusion method was used to perform an *in vitro* antimicrobial activity assay. Using Tripticase soy agar (TSA), the agar well diffusion method was used to test the antimicrobial activity of CuNPs. 15 mL of TSA was prepared and poured into sterile Petri plates, where it solidified for 5 minutes. At a concentration of 1×10^5 to 1×10^6 cfu/mL, 10 distinct bacterial pathogenic microorganisms and a yeast (*Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (709 Rome), *Bacillus subtilis* (ATCC 6633), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Aeromonas hydrophila* (ATCC 7966), *Pseudomonas aeruginosa* (ATCC 27853), *Vibrio anguillarum* (ATCC 43312), *Klebsiella pneumoniae* (ATCC 13883) and *Candida albicans* (ATCC 90028) were spread uniformly on the surface of a Tripticase soy agar (TSA) plate. After sowing, the plates were opened with 6 mm diameter wells, 3 mM CuNPs was added to 70 µL wells and after 24-48 hours of incubation, the zone diameters around the wells were measured in mm and the effect levels of CuNPs were determined. After the incubation period, the inhibition zones formed around the disc were measured using a high antibiotic zone scale. Additionally, antibiotic discs of ampicillin (10 µg) and Nystatin (200 µg) antibiotic discs were used as positive controls [28]. All tests were performed in triplicate.

2.6.2. Minimal inhibition concentration (MIC)

Minimum inhibition concentrations of CuNPs synthesized green by *V. album* ssp. *austriacum* were determined using sterile 96-well microplates. To carry out the study, the solutions taken from the cultures of 10 microorganisms in TSB were prepared with the McFarland 0.5 turbidity test as 1×10^6 cells/mL. The concentrations determined for silver nanoparticles with green synthesis are; 2, 4, 8, 16, 32, 64, 128, and 256 µL/mL. The prepared microplates were incubated in an oven at 37 °C for 24 hours. Plates taken after 24 hours were analysed in a spectrophotometer at 600 nm [29]. In the study, the operations with CuNPs were carried out in triplicate.

2.6.3. Antiquorum sensing analysis

In this study, antiquorum sensing (Anti-QS) analysis; carried out using macroscopic methods. CuNPs' suppression of the violacein pigment suggests that nanocolloids possess quorum quenching capabilities. In the macroscopic method, agar well diffusion method was used and *Chromobacterium violaceum* (ATCC 12472) was planted on the plates formed with Trypticase soy agar (TSA) and the zone diameters formed by CuNPs were calculated [30].

2.7. Statistical analysis

The number of samples for the parameters examined was determined to be three, and the means and standard deviations of the results were calculated using the Microsoft Excel program. The results of the study were expressed in tables and figures. A comparison of the antioxidant capacity results of the samples in the study with the standard ascorbic acid results was made with the single-sample Wilcoxon signed rank test. The results' significance level was expressed as $p < 0.05$.

3. Results and discussion

3.1. Green synthesis of copper nanoparticles from aqueous extracts of V. album ssp. austriacum plant parts

V. album ssp. *austriacum* plant sections were weighed and extracted with water solvent. The extracts were filtered. A 10 mM copper sulfate solution ($CuSO_4{\cdot}5H_2O$) (Merck) was prepared with distilled water. Then 10 mL of the plant samples was taken and mixed with 90 mL of $CuSO_4{\cdot}5H_2O$ (10 mM) solution [19]. The mixtures were wrapped in aluminum foil and mixed on a magnetic stirrer for 45 minutes at room temperature. Then, to obtain copper nanoparticles, the samples were placed in test tubes and centrifuged at 5.000 rpm for 30 minutes. The liquids in the tubes were discarded. The resulting nanoparticles were washed 2 or 3 times with pure water and centrifuged to purify them from plant extracts. The obtained CuNPs were placed in Eppendorf tubes. It was dried in an oven at 40 °C. The copper nanoparticles were ground to powder and kept in the refrigerator at +4 °C, wrapped in aluminium foil, until the day they were analysed.

3.2. The UV-Vis results of V. album ssp. austriacum leaf, fruit, branch parts, and copper nanoparticles

A reliable method to determine the surface plasmon resonance capabilities of nanoparticles is the UV-vis spectrometry method [20]. One of the most important techniques that demonstrate the formation of nanoparticles is the ultraviolet-visible (UV-vis) method. The surface plasmon bands seen in this wavelength range indicate the presence of copper nanoparticles. The UV-vis spectra of the plant extracts and their copper nanoparticles (CuNPs) in this study are shown in Figure 2. The differences in absorbances may have differed because they were different parts of the same plant. Extracts were obtained by weighing 10 grams of plant parts and extracting them with water. These are the absorbance values of the nanoparticle samples formed after adding $CuSO_4$ to the extracts.

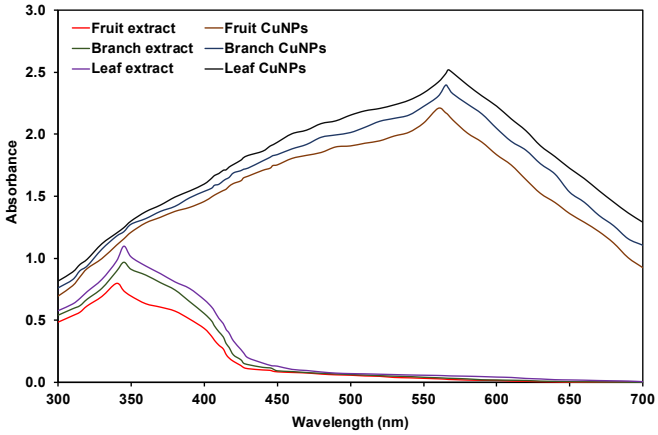

Figure 2. UV-vis spectrum of plant part extracts and copper nanoparticles.

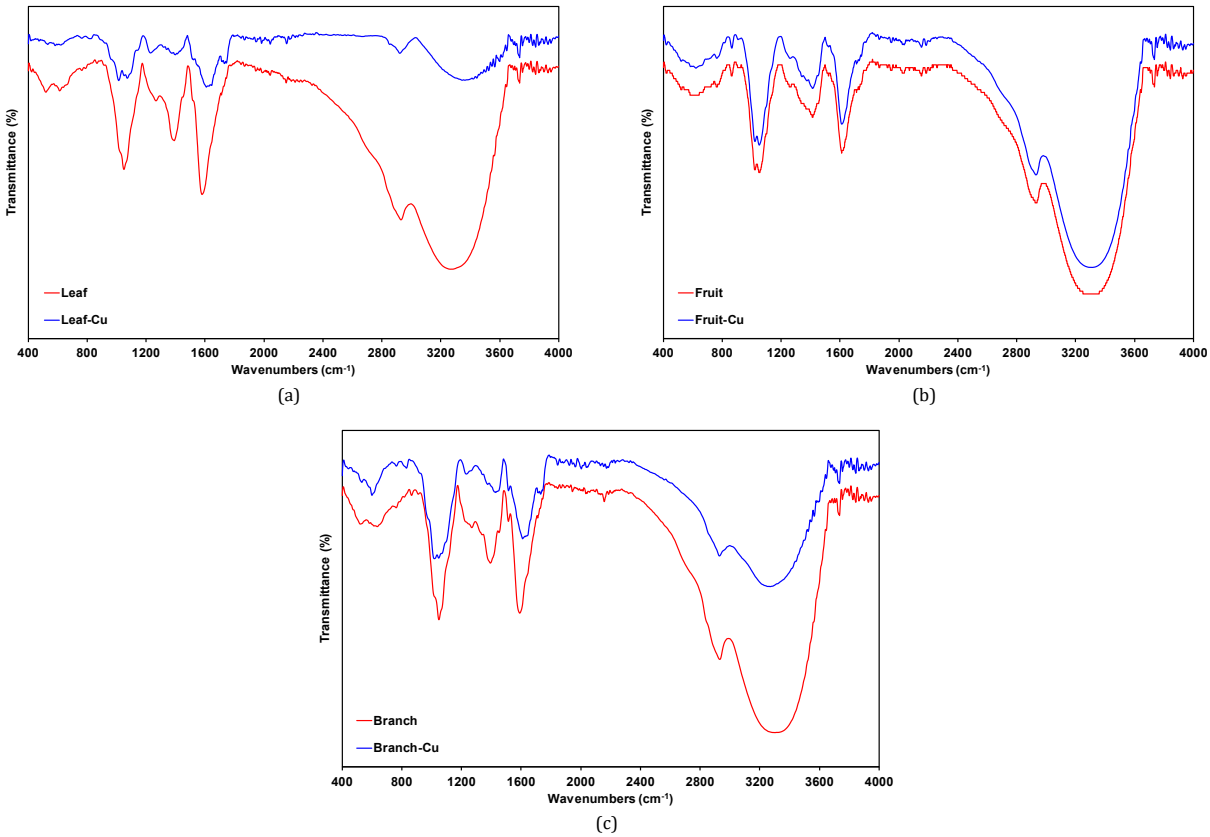

Figure 3. FT-IR spectra of (a) leaf extract and leaf CuNPs; (b) fruit extract and fruit CuNPs; and (c) branch extract and branch CuNPs.

It was determined that the absorption peaks of the leaf, fruit, and branch parts of the *V. album* ssp. *austriacum* plant were 345, 340, and 345 nm, respectively. The copper nanoparticles of the leaf, fruit, and branch of this plant were determined to be 567, 560, and 565 nm, respectively. Furthermore, when plant extracts were mixed with a $CuSO_4$ solution, it was observed that fruit copper nanoparticles formed in a light green color, while leaf and branch CuNP) were formed in a brown-dark green mixture color. The characteristic UV-specific absorption peak of CuNPs synthesized from *Eclipta prostrata* leaf extract was observed at 565 nm. This observed peak has been reported to be the surface plasmon band of Cu colloids of unoxidized CuNP [18]. In this study, it was determined that the aqueous extracts of the three parts of the plant showed maximum absorption peaks between 340-345 nm, and the copper nanoparticles synthesized from these extracts showed maximum absorption peaks between 560-567 nm. In general, nanosized copper particles typically exhibit a surface plasmon peaking around 556 to 580 nm [31]. The color changes and UV absorption peaks observed over time in CuNP samples synthesized from plant extracts confirmed the formation of CuNPs (Figure 2).

3.3. FT-IR results of V. album ssp. austriacum plant parts and copper nanoparticles

Fourier transform infrared spectroscopy (FTIR) is used to identify organic, inorganic, and polymeric samples of materials using infrared light. It is a spectroscopy technique that obtains the absorption and emission infrared spectra of samples.

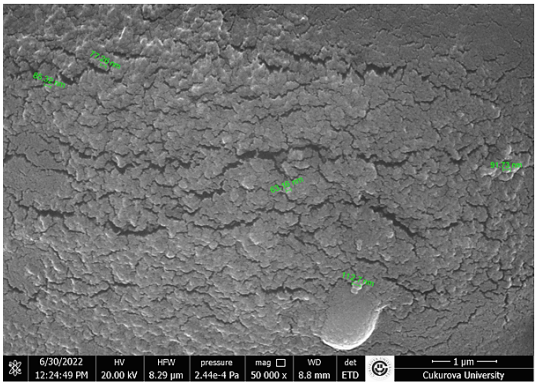

Figure 4. SEM image of leaf copper nanoparticles.

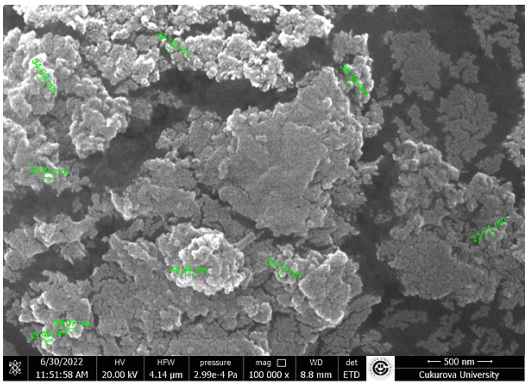

Figure 5. SEM image of fruit copper nanoparticles.

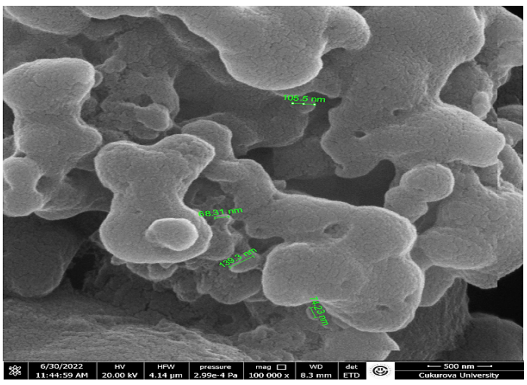

Figure 6. SEM image of branch copper nanoparticles.

Characteristic changes in absorption bands indicate changes in material composition [32,33]. The FTIR results of the plant part extracts and the copper nanoparticles (CuNPs) synthesized from these extracts are shown in Figure 3.

The FTIR diagrams of pine mistletoe, leaf, fruit, and branch extracts showed characteristic absorption peaks corresponding to OH and C-H stretching and NH intramolecular hydrogen bonding. Although the peaks of the leaf extract have been observed at 3266, 2929, 1579, 1390, 1049, and 518 cm^{-1}, the peaks of the leaf CuNPs have been found at 3345, 2921, 1730, 1608, 1398, 1229, 1072 and 1013 cm^{-1}. While the peaks of the fruit extract (3312, 2930, 1611, 1412, 1050, and 620 cm^{-1}) were observed, the peaks of fruit CuNPs (3265, 2918, 2153, 1627, 1429, 1019, and 596 cm^{-1}) were determined. Furthermore, peaks of branch extract (3301, 2931, 1589, 1392, 1048, and 633 cm^{-1}) were observed, while the peaks of branch CuNPs (3268, 2929, 1730, 1609, 1429, 1231, 1017, and 599 cm^{-1}) were determined. Phenolic compounds, especially those found in plant extracts, function to reduce copper ions [34]. The characteristic peak seen at 3200 and 2800 cm^{-1} is attributed to methylene stretching with the -OH group in the structure of the phenolic molecules. The peaks at 1600 to 1400 cm^{-1} are attributed to primary amine carbonyl group stretching mode and C=C stretching mode of the aromatic ring. The peaks between 1200 and 800 cm^{-1} may be due to C-OH stretching in the alcohol group or -C-N stretching in the structure of amines. In particular, the characteristic peak at 700-750 cm^{-1} may be caused by the interactions of the copper element with biomolecules in the structure of plant extracts [35-37]. When the FTIR spectra of plant extracts and copper nanoparticles are compared, it appears that some peaks have shifted or disappeared. FTIR analysis also confirmed this information and the formation of copper nanoparticles.

Table 1. IC_{50} values (DPPH• and $ABTS^{•+}$ radicals scavenging activities) of *V. album* ssp. *austriacum* plant part extracts, CuNPs and standard ascorbic acid.

Samples	IC_{50} (µg/mL)	
	DPPH•	$ABTS^{•+}$
Leaf extract	169.95	173.46
Leaf CuNPs	160.52	162.45
Fruit extract	157.94	163.23
Fruit CuNPs	151.41	160.43
Branch extract	172.14	176.39
Branch CuNPs	171.89	175.47
Standard (Ascorbic acid)	14.34	20.39

3.4. SEM results of V. album ssp. austriacum plant parts and copper nanoparticles

Scanning electron microscopy (SEM) is one of the leading tools used to examine and analyze the morphology and chemical characterization of nanosized structures [38]. SEM can identify and analyze surface fractures, examine surface contaminations, reveal spatial changes in chemical compositions, and determine crystal structures [39]. SEM images of copper nanoparticles (CuNPs) obtained from *V. album* ssp. *austriacum* plant parts are shown in Figures 4-6. The sizes of copper nanoparticles obtained from *V. album* ssp. *austriacum* leaf aqueous extract are: They varied between 63.16 and 112.70 nm. They were determined to be nano-sized and spherical in shape (Figure 4). The sizes of the copper nanoparticles obtained from the aqueous extract of the fruit were determined to be between 23.21 and 54.63 nm and spherical in shape (Figure 5). The sizes of copper nanoparticles obtained from the aqueous extract of *V. album* ssp. *austriacum* branch extract are: it was observed that they varied between 68.31 and 139.30 nm. They were determined to be nano-sized and spherical in shape (Figure 6). In the study, it was determined that the copper nanoparticle sizes obtained from the fruit aqueous extract were smaller than the nanoparticle sizes obtained from other leaf and branch extracts. Furthermore, it was observed that these copper nanoparticles obtained from the aqueous extracts of the leaves, fruits, and branches of this plant were spherical in shape (Figures 4-6).

3.5. Energy dispersive X-ray spectroscopy (EDS) results

It is used together with EDS to determine the compositional properties and orientation of the samples [40]. EDS is an important method to analyze and identify elements in the sample [41]. EDS is a fundamental analysis technique widely applied in a wide range of biological sciences, engineering, technology, and forensic research, based on the generation of characteristics [42]. The EDS results of CuNPs synthesized from *V. album* ssp. *austriacum* plant parts are shown in Figures 7-9. From the EDS analysis of CuNPs obtained from leaf extract, it was determined that 20% copper nanoparticles were formed. Furthermore, EDS peaks of C (36%), O (29%), and S (15%) from leaf extract were observed (Figure 7). From the EDS analysis of CuNPs synthesized from aqueous fruit extract, it was determined that 23% copper nanoparticles were formed. Especially elemental EDS peaks C (22%), O (26%), and S (30%) from the fruit extract were observed (Figure 8). Taking into account the EDS analysis of the copper nanoparticles synthesized from branch aqueous extract, it was observed that 19% CuNPs were formed. Furthermore, EDS peaks of elements C (40%), O (25%), and S (16%) were observed from the aqueous branch extract (Figure 9).

3.6. Antioxidant capacity results of samples

In the study, two different radicals were used to determine the antioxidant capacities of *V. album* ssp. *austriacum* plant extracts and copper nanoparticles. For this purpose, samples of all samples were prepared at concentrations of 25, 50, and 100 µg/mL. These radical scavenging activities of the samples were examined. For this purpose, the percentages of inhibition of these radicals and the IC_{50} values of the samples were determined. The percentage of inhibition results of the samples in the study against DPPH• and $ABTS^{•+}$ radicals is shown (Figure 10).

Antioxidant capacity analyzes frequently use the DPPH• radical. The reason for this is that this radical has the ability to react with antioxidants that are very weak, lipophilic, and hydrophilic. For this reason, it is highly preferred in antioxidant capacity analyses [43]. Another radical used in antioxidant capacity analyzes is the $ABTS^{•+}$ radical. This radical can also dissolve in both inorganic and aqueous environments. Therefore, it is used in the antioxidant analysis of lipophilic and hydrophilic substances. It cannot be prepared as easily as the DPPH• radical [44]. DPPH• radical inhibition percentages of samples at 100 µg/mL concentrations were obtained as: standard ascorbic acid (83.25), fruit CuNPs (38.25), fruit extract (36.93), leaf CuNPs (36.16), branch CuNPs (34.47), leaf extract (34.13), and branch extract (33.51). $ABTS^{•+}$ radical inhibition percentages were determined as ascorbic acid (79.95), fruit CuNPs (36.99), fruit extract (36.11), leaf CuNPs (35.12), leaf extract (33.11), branch CuNPs (32.62), and branch extract (32.16) ($p < 0.05$). The IC_{50} value (inhibitory concentration), which indicates antiradical activity, is expressed as the amount of antioxidant substance required to reduce the initial radical concentration by 50% [45]. Additionally, in this study, DPPH• and $ABTS^{•+}$ radical inhibition percentages as well as IC_{50} values of plant part extracts and CuNPs synthesized from these extracts were calculated. The results are shown in Table 1. A lower IC_{50} value indicates greater antioxidant activity. The DPPH• radical IC_{50} values of the samples were determined as standard ascorbic acid (14.34 µg/mL) > fruit CuNPs (151.41 µg/mL) > fruit extract (157.94 µg/mL) > leaf CuNPs (160.52 µg/mL) > leaf extract (169.95 µg/mL) > branch CuNPs (171.89 µg/mL) > branch extract (172.14 µg/mL) ($p < 0.05$). Again, the $ABTS^{•+}$ radical IC_{50} values of the samples are, respectively, standard ascorbic acid (20.39 µg/mL) > fruit CuNPs (160.43 µg/mL) > leaf CuNPs (162.45 µg/mL) > fruit extract (163.23 µg/mL) > leaf extract (173.46 µg/mL) > branch CuNPs (175.47 µg/mL) > branch extract (176.39 µg/mL), determined ($p < 0.05$).

In the literature review, no copper nanoparticle synthesis was found from Viscum species extracts using the green synthesis method. There are many studies on the synthesis of CuNPs from different plant extracts and the antioxidant capacities of these nanoparticles. In a study in which copper nanoparticles were synthesized using the *Anonnaceae squamosa* plant, the antioxidant capacity of CuNPs was determined using the DPPH• radical. As a result of the study, the IC_{50} value of CuNPs was determined to be 257.05 µg/mL [46]. Again, the antioxidant capacity of CuNPs synthesized from *Athrixia phylicoides* DC plant leaf extract was examined, and the IC_{50} value of the copper nanoparticle was reported to be 10.68±0.03 µg/mL [47]. In a study, copper nanoparticles were synthesized using *Impatiens chinensis* L. plant leaf extract, and the ABTS radical scavenging activity was investigated in antioxidant capacity analyses. As a result, the ABTS•+ radical IC_{50} value of CuNP obtained from this plant was determined to

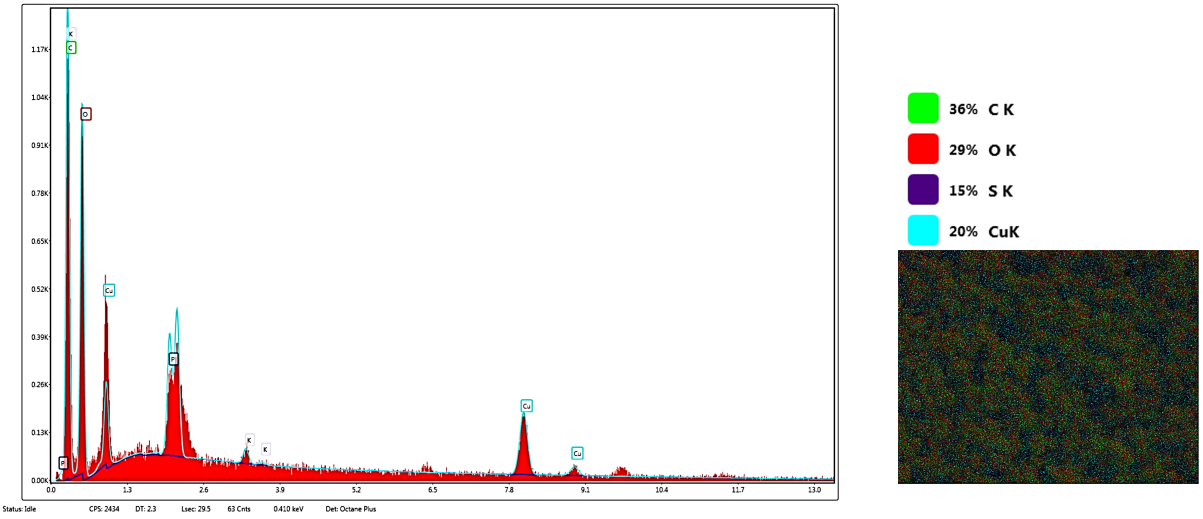

Figure 7. EDS results of leaf copper nanoparticles.

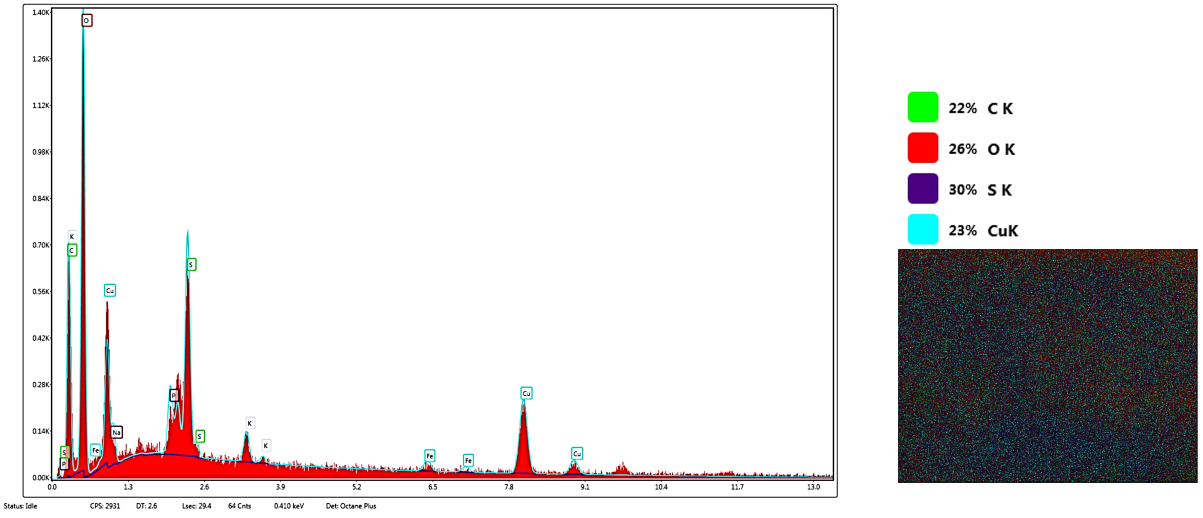

Figure 8. EDS results of fruit copper nanoparticles.

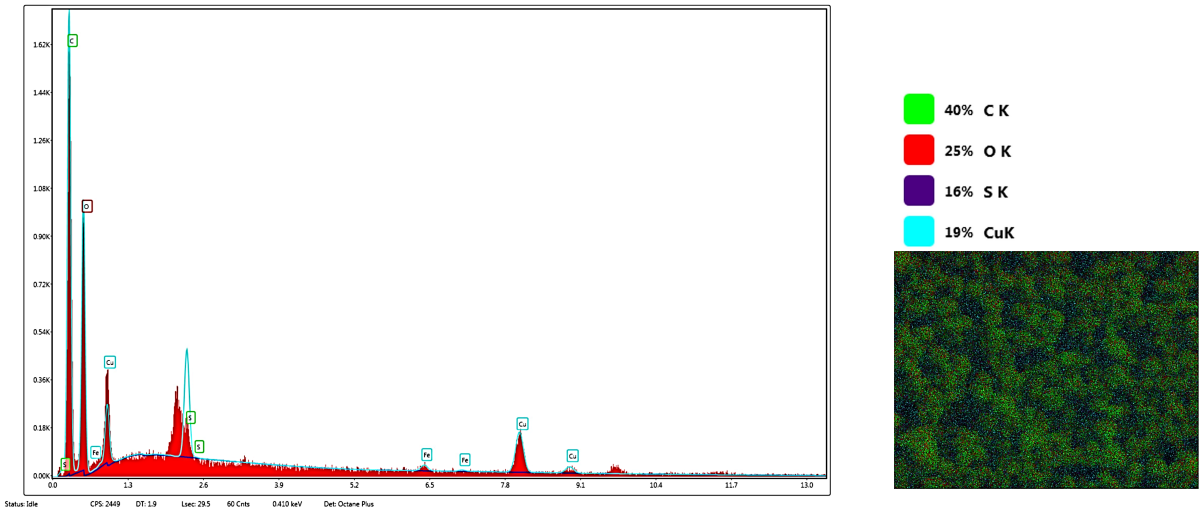

Figure 9. EDS results of branch copper nanoparticles.

Table 2. The diameters of the antimicrobial activity zone (mm) of the copper nanoparticles synthesized by *V. album* ssp. *austriacum* (mm).

Microorganisms	Leaf (CuNPs)	Fruit (CuNPs)	Branch (CuNPs)	*V. album* extract (mm)			Control			
				Leaf	Fruit	Branch	Ampicillin 10 µg	Nystatin 200 µg	$CuSO_4$ 10 mM	Distilled water
S. aureus (ATCC 25923)	15	16	13	12	14	10	18	_	6	_
B. cereus (709 Roma)	_	12	8	_	13	15	17	_	6	_
B. subtilis (ATCC6633)	_	16	_	_	_	_	_	_	6	_
E. faecalis (ATCC29212)	14	22	18	13	12	13	15	_	5	_
E. coli (ATCC 25922)	_	15	14	12	14	_	15	_	6	_
A. hydrophila (ATCC7966)	14	15	8	13	_	_	_	_	7	_
P. aeruginosa (ATCC 27853)	_	25	8	_	_	13	_	_	6	_
V. anguillarum (ATCC 43312)	_	16	12	_	13	_	15	_	7	_
K. pneumoniae (ATCC 13883)	15	26	15	14	_	_	_	_	6	_
C. albicans (ATCC 90028)	15	8	13	_	_	_	_	18	5	_

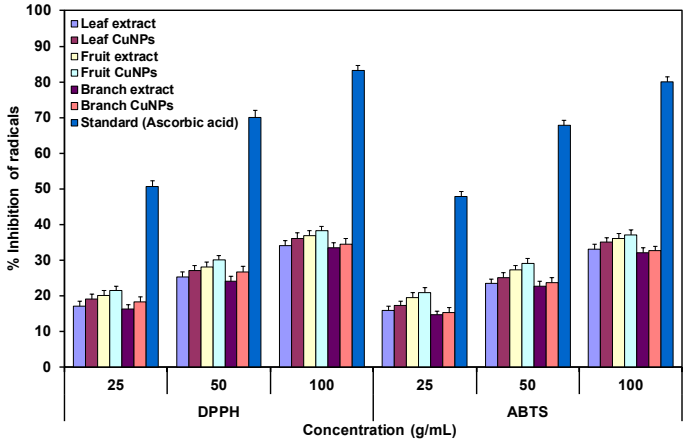

Figure 10. Inhibition percentages of plant parts and copper nanoparticles against DPPH and ABTS radicals.

be 85.23 µg/mL [48]. The results of this study are compatible with the literature. When the results of the study are compared with standard ascorbic acid, it can be said that both plant extracts and copper nanoparticles have antioxidant properties. In particular, it was determined that fruit extract and copper nanoparticles synthesized from this extract had the highest radical scavenging activities ($p < 0.05$).

3.7. Antimicrobial activity of copper nanoparticles

Within the scope of the study, the antimicrobial activities of CuNPs (10 mM) synthesized from *V. album* ssp. *austriacum* on pathogenic microorganisms were investigated. The diameters of the zones related to the antimicrobial activities of the synthesized CuNP are given in Table 2 in millimeters.

In the leaf of the *V. album* ssp. *austriacum,* copper nanoparticles were biosynthesized. These particles showed activity against *S. aureus* (15 mm), *E. faecalis* (14 mm), *A. hydrophila* (14 mm), *K. pneumoniae* (15 mm), and *C. albicans* (15 mm), but not against *B. cereus, B. subtilis, E. coli, P. aeruginosa,* and *V. anguillarum.* Copper nanoparticles biosynthesized from the fruit of *V. album* ssp. *austriacum,* it exhibited good antimicrobial activity against *E. faecalis, P. aeruginosa*, and *K. pneumoniae*. (22 mm, 25 mm and 26 mm, respectively). It was determined that copper nanoparticles synthesized from *V. album* fruit extract formed a zone against *E. faecalis* (22 mm), *P. aeruginosa* (25 mm), and *K. pneumoniae* (26 mm) microorganisms. In addition, it was also determined that copper nanoparticles synthesized from plant branch extract formed a zone against *S. aureus* (13 mm), *E. faecalis* (18 mm), *E. coli* (14 mm), *V. anguillarum* (12 mm), *K. pneumoniae* (15 mm), and *C. albicans* (13 mm). It was observed that nanoparticles synthesized from branch extract showed good antibacterial activity. Less activity against bacteria and yeast was observed in extracts (leaf, fruit, and branch) with pure water Table 2.

3.7.1. Minimal inhibition concentrations (MIC) of copper nanoparticles synthesized by V. album ssp. austriacum

Within the scope of the study; Minimum inhibition concentrations (MIC) of copper nanoparticles with antimicrobial activity are shown in Table 3. The MIC value of CuNPs was determined using 96 microplates containing nanoparticles in concentrations ranging from 2 to 256 µL/mL. It can be concluded that *P. aeruginosa and K. pneumoniae* are effective in the MIC evaluation of CuNPs synthesized from fruit at a concentration of 4 µL/mL. The branch analysis shows that *B. subtilis* is effective at a concentration of 4 µL/mL (Table 3). According to the antimicrobial activity results, copper nanoparticles are effective antimicrobial agents against pathogens. The potential antimicrobial activity of the synthesized copper nanoparticles could be attributed to the nanoparticles' large surface-to-volume ratio. In addition to being generally safe for humans, soluble copper compounds have been shown to have strong antibacterial action against a variety of microorganisms, including bacteria, fungi, algae, and

Table 3. Minimal inhibition concentrations of copper nanoparticles synthesized by *V. album* ssp. *austriacum* (µL/mL).

Microorganisms	Leaf (CuNPs)	Fruit (CuNPs)	Branch (CuNPs)	*V. album* extract			Control			
				Leaf	Fruit	Branch	Ampicillin 10 µg	Nystatin 200 µg	$CuSO_4$ 10 mM	Distilled water
S. aureus (ATCC 25923)	16			_	32	_				
B. cereus (709 Roma)	256	64	256	_	16	8	16	_	128	_
E. faecalis (ATCC29212)	_	32	_	32	64	16	32	_	128	_
B. subtilis (ATCC6633)	32	16	4	_	_	_	_	_	256	_
E. coli (ATCC 25922)	128	64	32	64	32	_	32	_	128	_
A. hydrophila (ATCC7966)	32	32	256	32	32	_	_	_	32	_
P. aeruginosa (ATCC 27853)	_	4	256	_	16	16	_	_	64	_
V. anguillarum (ATCC 43312)	256	16	32	_	_	_	32	_	128	_
K. pneumoniae ,(ATCC 13883)	16	4	16	16	_	_	_	_	256	_
C. albicans (ATCC 90028)	16	512	16	_	_	_	_	16	_	_

Table 4. Zone diameters of antiquorum sensing activities of CuNPs synthesized by *V. album* ssp. *austriacum*.

Concentration (µL/mL)	Copper nanoparticles			*V. album* extracts			$CuSO_4$
	Leaf	Fruit	Branch	Leaf	Fruit	Branch	
5	23	30	27	11	12	13	-
2.5	20	28	22	10	11	12	-
1.25	18	25	18	8	10	11	-
0.625	14	23	14	5	7	6	-

viruses [49]. According to several studies, copper appears to kill by producing reactive hydroxyl radicals that can oxidize proteins, cleave DNA and RNA molecules, and damage membranes due to lipid peroxidation [50,51]. Gram-negative *E. coli,* Gram-positive *S. aureus*, and yeast *S. cerevisiae* are the most typical microorganisms used in copper nanoparticle bioactivity tests to assess the biological activity of antimicrobial drugs. However, it should be emphasized that Cu-nanoantimicrobial capabilities have frequently been investigated on a variety of different species with an eye toward practical applications [52]. Cu nanoantimicrobials have been shown to efficiently kill or severely inhibit the growth of all known types of microorganisms.

3.7.2. Antiquorum sensing analysis results

Zone diameters of the copper nanoparticles made with the anti-majority detection activity (Anti-QS) agar well diffusion method was calculated in mm (Table 4). The loss of pigment in *C. violaceum* bacteria is an indication that the applied material (CuNPs) causes Quorum-Sensing (QS) inhibition. As stated in Table 4; it was determined that all synthesized copper nanoparticles were effective in the agar diffusion method for anti-QS. The inhibition zones formed were determined as the regions where the *C. violaceum* bacteria could not produce pigment. Copper nanoparticles inhibit the production of acyl homoserine lactone (AHL) by *C. violaceum,* which prevents the bacteria from coming together and therefore has anti-QS activity. The *C. violaceum* assay was carried out with different concentrations (5, 2.5, 1.25, 0.625 µL/mL) of CuNPs synthesized from *V. album* ssp. *austriacum* via disc diffusion using the bioreporter strain CV12472. The loss of purple pigment in CV 12472 cultures with exogenous AHL is indicative of quorum sensing inhibition by *V. album* ssp. *austriacum*. A clear halo zone of inhibition around the wells of varying diameter indicates that the quorum sensing inhibition effect was proportional to the amount of copper nanocolloids added. In this study, the antiquorum detection activity of CuNPs synthesized by *V. album* ssp. *austriacum* was published for the first time. Antiquorum sensing activities of CuNPs synthesized by *V. album* ssp. *austriacum* have not been directly reported in the reviewed literature. Although there are many antimicrobial studies on *V. album* ssp. *austriacum* in the literature, no study has been conducted on antiquorum sensing activity CuNPs synthesized by *V. album* ssp. *austriacum.* However, extracts of *Carica papaya* L., *Cocos nucifera* L., *Balanites aegyptiaca* and *Terminalia macroptera* have shown potential to treat microbial infections by inhibiting QS [53,54].

4. Conclusions

Plant parts (leaf, fruit, and branch) were used to synthesize CuNPs under room conditions. The absorption peaks of CuNPs in the leaf, fruit, and branch of the plant were detected at 567, 560, and 565 nm, respectively. SEM analysis was used to determine the morphology and size of the particle. It was determined that the copper nanoparticles obtained from fruit aqueous extracts were smaller and larger in quantity than the other nanoparticles obtained. The copper nanoparticles obtained from all three plant extracts were determined to be spherical in shape. FTIR studies revealed that copper nanoparticles were biologically produced by the action of various phytochemicals with various functional groups present in the extract. Nowadays, especially when leaf and branch extracts of *V. album* ssp. *austriacum* species are widely used, and it can be said that fruit extracts can also be used successfully in nanoparticle synthesis. In addition, copper nanoparticles obtained from this plant fruit extract have therapeutic potential in the treatment of free radical-induced diseases. It was also observed that biosynthesized copper nanoparticles had a potential antibacterial effect against infections. Furthermore, this study includes for the first time, the synthesis, characterization and antimicrobial research of CuNPs from all three parts of the plant: the leaf, fruit, and branch parts. In this respect, it is an innovative work. As a result of this study, it can be said that the copper nanoparticles were obtained from *V. album* ssp. *austriacum* can be used as an effective antibacterial agent in the food, textile, and medical industries. It is thought that nanomaterials synthesized by the green synthesis method will contribute to the literature on

plants. The results of the study will contribute to the fields of nanotechnology and nanomedicine.

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Disclosure statement

Conflict of interest: The authors declare that they have no conflict of interest.
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Sample availability: Samples of the compounds are available from the author.

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