Avrupa Bilim ve Teknoloji Dergisi
Özel Sayı 36, S. 139-142, Mayıs 2022

Araştırma Makalesi

www.ejosat.com ISSN:2148-2683

European Journal of Science and Technology
Special Issue 36, pp. 139-142, May 2022

Research Article

Antibacterial Activity of Copper Nanoparticles Synthesized by Using *Peumus boldus* Leaf Extract

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(1st International Conference on Engineering and Applied Natural Sciences ICEANS 2022, May 10-13, 2022)

(**DOI:** 10.31590/ejosat.1110891)

ATIF/REFERENCE: Kamçı, H., Taş. R. & Çelebioğlu, H. U. (2022). Antibacterial Activity of Copper Nanoparticles Synthesized by Using Peumus boldus Leaf Extract. *European Journal of Science and Technology*, (36), 139-142.

Abstract

The green synthesis method, which is one of the methods of obtaining nanoparticles; It is a preferred method because it is simpler, environmentally friendly, economical and turns into a product in a short time. In this study, it was aimed to investigate CuNp synthesis possibilities and antimicrobial activity of this Np by using the extract of *Peumus boldus*, an endemic plant. The synthesized CuNps were characterized by scanning electron microscopy (SEM), Fourier transform infrared spectrophotometer (FTIR) and X-ray diffraction (XRD). It was determined that the NPs synthesis was successful, and the overall geometry was spherical; besides, the diameters were less than 50 nm. The antibacterial activity of CuNps was tested by the broth dilution method. It was observed that CuNps synthesized with *Peumus boldus* extract used at 125 to 250 µgr/ml concentrations suppressed the viability of *E. coli* and *S. aureus* by 60%.

Keywords: Biogenic synthesis, CuNps, *Peumus boldus*, Antibacterial activity.

Peumus boldus Yaprağı Ekstresi Kullanılarak Sentezlenen Bakır Nanopartiküllerin Antibakteriyel Aktivitesi

Öz

Nanoparçacık elde etme yöntemlerinden biri olan yeşil sentez yöntemi, daha basit, çevreci, ekonomik olması ve kısa sürede ürüne dönüşmesi nedeniyle tercih edilen bir yöntemdir. Bu çalışmada, endemik bir bitki olan *Peumus bolus*'un ekstresi kullanılarak bakır nanopartikül (CuNp) sentez olanaklarının ve antimikrobiyal aktivitesinin araştırılması amaçlanmıştır. Sentezlenen CuNp'ler, taramalı elektron mikroskobu (SEM), Fourier transform kızılötesi spektrofotometre (FTIR) ve X-ışını kırınımı (XRD) ile karakterize edilmiştir. Başarılı bir şelilde sentezlenen NP'lerin 50 nm den küçük çapta olduğu küre şeklinde olduğu belirlenmiştir. CuNp'lerin antibakteriyel aktivitesi, *broth seyreltme* yöntemiyle test edilmiştir. Sonuçta *Peumus boldus* ekstraktı ile senetzlenen CuNp'lerin 125 ila 250 µgr/ml konsantrasyonlarda kullanıldığında *E. coli* ve *S. aureus* canlılıklarını %60 oranında baskıladığı görülmüştür.

Anahtar Kelimeler: Bioyogenik sentez, CuNps, *Peumus boldus*, Antibakteriyel aktivite.

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1. Introduction

Although nanotechnology is a new and developing science that is carried out at the nanoscale level, it is developing especially in the fields of engineering, biotechnology and medicine. The products of nanotechnology are called nanoparticles (NPs) or nanomaterials, which are in the range of 10−9 nm and 1-100 nm in size. NPs are divided into three types: natural nanoparticles, anthropogenic nanoparticles, and engineered nanoparticles. The large surface/volume ratio of nanoparticles, their ability to interact easily with other particles, and many other properties have led to an increased interest in them in various fields. NPs are widely used in electronics, cosmetics, biomedical and biotechnological applications. The effective crystallographic and physicochemical properties of NPs make nanotechnology an excellent field to focus on (Buzea et al., 2007). Synthesis of NPs can be achieved by some physical and chemical methods. The traditional and widely used method for nanoparticle synthesis is the wet method. In chemical synthesis, nanoparticles are obtained by developing them in a liquid medium containing various reducing agents, especially sodium borohydride, potassium bitartartrate, methoxypolyethylene glycol or hydrazine (Kim et al., 2007). Some stabilizing agents such as sodium dodecyl benzyl sulfate or polyvinyl pyrrolidone are added at the reaction stage to prevent aggregation of metallic nanoparticles (Lee et al., 2008). The most widely used chemical methods are chemical reduction, electrochemical techniques, and photochemical reactions in reverse micelles (Taleb et al., 1997). Commonly used physical methods are attrition and pyrolysis. So far, some plant extracts, bacteria, fungi, enzymes and algae were used for the synthesis of NPs (Saifuddin et al., 2009). Nanoparticles of a wide variety of materials can be prepared by various methods. In general, production techniques fall into two categories: "bottom-up" and "top-down" approaches. The bottom-up approach is expressed in the "bottom-up", that is, the accumulation of an atomic-sized material. Molecule molecule or cluster cluster: nanolithography and nanomanipulation techniques are also examples of the bottom-up approach. The top-down approach starts with a block of bulk material and involves designing or grinding it to the desired shape. Both approaches play crucial roles in modern industry and most likely nanotechnology. Metal nanoparticles are obtained by various physical (physical vapor deposition, laser ablation and ion implantation, etc.), chemical (colloidal and can be synthesized with sol-gel) and biological (green synthesis using microorganisms, plant and fruit extracts) methods (Kathiravan et al., 2014). In this study, the synthesis of copper nanoparticles in accordance with the principles of green chemistry was carried out. In addition, the antibacterial properties of the obtained copper nanoparticles were also investigated.

2. Material and Method

2.1. Preparation of the Plant Extracts

In this study, a 2-necked 500 mL balloon was used to obtain the extract. Thermometer is fixed on one neck of the balloon with a back cooler on the other neck. A certain amount of ultrapure water was put into the balloon and the heating plate was operated. It was waited until the water in the balloon boiled, then 10 g of *Peumus boldus* and 100 ml of distilled water were added to it and left to boil for 5 minutes. The heating plate was then turned off and allowed to cool at room temperature. The extract obtained after cooling was first passed through ordinary filter paper, and then the filtrate was passed through blue band filter paper. The extract obtained was wrapped with aluminum foil and stored in closed polyethylene containers in the refrigerator.

2.2. Synthesis of Copper Nanoparticles

For the preparation of target copper nanoparticles, 20 mL of each plant extract, which was first cooled to room temperature, was added to 80 mL of 1 mM CuNO3 solution and incubated at room temperature for 45 min. mixed with time. In this process, a significant color change was observed in the solution, which is a phenomenon known in the literature and explained by the formation of copper nanoparticles (Asha, 2016). The resulting solution was stirred at 8,000 rpm for 150 min. The nanoparticles were precipitated by centrifugation throughout. Particles were collected in Eppendorf tubes and washed 3 times with distilled water. Finally, it was dried in an oven at 65 ºC and stored for characterization.

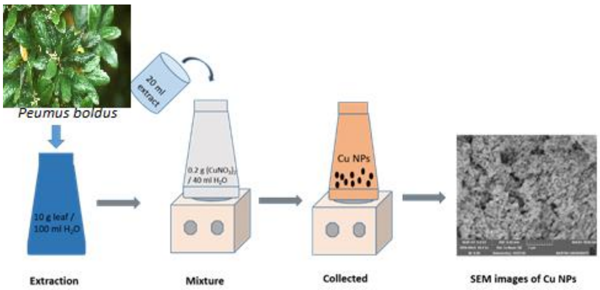

Figure 1. Schematic representation of copper nanoparticle synthesis.

2.3. Characterization of Copper Nanoparticles

Fourier transform infrared spectroscopy (FT-IR) was used to obtain information about the functional groups of Cu nanoparticles synthesized by biosynthesis method using Peumus boldus extract as reducing agent. In addition, X-ray diffractometry (XRD) was preferred to determine crystal structures in certain ranges. In addition, SEM images of nanoparticles were taken with Scanning Electron Microscope (SEM) and their surface morphologies were determined. The amounts of Cu in the structure were determined with the EDX (Energy Dispersive X-Ray Spectroscopy) spectrum.

2.4. Anti-bacterial Effects of Copper Nanoparticles

For investigation of antibacterial activities of the copper nanoparticles synthesized using *Peumus boldus*, Broth Micro-dilution Assay was used. Briefly, Gram-negative Escherichia coli and Gram-positive Staphylococcus aureus bacterial cultures were inoculated into Nutrient Broth (NB, 24 h, 37ºC) and prepared until 0.5 McFarland Unit. In total 200 µL in microtiter plate wells, 10 µL of bacterial cultures were inoculated with NB containing different concentrations of the nanoparticle suspended in glycerol (0-500 µg/mL). Negative controls were prepared using NB and glycerol without bacteria. On the other hand, positive controls did not contain nanoparticles, but a respective amount of glycerol. Absorbances of microtiter plates were measured at 600 nm using a microplate reader before (0th h) and after (24th h) the incubation at 37ºC. Bacterial viability was measured as a percentage of compound-treated bacterial groups to the positive control (bacterial viability of positive control was taken as 100%). One-way ANOVA with Tukey's test was used to analyze the data and $p<0.05$ was considered as statistically different.

3. Results and Discussion

3.1. Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR analysis performed to characterize the surface structure of CuNp is shown in Figure 2. Visible bands are lattice vibrational modes showing functional groups of biomolecules adsorbed on Nps. FTIR spectra of CuNps exhibited vibrations in the region of 700-1000 cm-1, which can be attributed to Cu vibrations confirming the formation of CuNps. Depending on the vibrations of Cu, an absorption band was observed at 729 cm-1. A dense and wide band of 3385 cm-1 appeared in the region corresponding to the stretching movements of the hydroxyl functional groups (Veisi et al., 2016). In the FTIR analysis for CuNp, peaks showing numerical values of 729, 1049, 1421, 1450, 1728, 2139, 2918 and 3385 cm-1 were observed.

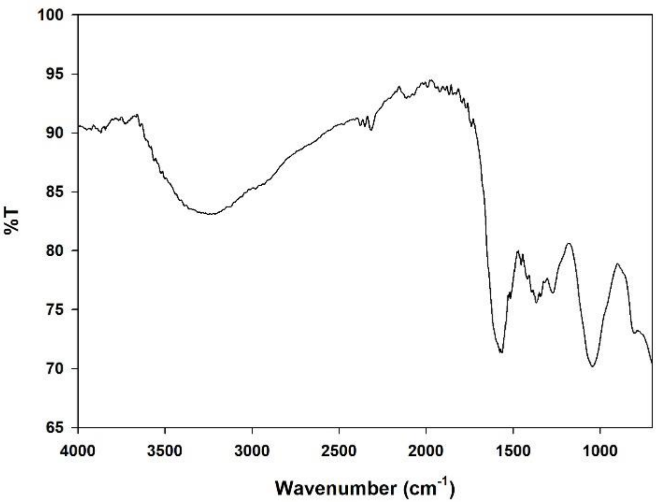

Figure 2: FTIR spectra of CuNps synthesized with *Peumus boldus* extract.

3.2. XRD analysis

CuNps have been identified with XRD, which is considered a very important key tool to evaluate the crystallinity and tertiary structures of particles at molecular levels (Sapsford et al., 2011). By X-ray diffraction, distinct crystalline phases associated with CuNps: metallic Cu, cuprite (Cu_2O) and tenorite (CuO) were observed. X-ray diffraction patterns of CuNp were obtained in the angle range of $2\theta = 20–80$. Diffraction peaks were observed at 36.49° (111), 39.90° (111), 49.5° (200), 62.49° (111) and 73.15° (200) (Figure 3).

3.3. Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDX) Study of CuONPs XRD Analysis

SEM images of CuNp obtained from black mulberry by green synthesis method are given in Figure 4. From the SEM images, it is seen that the particles have different diameters and sizes. It has been determined that the shapes of CuNp are spherical and their dimensions are <50 nm.

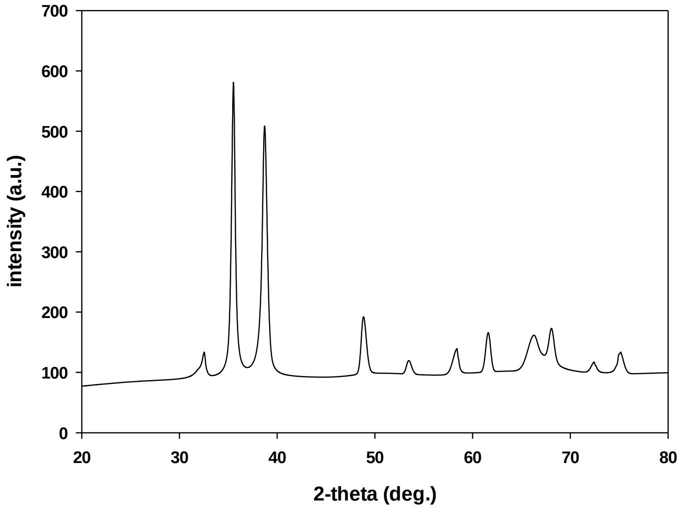

Figure 3. XRD diffraction pattern of CuNp biosynthesized by *Peumus boldus* extract.

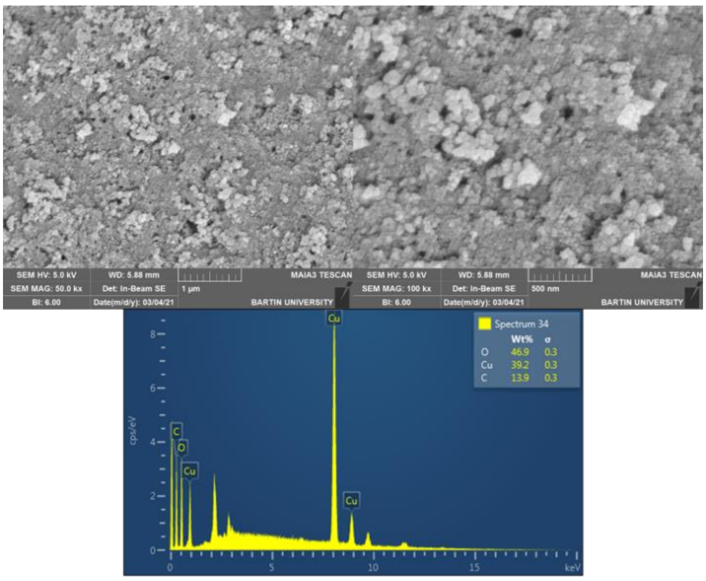

Figure 4. SEM and EDX images of Cu nanoparticles at different magnifications.

3.3. Antibacterial Activity of the Nanoparticles

Different concentrations ranging from 0-500 µg/mL were used to assess potential antibacterial effects of copper nanoparticles synthesized using *Peumus boldus*. The results showed that these nanoparticles had significant antibacterial effect against Gram negative bacteria *E. coli* (Figure 5) and Gram positive bacteria *S. aureus* (Figure 6). Viability scores of *E. coli* was dropped by approximately 60% when final concentration of 250 µgr/ml NP was used whereas around 60% drop in viability score was achieved with *S. aureus* when 250 µgr/ml NP concentration was used. It is evident from the results that at some elevated dosages the synthesissed Green-CuNps show its' antibacterial effect irrespective of the type and strain of the bacterial sepcies.

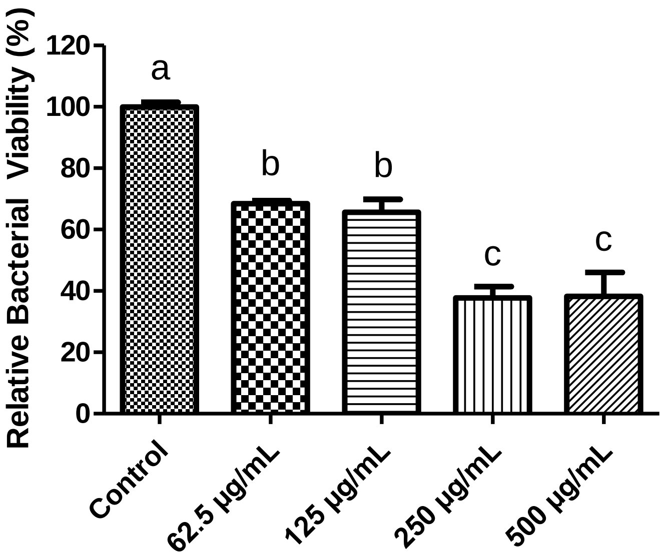

Figure 5. Antibacterial activity of copper nanoparticles synthesized using *Peumus boldus* on *E. coli*. Different lower letters indicate $p<0.05$ according to Tukey's test.

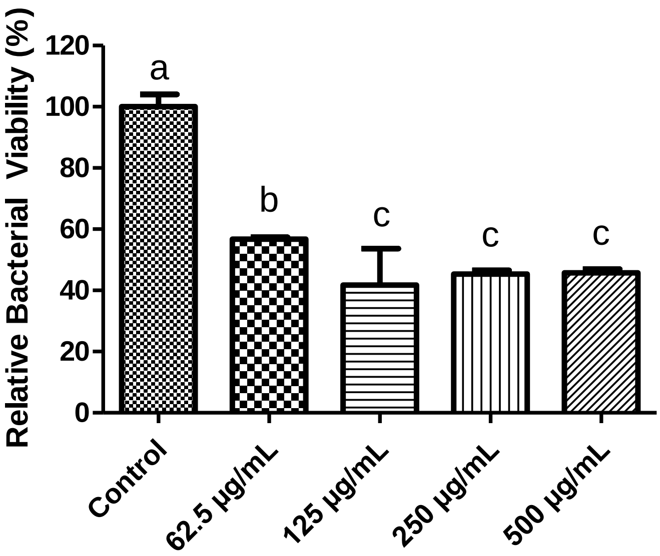

Figure 6. Antibacterial activity of copper nanoparticles synthesized using *Peumus boldus* on *S. aureus*. Different lower letters indicate $p<0.05$ according to Tukey's test.

4. Conclusions and Recommendations

For the synthesis of Np, physical and chemical methods that pose potential environmental and biological risks, including external reducing agents and toxic organic solvents, have been replaced by the green synthesis method, which is a fast, clean, non-toxic, cost-effective and environmentally friendly approach in recent years. The use of plant extracts in the green synthesis method is a more preferred approach. Because plants, together with the flavonoids, terpenoids, tannins, alkaloids, proteins and other phytochemicals they contain, can reduce metal ions and act as stabilizers that prevent the size of Np from growing. The structural, morphological, optical properties of the nanoparticles synthesized in the research were characterized using SEM, FTIR and XRD. The size measurements and shape characterization of CuNp were investigated using SEM and the results showed that CuNp smaller than 50 nm with spherical morphology was formed. The results obtained in this study show that the antimicrobial efficacy of CuNp synthesized from black pepper against pathogenic bacteria is at a sufficient level. These results show that CuNps can be an alternative to existing antibiotics/antimicrobial products to overcome the antibiotic resistance crisis that has been a serious problem in recent years.

5. Acknowledge

This study was partly supported by Bartin University.

References

Asha, A. (2016). Green Synthesis of Silver Nanoparticle from Different Plants– A Review. *International Journal of Pure & Applied Bioscience*, *4*(2), 118–124. https://doi.org/10.18782/2320-7051.2221

Buzea, C., Pacheco, I. I., & Robbie, K. (2007). Nanomaterials and nanoparticles: Sources and toxicity. *Biointerphases*, *2*(4), MR17–MR71. https://doi.org/10.1116/1.2815690

Kathiravan, V., Ravi, S., & Ashokkumar, S. (2014). Synthesis of silver nanoparticles from Melia dubia leaf extract and their in vitro anticancer activity. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, *130*, 116–121. https://doi.org/10.1016/j.saa.2014.03.107

Kim, J. S., Kuk, E., Yu, K. N., Kim, J. H., Park, S. J., Lee, H. J., Kim, S. H., Park, Y. K., Park, Y. H., Hwang, C. Y., Kim, Y. K., Lee, Y. S., Jeong, D. H., & Cho, M. H. (2007). Antimicrobial effects of silver nanoparticles. *Nanomedicine: Nanotechnology, Biology, and Medicine*, *3*(1), 95–101. https://doi.org/10.1016/j.nano.2006.12.001

Lee, C., Kim, J. E. E. Y., Lee, W. O. N. I. L., & Nelson, K. L. (2008). *Bactericidal Effect of Zero-Valent.pdf*. 4927–4933.

Saifuddin, N., Wong, C. W., & Yasumira, A. A. N. (2009). Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave irradiation. *E-Journal of Chemistry*, *6*(1), 61–70. https://doi.org/10.1155/2009/734264

Sapsford, K. E., Tyner, K. M., Dair, B. J., Deschamps, J. R., & Medintz, I. L. (2011). Analyzing nanomaterial bioconjugates: A review of current and emerging purification and characterization techniques. *Analytical Chemistry*, *83*(12), 4453–4488. https://doi.org/10.1021/ac200853a

Taleb, A., Petit, C., & Pileni, M. P. (1997). Synthesis of Highly Monodisperse Silver Nanoparticles from AOT Reverse Micelles: A Way to 2D and 3D Self-Organization. *Chemistry of Materials*, *9*(4), 950–959. https://doi.org/10.1021/cm960513y

Veisi, H., Rashtiani, A., & Barjasteh, V. (2016). Biosynthesis of palladium nanoparticles using Rosa canina fruit extract and their use as a heterogeneous and recyclable catalyst for Suzuki-Miyaura coupling reactions in water. *Applied Organometallic Chemistry*, *30*(4), 231–235. https://doi.org/10.1002/aoc.3421