GREEN SYNTHESIS OF COPPER OXIDE NANOPARTICLE FROM *DODONAEA VISCOSA* EXTRACT AND ITS ANTIBACTERIAL ACTIVITY

S. K. Periyasamy[1]*, R. Ramkumar[1]

ABSTRACT

Objectives: The current work concentrated on the characterization of , *Dodonaea Viscosa* extract, investigations into its antibacterial properties, and the synthesis of copper oxide nanoparticles (CuO NPs).

Methods: Under ideal circumstances (pH=11), 1 mM copper(II) sulfate pentahydrate was combined with 2% (m/v) aqueous , Dodonaea Viscosa extract of Dodonaea viscosa to create the CuO NPs.

Results: The color shift from colorless to light yellow and finally to brownish has been used to first confirm the formation of CuO NPs. The kinetics of the reaction were examined with a UV-Visible spectrophotometer, revealing surface plasmon resonance at 382 nm. The calculated values of zeta potential and particle size were −15.2 mV and 197 nm, respectively. Gram-positive and gram-negative cultures were used to test CuO NPs' antibacterial activity, and the results demonstrate desirable activity.

Conclusion: The study's conclusion shows that Dodonaea Viscosa extract converts Cu2+ metallic ions into CuO NPs by acting as a reducing and stabilizing agent. Following a successful green synthesis of the CuO NPs using Dodonaea Viscosa extract, the antibacterial activity, characterization, and activity against gram-positive (Staphylococcus aureus) and gram-negative (Escherichia coli) bacteria were examined.

Keywords: Copper oxide, Nanoparticles, Green synthesis, Antibacterial activity, Dodonaea Viscosa extract.

[1]*PG & Research Department of Chemistry, Jamal Mohamed College (Autonomous) Affiliated to Bharathidasan University, Tiruchirappalli-620 020.

***Corresponding Author:** S. K. Periyasamy
*Email: skps@jmc.edu

DOI: 10.53555/ecb/2024.13.04.12

INTRODUCTION

Because of their unique qualities, nanotechnology and nanoscience have a significant impact on food, medicine, agriculture, cosmetics, and human health. Particles with a size range of 1–100 nm are known as nanoparticles (NPs). Over the past ten years, a great deal of research has been done on the synthesis and application of NPs. Because of their special qualities and potential uses, which are heavily impacted by their size, shape, and structure, nanoparticles (NPs) have garnered a lot of attention [1]. There are several ways to synthesize NPs, including chemical, biological, and physical techniques. The biological method is a safer, more affordable, nontoxic, and environmentally friendly way to produce NPs. To raise living standards, metal nanoparticles (NPs) such as copper, gold, platinum, silver, and gold are frequently used in a variety of industries. Metal nanoparticle (NP) stabilization and reduction are achieved by employing pharmacologically significant plant and microbial extract compounds [2, 3].

In recent times, various plant parts such as stems, fruits, roots, calluses, peel, seeds, flowers, and leaves have been employed to prepare metal oxide nanoparticles (NPs) in various forms and dimensions through the use of biological systems [4]. Within the current research space, there are limited reports on copper NPs, despite the fact that gold and silver NPs have been studied extensively. Because of their superior electrical, conductivity, catalytic, and antimicrobial qualities, copper oxide NPs (CuO NPs) have attracted attention [2]. For the synthesis of CuO NPs, a number of physio-chemical techniques have been reported, including precipitation, sonochemistry, microwave radiation, electrochemical reduction, etc. [3,5,6]. While there are a number of practical ways to create CuO NPs, the field of green synthesis of NPs is a rapidly developing field of study that uses plant extracts. Certain experimental parameters, such as the mass ratio of extract to copper salt, the type of plant extract, temperature, and reaction time, have been found to have a direct impact on the morphology of CuO nanoparticles when utilizing the plant extract mediated synthesis method [1].

CuO NPs made chemically limit their use in biological systems and contaminate the ecosystem by releasing harmful process byproducts into the atmosphere [7]. Researchers concentrated on creating an environmentally friendly biological synthesis of nanoparticles after realizing the drawbacks of physiochemical methods [8]. Although many microorganisms find copper toxic, mammals do not [9] find it to be a toxic metal. This opens up new possibilities for antimicrobial treatments. Several reports on the antimicrobial properties of Copper NPs are available based on the literature [10–13]. Strong antibacterial activity of CuNPs against strains of Salmonella choleraesuis, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, and Escherichia coli has been demonstrated [14]. Numerous studies have been conducted on the bioreduction of metal ions to form nanoparticles (NPs) using phytochemicals and compounds found in plant extracts, including those from Momordica charantia [17], Gum karaya [15], Ocimum basilicum [16], and Leucaena leucocephala L. [18].

The goal of the current study was to evaluate the antibacterial activities of the synthesized CuO NPs against specific pathogenic organisms using the disc diffusion method, as well as to investigate the potential applications of Dodonaea Viscosa extract as a capping and reducing agent for the CuO NPs [19]. This was accomplished by green synthesis of the CuO NPs using this extract.

METHODS

Every chemical was of the analytical variety. The following materials were obtained from Loba Chemie Pvt. Ltd. in India: Benedict's solution, iodine solution, hydrochloric acid (HCl), sulfuric acid (H_2SO_4), ethanol (C_2H_5OH), ferric chloride ($FeCl_3$), sodium hydroxide (NaOH), deionized water, and copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$).

Bacterial strain

For this experiment, strains of both Gram-positive and Gram-negative bacteria were used.

Collection of Dodonaea Viscosa

Dodonaea viscosa was gathered from nearby regions. The Dodonaea viscosa was then cleaned, allowed to air dry, and ground into a fine powder, yielding about 100 g of powder.

Preparation of Dodonaea Viscosa extract

The decoction method was used to carry out the extraction. After that, it was permitted to reach room temperature. Using Whatman filter paper, the mixture was first filtered. The filtrate was then collected and stored at 4°C to facilitate the further synthesis of CuO NPs.

Phytochemical screening

Test for alkaloids (Wagner's test: Iodine-potassium iodide solution)

A solution of 100 milliliters was created by mixing 1.2 g of iodine with 2 mL of H_2SO_4. 1.5% (v/v) of

HCl was added to 10 mL of the alcoholic extract to acidify it, and then a few drops of Wagner's reagent were added. To verify the presence of alkaloids, the formation of brown or yellow precipitates was evaluated.

Test for glycosides
A small amount of the alcoholic extract was combined with the distilled water, and then aqueous NaOH solution was added. This solution was diluted in one milliliter of distilled water. The development of a reddish brown hue was interpreted as a glycoside presence indicator.

Test for tannins ($FeCl_3$ test)
When one milliliter of extract and one milliliter of $FeCl_3$ solution were combined, a greenish-black precipitate formed, which suggested the presence of tannins.

Test for flavonoids
2 mL of 10% (m/v) $FeCl_3$ solution were combined with 1 mL of extract, and the mixture was shaken. The presence of flavonoids was indicated by a brownish, woody precipitate.

Test for phenols
A solution containing 3-5 drops of $FeCl_3$ was added to the extract. After that, it was allowed to develop a bluish-black hue, which is indicative of the presence of phenols.

Test for carbohydrates (benedict test and iodine test)
The plant extract was mixed with a few drops of Benedict solution, and its ability to produce the color brick red—which indicates the presence of glucose—was observed. When a few drops of iodine were added to the other extract, starch was confirmed to be present by the formation of a dark blue color.

Green synthesis of CuONPs
Two milliliters of 2% (m/v) Dodonaea Viscosa extract were added to a 1 mM $CuSO_4$.$5H_2O$ solution, which was then magnetically stirred at room temperature until the light blue hue turned light yellow. After that, the mixture was heated for 30 minutes at 80°C. After that, it was given time to cool. The mixture was then subjected to a drop-by-drop treatment with a 1 M NaOH solution until the pH reached 11. After centrifuging the mixture for 15 minutes at 10,000 rpm, it was dried for 24 hours at 100°C. Using UV-visible spectroscopy and visual observation, the synthesized CuO NPs were characterized [20].

Characterization
Visual observation
When copper NPs are formed during the bio-reduction of the copper sulfate aqueous solution using extract, a brown color appears.

UV-Vis spectroscopy
Monitoring the reduction of copper sulfate to copper was done by taking UV-Vis spectra at 200–700 nm in wavelength. The measurements were made using a lambda 25 spectrometer made by Perkin Elmer.
pH analysis. A microprocessor pH meter (ESICO model 1010) was used to measure the pH of the extract, precursor, and final combination after NaOH was added

Particle size and zeta potential measurement
Using the Malvern Zetasizer instrument, the dynamic light scattering (DLS) method was used to determine the particle size and zeta potential.

Antibacterial activity of CuO NPs using the disc diffusion method
Double-distilled water, 2% (m/v) Dodonaea Viscosa extract, 1 mM $CuSO_4$.$5H_2O$ solution, and solutions containing CuO NPs of each kind individually were all soaked into the discs. Tetracyclin served as a positive control and was positioned in the middle of the plates. Subsequently, the discs underwent sterile air drying. The microbial cultures (S. aureus and E. coli) were swabbed onto the plates to prepare them for nutrient agar media. Every section of the plate had a disc that had been previously prepared on it. The order of the discs was as follows: As a negative control, a disc was soaked in double-distilled water; solutions containing extract-mediated CuO NPs in ratios of 1:2 and 1:3; and a disc soaked in Dodonaea Viscosa extract. For a full day, the plates were incubated at 37°C. Lastly, a measurement and observation of the zone of inhibition were made against every variety of test microorganism [21].

RESULTS AND DISCUSSION
Phytochemical test
The results of the phytochemical analysis of the extract are shown in Table 1, which revealed the presence of secondary metabolites.

Table 1: The result of phytochemical screening test

Test	Result
Alkaloids	+
Glycoside	−
Tannins	+
Phenols	+
Flavonoids	+
Carbohydrate	−

Visual observation

• The formation of CuO NPs is indicated by the color changes that result from the metal NPs' surface plasmon resonance being excited.

• As soon as the Dodonaea Viscosa extract was added, the colorless 1 mM $CuSO_4$.$5H_2O$ solution began to turn pale yellow. The solution had a brownish hue when the NaOH solution came into contact with it, which suggested that CuO NPs were forming.

UV-Vis analysis

The pure Dodonaea Viscosa extract displayed a strong absorbance at 294 nm, while the UV-Vis spectra result indicated a strong absorbance at 382 nm, suggesting the formation of CuO NPs.(Fig .1) This outcome is unquestionably consistent with the 200–400 nm range of εmax values of the CuO NPs found in various earlier studies employing plant extracts other than cinchona. The measurements were made using a lambda 25 PerkinElmer spectrometer.

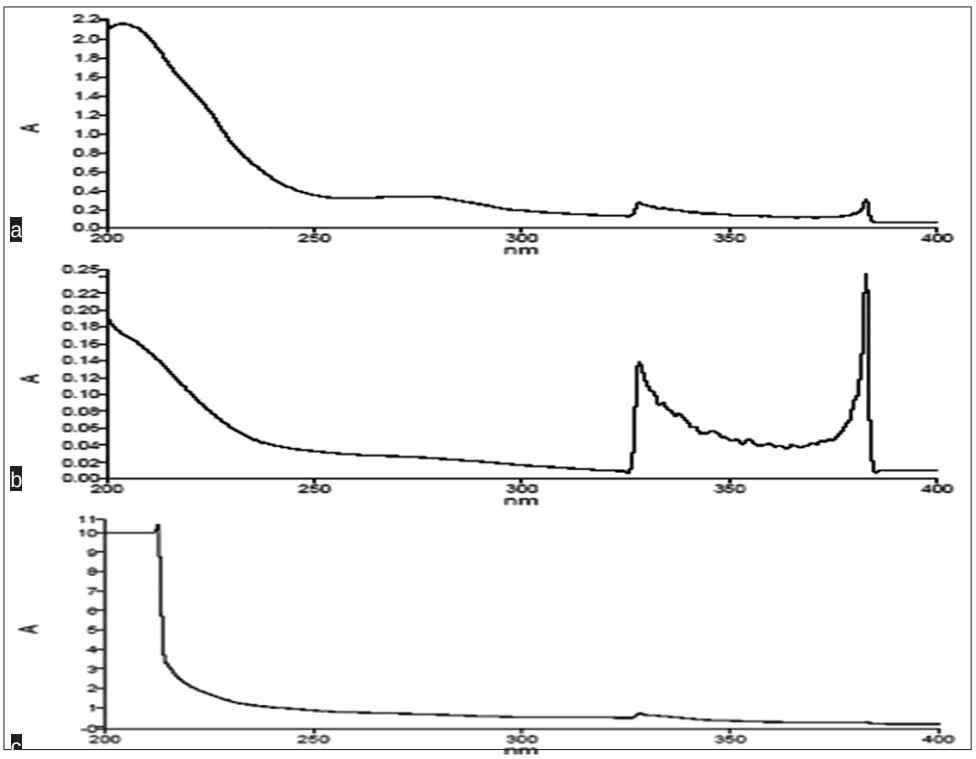

Fig. 1: UV-visible absorption spectra of copper oxide nanoparticles synthesized by using Dodonaea Viscosa extract as shown in
(a) UV-Visible spectrum of Dodonaea Viscosa, (b) UV-visible spectrum of $CuSO_4$, (c) UV-visible spectrum for the formation of CuONPs under optimum conditions

Optimization of precursor concentration

Two procedures were followed in order to maximize the precursor's concentration. The initial phase occurred when pH was not changed. In this case, the CuO NP peaks were all closer to the pure extract peak, which is located at 294 nm. Several earlier studies on the synthesis of CuO NPs indicated that the media needed to be changed to a basic condition by adding NaOH solution in order to adjust the pH. Therefore, the next step was to add a NaOH solution with the recommended pH=11 (prior to optimization). Upon its addition, there was an immediate and notable shift in the peaks' positions [20].

Ratio of volume of extract: $CuSO_4$, CuO NPs were synthesized for 1:3 ratios of extract to copper sulfate at various pH values, which were 4, 5, 6, 7, 8, 9, 10, and 11. The reaction mixture's UV-visible spectrum and the amount of time it took for the color to change were both observed. An increase in precursor salt concentration was associated with a blue shift in wavelength from 382 to 328 nm. This shift can be explained by a higher nucleation rate brought on by more Cu^{2+} ions in the solution as well as the production of smaller nanoparticles. However, a red shift from 328 to 294 nm was seen in the SPR with an additional increase in the precursor ion from 1:2 to 1:3. Particle growth may result from smaller NPs colliding with one another [22]. The brown hue was identified as the ideal concentration of precursor and extract, resulting in the highest quantity of copper nanoparticles in the aqueous medium. (1:3). Since the biosynthesized NPs exhibited maximum absorption at 382 nm, it

was determined that this ratio was optimal.Fig. 2

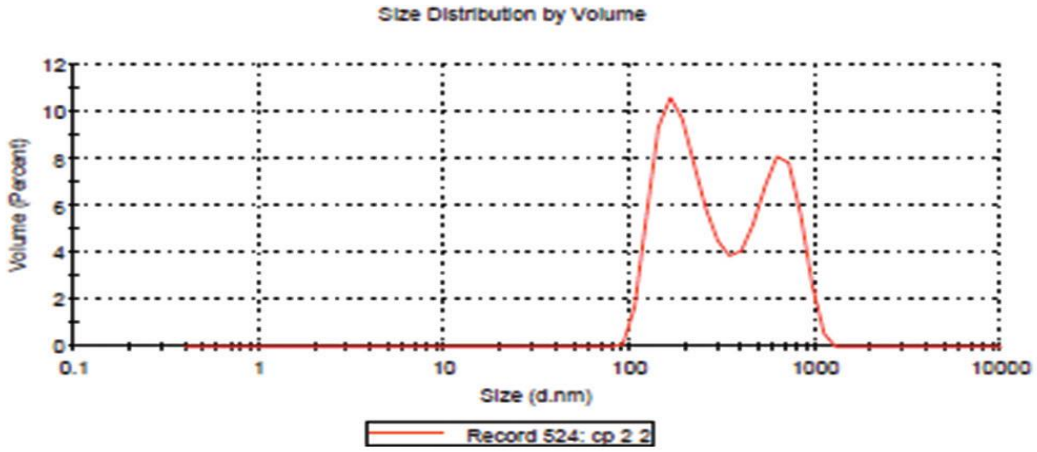

Fig. 2: Particle size distribution of Copper(II) Sulphate pentahydrated measured by Dynamic light scattering

Effect of temperature on biosynthesis of CuONPs

The study examined the impact of temperature on the rate of CuO NP formation in the extract and CuSO4 solution with a 1:3 composition. At 80°C, the CuO NPs formed in less than 30 minutes. However, the formation of CuO NPs took place after 1 day and 2 hours at room temperature and 60°C, respectively, and above 80°C under boiling conditions, the solution burns and no particle formation is observed. Therefore, the biosynthesis of CuO NPs using extract is favored by the reaction at 80°C.

Optimization of the pH of the mixture of extract and the precursor

The pH values of the precursor, the extract, and the NPsPrior to pH adjustment, the precursor, extract, and NPs had pH values of 4, 5, and 5.4, respectively. CuO NPs that were synthesized at various pH values (6, 7, 8, 9.5, 10, 11, and 12) were found to be optimal based on the λmax values' red shift and peak intensity. At pH=11, the synthesis of CuO NPs produced the best results.

This demonstrates that the synthesis of CuO NPs can be done effectively using a more basic medium. It is anticipated that the additional NaOH solution will catalyze the formation of the nanoparticle by causing particles to collide and bond with one another to create homodispersed NPs [23]. However, raising the pH above 12 may prevent CuO NPs from forming. By comparing the UV-Vis spectra of the synthesized CuO NPs at various ages, the stability of the material was evaluated [24].

Particle size and zeta potential measurement

The DLS method was used to determine the particle size and zeta potential. The Malvern Zetasizer instrument measured 197 nm and -15.2 mV, in that order.Fig.3

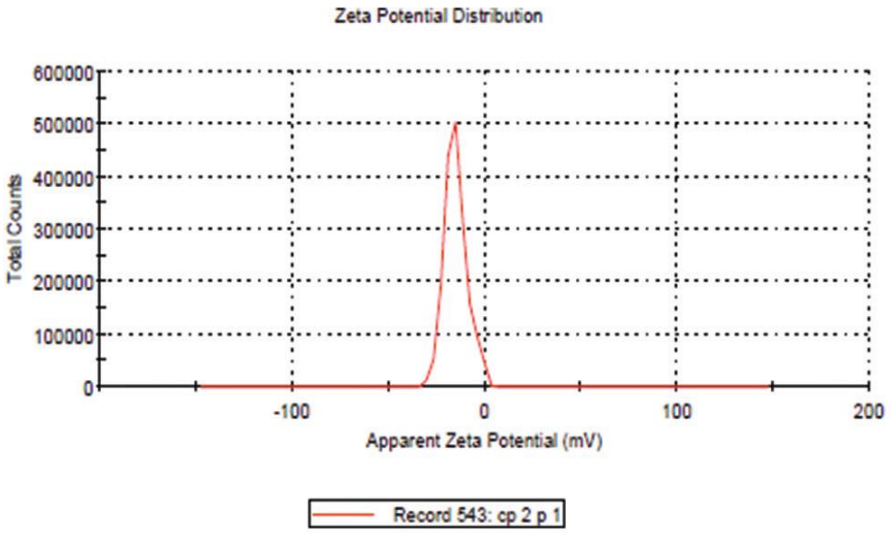

Fig. 3: Zeta potential of precursor

Antibacterial activity of CuO NPs

The zone of inhibition served as the basis for the analysis of copper NPs' antibacterial activity.(Fig.4) Using the disc diffusion method, CuO NPs demonstrated antibacterial activity against gram-positive and gram-negative bacteria, including S. aureus, B. subtilis, E. coli, and P. aeruginosa. Table 2 displays the effects of the synthesized CuO NPs against gram positive and gram negative bacteria, with a zone of inhibition of 12 mm and 10 mm, respectively. The outcomes of conventional antibiotics and these ones were also contrasted.

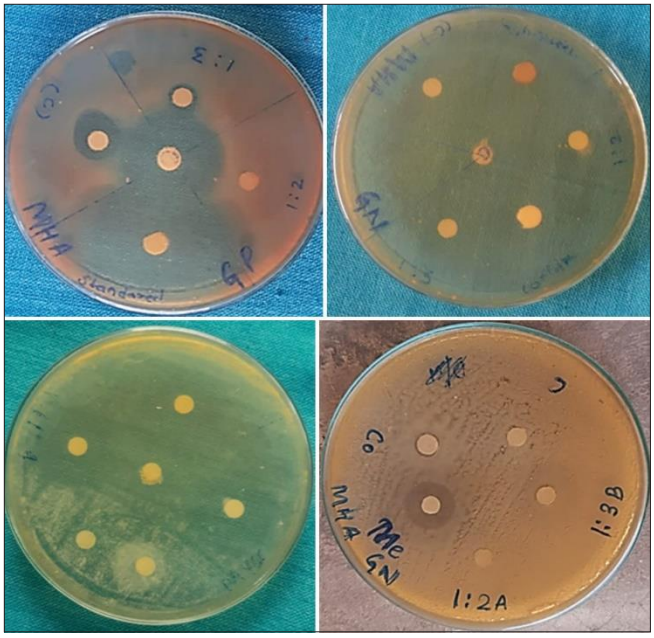

Fig. 4: Zone of inhibition produced by copper oxide nanoparticles

Table 2: Antibacterial activity of CuO NPs using some human pathogenic bacteria by disc diffusion method

Test organism	Zone of inhibition (mm)
Staphylococcus aureus	12
Escherichia coli	10

CONCLUSION

CuO NPs can now be produced in an easy, straightforward, economical, and ecologically friendly manner. Dodonaea Viscosa extract converts Cu^{2+} metallic ions into CuO NPs and acts as a reducing and stabilizing agent. CuO NPs were successfully synthesized using Dodonaea Viscosa extract in a green manner, and their antibacterial activity was determined. By measuring the zone of inhibition, the antibacterial activities of the synthesized CuO NPs against gram-positive (S. aureus) and gram-negative (E. coli) bacterial strains were assessed. Gram-positive (S. aureus) bacteria demonstrated greater activity than gram-negative (E. coli) bacteria.

Acknowledgement

The authors thank the Management and Principal Jamal Mohamed College (Autonomous), Tiruchirappalli, Tamil Nadu, India for providing necessary facilities and encouragement

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS FUNDING

No funding has been received for this research work.

REFERENCES

1. Khan SA, Shahid S, Sajid MR, Noreen F, Kanwal S. Biogenic synthesis of CuO nanoparticles and their biomedical applications: A current review. Int J Adv Res 2017;5:925-46. doi: 10.21474/IJAR01/4495

2. Abboud Y, Saffaj T, Chagraoui A, El Bouari A, Brouzi K, Tanane O, *et al.* Biosynthesis, characterization and antimicrobial activity of copper oxide nanoparticles (CONPs) produced using brown alga extract (*Bifurcaria bifurcata*). Appl Nanosci 2014;4:571-6.
3. Yang C, Xiao F, Wang J, Su X. 3D flower-and 2D sheet-like CuO nanostructures: Microwave-assisted synthesis and application in gas sensors. Sens Actuators B Chem 2015;207:177-85.
4. Mittal AK, Chisti Y, Banerjee UC. Synthesis of metallic nanoparticles using plant extracts. Biotechnol Adv 2013;31:346-56. doi: 10.1016/j. biotechadv.2013.01.003
5. Yang XD, Jiang LL, Mao CJ, Niu HL, Song JM, Zhang SY. Sonochemical synthesis and nonlinear optical property of CuO hierarchical superstructures. Mater Lett 2014;115:121-4. doi: 10.1016/j.matlet.2013.10.037
6. Mandke MV, Pathan HM. Electrochemical growth of copper nanoparticles: Structural and optical properties. J Electroanal Chem 2012;686:19-24. doi: 10.1016/j.jelechem.2012.09.004
7. Zhou Y, Lin W, Huang J, Wang W, Gao Y, Lin L, *et al.* Biosynthesis of gold nanoparticles by foliar broths: Roles of biocompounds and other attributes of the extracts. Nanoscale Res Lett 2010;5:1351-flowerlike architectures exhibiting antimicrobial activity. J Phys Chem B 2006;110:205-10. doi: 10.1021/jp054827x, PMID 16471522
10. Lee HJ, Song JY, Kim BS. Biological synthesis of copper nanoparticles using *Magnolia kobus* leaf extract and their antibacterial activity. J Chem Technol Biotechnol 2013;88:1971-7. doi: 10.1002/jctb.4052
11. Sutradhar P, Saha M, Maiti D. Microwave synthesis of copper oxide nanoparticles using tea leaf and coffee powder extracts and its antibacterial activity. J Nanostruct Chem 2014;4:86.
12. Ahamed M, Alhadlaq HA, Khan MA, Karuppiah P, Al-Dhabi NA. Synthesis, characterization, and antimicrobial activity of copper oxide nanoparticles. J Nanomater 2014;2014:637858. doi: 10.1155/2014/637858
13. Awwad A, Albiss BA, Salem NM. Antibacterial Activity of Synthesized Copper Oxide Nanoparticles using *Malva sylvestri*s Leaf Extract; 2015. p. 90-101.
14. Usman MS, El Zowalaty ME, Shameli K, Zainuddin N, Salama M, Ibrahim NA. Synthesis, characterization, and antimicrobial properties of copper nanoparticles. Int J Nanomedicine 2013;8:4467-79. doi: 10.2147/IJN.S50837, PMID 24293998
15. Padil VV, Černík M. Green synthesis of copper oxide nanoparticles using gum karaya as a biotemplate and their antibacterial application. Int J Nanomedicine 2013;8:889-98. doi: 10.2147/IJN.S40599, PMID 23467397
16. Altikatoglu M, Attar A, Erci F, Cristache CM, Isildak I. Green synthesis of copper oxide nanoparticles using *Ocimum basilicum* extract and their antibacterial activity. Fresenius Environ Bull 2017;25:7832-7.
17. David SA, Rajadurai SI, Kumar SV. Biosynthesis of Copper Oxide Nanoparticles using *Momordica charantia* Leaf Extract and their Characterization. In: One Day National Conference on Recent Innovations in Science, Technology and Engineering. Vol. 31; 2017. p. 333-40.
18. Aher YB, Jain GH, Patil GE, Savale AR, Ghotekar SK, Pore DM, *et al.* Biosynthesis of copper oxide nanoparticles using leaves extract of *Leucaena leucocephala* L. and their promising upshot against diverse pathogens. Int J Mol Clin Microbiol 2017;7:776-86.
19. Andualem WW, Sabir FK, Mohammed ET, Belay HH, Gonfa BA. Synthesis of copper oxide nanoparticles using plant leaf extract of *Catha edulis* and its antibacterial activity. J Nanotechnol 2020;2020:2932434. doi: 10.1155/2020/2932434
20. Gebremedhn K, Kahsay MH, Aklilu M. Green synthesis of CuO nanoparticles using leaf extract of *Catha edulis* and its antibacterial activity. J Pharm Pharmacol 2019;7:327-42. doi: 10.17265/2328- 2150/2019.06.007
21. Banerjee P, Satapathy M, Mukhopahayay A, Das P. Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: Synthesis, characterization, antimicrobial property and toxicity analysis. Bioresour Bioprocess 2014;1:3.
22. Law M, Greene LE, Johnson JC, Saykally R, Yang P. Nanowire dye- sensitized solar cells. Nat Mater 2005;4:455-9. doi: 10.1038/nmat1387, PMID 15895100
23. Chauhan RP, Gupta C, Prakash D. Methodological advancements in green nanotechnology and their applications in biological synthesis of herbal nanoparticles. Int J Bioassays 2012;1:6-10.
24. Lebaschi S, Hekmati M, Veisi H. Green synthesis of palladium nanoparticles mediated by black tea leaves (*Camellia sinensis*) extract: Catalytic activity in the reduction of 4-nitrophenol and Suzuki-Miyaura coupling

reaction under ligand-free conditions. J Colloid Interface Sci 2017;485:223-31.