Peer Reviewed and Refereed Journal 2023 (ISSN : 09726330)

Rashtrasant Tukadoji Maharaj Nagpur University
SCIENCE JOURNAL 2023
Vol.: XVI

Green synthesis of copper oxide nanoparticle using leaf extract of Cassia tora and its antibacterial assays

Shivani R. Sharma[1]*, Nilima M. Dhote[1], Mamta S. Wagh[1], Shamli S. Gupte[2], Sanjay J. Dhoble[2]

[1]Department of Chemistry, Kamla Nehru Mahavidyalaya, Nagpur-440024, India
[2]Department of Physics, R.T.M. Nagpur University, Nagpur-440033, India

Corresponding author email:
shivanisharma5881@gmail.com

ABSTRACT

A facile and green strategy is reported here to synthesize *Cassia tora* mediated copper oxide nanoparticles (Ca CuNPs). *Cassia tora* leaves extract acts as both reducing and capping agent. Preliminary characterization of Ca CuNPs was done: UV Vis analysis, XRD, FTIR and SEM. The antibacterial potential of green synthesized nanoparticles was checked on gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria. The UV Vis analysis of these nanoparticles showed two absorption peaks: a minor peak around 400 nm and a broad peak at 805 nm. According to XRD results the synthesized Ca CuNPs were crystalline in nature. The SEM images revealed that these nanoparticles were agglomerated and spherical in shape. The FTIR data showed the presence of O-H group along with thiol and nitro groups. These Ca CuNPs were found to be effective in inhibiting the growth of both gram- positive and gram-negative bacteria. However, these nanoparticles were found to be more effective against gram-negative (*Escherichia coli*) bacteria.

Keywords : Green synthesis, *Cassia tora*, copper oxide nanoparticles, antibacterial activity, *Staphylococcus aureus* and *Escherichia coli*

Introduction

Nanotechnology is an area of science which deals with synthesis, characterization and application of nano scaled particles. Nanoparticles have an extensive use in the field of biomedical application[1]. It has been reported that the nanoparticles such as silver (Ag), gold (Au), platinum (Pt), copper (Cu) have excellent antibacterial properties. Among all the metallic nanoparticles, Cu has drawn attention of researchers due to its excellent physiochemical properties. Cu oxide nanoparticles (CuNPs) can be synthesized using chemical and physical methods. However, these methods are tedious and involves use of harsh and toxic chemicals. In contrast to this, green synthesis or phytofabrication of CuNPs is cost effective and is relatively less toxic [2].

Cassia tora (Ca) is commonly consumed leafy vegetable in India, it belongs to the Leguminosae family. It is the well documented plant of *"Ayurveda"* and in traditional medical system it is used in treatment of leprosy, bronchitis, cardiac disorder, ringworms and it is also used as laxative [3]. These pharmacological activities are due to presence of various bioactive compounds such as: emodin, anthraquinones, apigenin and chrysophanol [4]. In present study, an attempt has been made to synthesis *Cassia tora* mediated copper oxide nanoparticles and its antibacterial efficacy will be checked on gram- positive and gram-negative bacteria.

2. Materials and methods

2.1. Collection of plant sample and extraction

The *Cassia tora* leaves were collected from the rural areas of Nagpur (Hudkeshwar area). The leaves were identified with the help of a taxonomist and an herbarium was submitted to the Department of Botany, RTM University, Nagpur (accession no. 10921). The leaves were washed, dried and grinded. The grinded powder was sieved, the fine powder was collected and was used for further extraction process. For extraction 1 g of powder was added to 100 ml of distilled water in 250 ml of beaker, the mixture was stirred for 1 hour at 85°C. The mixture was allowed to cool at room temperature and was filtered using Whatman's filter paper. The extract was collected and was used for further use.

2.2. Green synthesis of copper oxide nanoparticles

For *Cassia tora* mediated copper oxide nanoparticle (Ca CuNPs) synthesis 10 ml of leaves extract was added to 100ml of copper sulphate ($CuSO_4.5H_2O$) (0.5 M), the solution was continuously stirred for 3 hours at 90°C, the color change from green to bluish ascertains the synthesis of Ca CuNPs. The colloidal solution of nanoparticles was centrifuged at 5000 rpm for 10 minutes and were washed with ethanol followed by acetone. The precipitate was transferred into crucibles and were calcinated in muffle furnace for 2 hours at 400°C. Subsequently, light blue colored nanoparticles were collected.

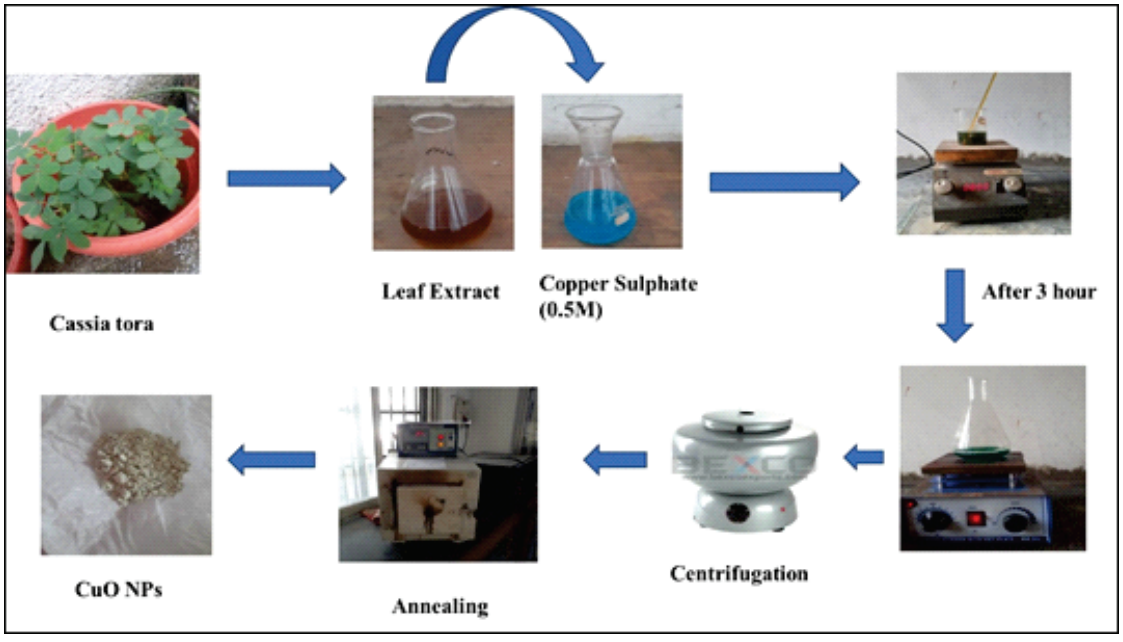

Fig1:Synthesis of *Cassia tora* copper oxide nanoparticles (Ca CuNPs)

2.3. Preliminary characterization

The change in color from green to bluish at the end of incubation suggested the phyto fabrication of *Cassia tora* mediated copper oxide nanoparticles (Ca CuNPs). The aliquot of these nanoparticles was further examined using UV Vis spectrophotometer and its absorbance peak of noted. The crystalline nature of nanoparticles was determined through XRD and for determination of functional groups FTIR technique was used. The morphology of nanoparticles was studied through SEM images

2.4. Antibacterial studies

The antibacterial efficacy of Ca CuNPs was studied on gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria. The bacterial colonies were cultured on Muller Hinton agar. The bacterial inoculum was spread on agar plates with sterile L-shaped glass rod and the agar plates were incubated overnight. 5mm wells were made in agar plates with help of well borer and different concentration of Ca CuNPs were added to the well. 4 different concentrations of

Ca CuNPs were taken: (50µg/well, 100µg/well, 1mg/well and 2mg/well). The plates were again incubated at 37°C, for 24 hours and the zone of inhibition was calculated at the end of incubation.

3. Results

3.1. UV Vis analysis

The UV Vis absorption spectra Ca CuNPs was recorded at different wavelengths from 200 nm to 1100 nm. Two absorption peaks were noted around 400 and 800 nm. The peak around 400 nm was a minor peak (Fig 2). A broader peak was observed around 800 nm, the broadened surface plasmon resonance peak of Ca CuNPs indicates that the presence of polydisperse nanosized particles.

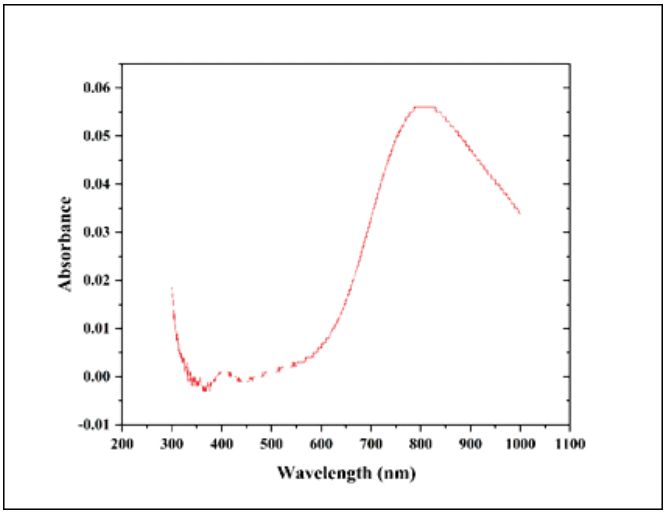

Fig2: UV Vis analysis of *Cassia tora* copper oxide nanoparticles (Ca CuNPs)

3.2. Fourier-transform infrared spectroscopy analysis

The FTIR spectra of *Cassia tora* copper nanoparticles (Ca CuNPs) is represented in fig 3. The presence of different functional groups is determined by FTIR analysis. In present FTIR results different peaks were noted at 3321.56, 3238.62, 2481.53, 2089, 1655, 1533.47, 1197.85, 978.92, 862.22, 646, 492.83, 412.78. The peaks 3321.56, 3238.62 represents strong O-H stretching which indicates presence of polyphenolic compounds. Peaks 2481.53, 2089,1655, 1533.47, 978.92 represents S-H stretching, N=C=S stretching, C-H bending, N-O stretching and C=C bending respectively.

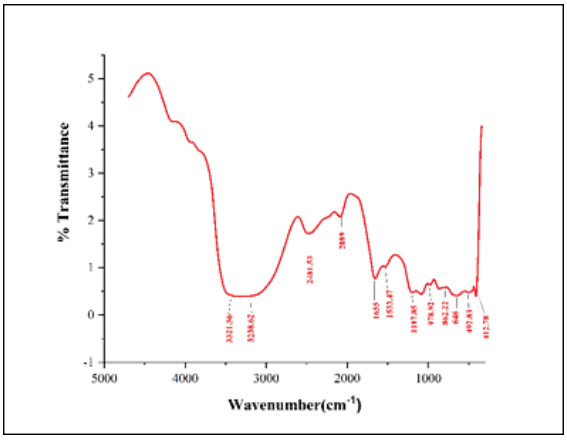

Fig3: FTIR analysis of *Cassia tora* copper oxide nanoparticles (Ca CuNPs)

3.3. X ray diffraction analysis

The X ray diffraction pattern of Ca CuNPs is shown in fig 4. The XRD pattern of nanoparticles reveals the crystalline or amorphous nature of nanoparticles. In present study several peaks were noted along with peaks at 200 and 220. According to JCPDS file no 89-7102 peaks at 200 and 220 indicates the crystalline nature of Ca CuNPs.

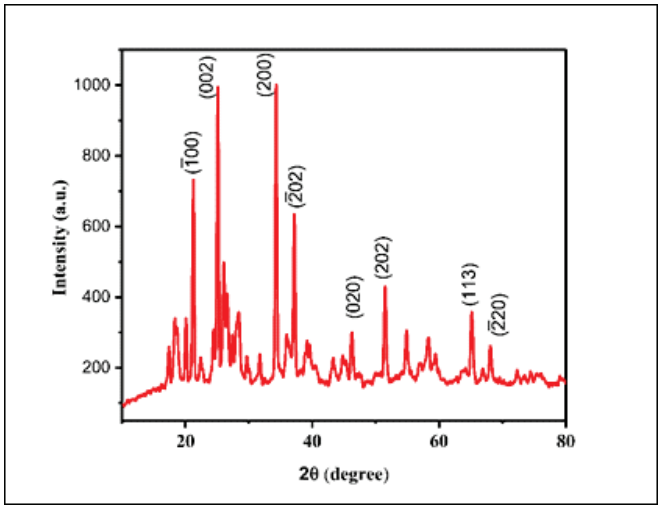

Fig4: XRD analysis of *Cassia tora* copper oxide nanoparticles (Ca CuNPs

3.4. Scanning electron microscope image

The morphology of nanoparticles is studied by scanning electron microscope. In present experiment it was noted that the green synthesized Ca CuNPs were highly agglomerated. However, in few images the spherical shape of nanoparticles was visible (fig 5, 6).

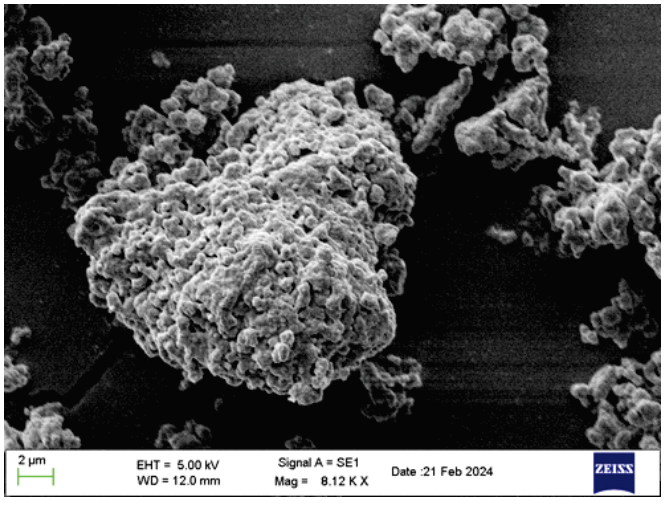

Fig5: *Cassia tora* copper oxide nanoparticles (Ca CuNPs)

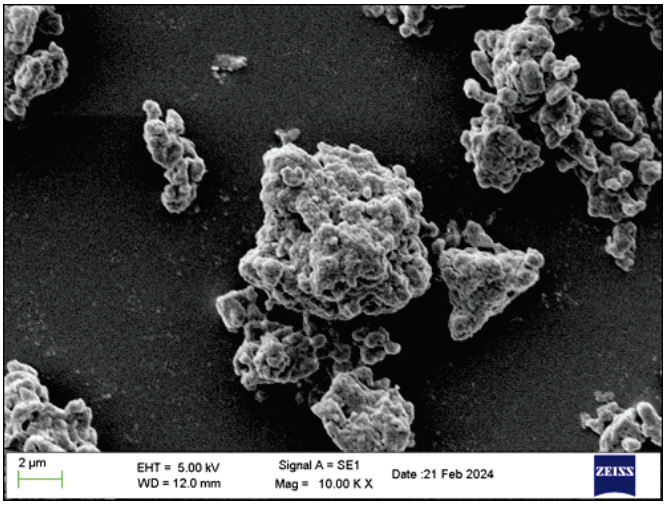

Fig6: *Cassia tora* copper oxide nanoparticles (Ca CuNPs)

3.5. Antibacterial assay

The antibacterial efficacy of *Cassia tora* mediated copper oxide nanoparticles was checked on gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria. These nanoparticles were able to inhibit the growth of both bacteria in dose dependent manner. In case of *E.coli* maximum inhibition (23 mm) was noted at the highest dose (2mg/well). Similarly, in case of *S. aureus* maximum inhibition (20 mm) was also noted at the highest dose (2mg/well). However, the Ca CuNPs showed more efficacy against gram-negative (*E. coli*) bacteria as compared to gram-positive (*S. aureus*) bacteria (fig 7,8).

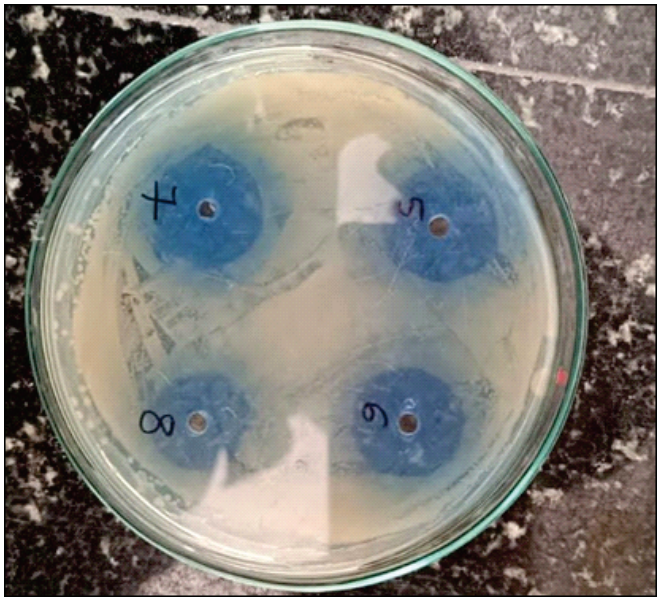

Fig7: Bacteriostatic effect of *Cassia tora* copper oxide nanoparticles (Ca CuNPs) on gram-positive (*Staphylococcus aureus*) bacteria

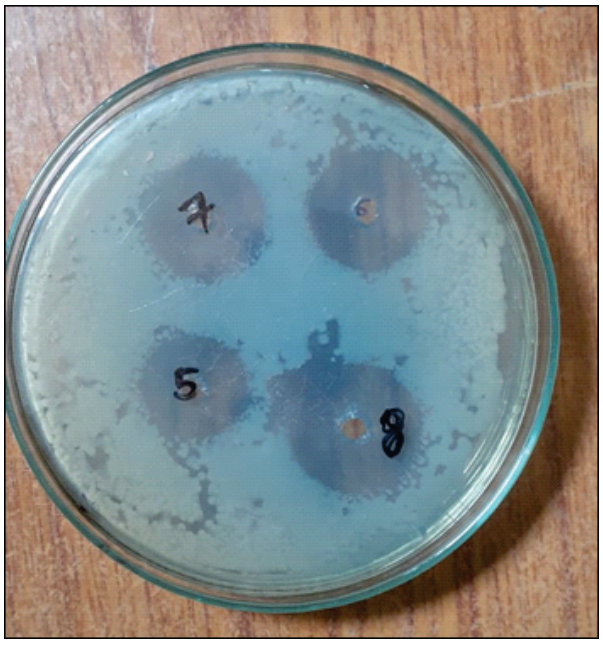

Fig8: Bacteriostatic effect of *Cassia tora* copper oxide nanoparticles (Ca CuNPs) on gram-negative (*Escherichia coli*) bacteria

4. Discussion

Metallic nanoparticles are widely used in biomedical and pharmaceutical industries due to its typical physiochemical properties and surface to volume ratio. The metallic nanoparticles are commonly used as an antibacterial agents [5]. There are several metallic nanoparticles that are used as antibacterial agent. However, copper oxide nanoparticles (CuNPs) have drawn much attention due to its relatively low toxicity and cost effectiveness [2]. CuNPs are synthesized by physical, chemical and biological method. The biogenic synthesis of nanoparticles is preferred over physical and chemical method as the biogenic synthesis is nontoxic and ecofriendly. In biogenic synthesis plant extracts are used as reducing and capping agents [6].

In present experiment, an attempt has been made to study the antibacterial efficacy of copper oxide nanoparticles synthesized from *Cassia tora* leaves extract. The green synthesized Ca CuNPs were characterized by: UV Vis spectrophotometry, FTIR analysis, X ray diffraction technique and the morphology was studied with the help of scanning electron microscope images.

The *Cassia tora* leaves extract was added to copper sulphate solution and the mixture was incubated at 90°C for 3 hours. The change in color from green to bluish was observed by naked eyes. This color change indicated the synthesis of Ca CuNPs. Further, these Ca CuNPs were subjected to UV Vis analysis, where two different peaks were noted first a minor peak around 400 nm and a broader second peak at 805 nm. Similarly, Thien and group also reported an absorption peak at 805 nm for chitosan mediated copper oxide nanoparticles [7]. The absorption peak and its width depends upon the size of metallic nanoparticles and nature of reducing agents [8]. According to the broad absorbance peak indicates the polydispersity of nanoparticles.

The presence of functional groups in the Ca CuNPs was determined by FTIR. The presence of O-H stretching, S-H stretching, N=C=S stretching, C-H bending, N-O stretching and C=C bending was observed. These bond pattern indicates the presence of phenolic compounds, carboxylic acid, alkene, thiol and nitro groups. Various studies have reported the presence of polyphenolic compounds in *Cassia tora* extract, these polyphenolic compounds act as reducing and capping agent thereby facilitating the synthesis of Ca CuNPs. [10] and team synthesized *Cassia tora* mediated silver nanoparticles and observed similar results.

The crystalline nature of Ca CuNPs was confirmed by XRD analysis. The XRD results of Ca CuNPs showed at total of 8

peaks. These results were compared with three different JCPDS files (JCPDS file no. 04-0836; 48-1548; 89-7102). It was observed that our results showed slight variation from the standard files. However, peaks at 200, 202, 113 and 220 aligned with the JCPDS files (04-0836; 48-1548; 89-7102), from these results we can say that the green synthesized Ca CuNPs were crystalline in nature and had face centered cubic phase. According to [5] and the green synthesized CuNPs have face centered cubic phase and are crystalline. [10]. The SEM images revealed that the green synthesized Ca CuNPs were agglomerated. However, a few spherical nanoparticles were also noted. The agglomeration of nanoparticles occurs due to interaction between the bio organic capping of two adjacent nanoparticles [8,11] and team also reported the agglomeration of CuNPs synthesized from *Ziziphus spina-christi* fruit extract. According to them the O-H group present on the surface of CuNPs was responsible for agglomeration.

The antibacterial activity of Ca CuNPs was studied on gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria. Ca CuNPs showed bacteriostatic effect on both *Staphylococcus aureus* and *Escherichia coli*, According to [9], the CuNPs produces free radical that binds with macromolecules such as protein and nucleic acids, thereby hindering its function. These free radicals also bind with cell membrane and breaches its integrity. Our results align with [12] and team, who reported the antibacterial activity of CuNPs synthesized using *Piper retrofractum Vahl* extract. However, it was observed that these nanoparticles were more effective against gram negative (Escherichia *coli*) bacteria. The difference in antibacterial effect of Ca CuNPs on both the bacteria could be because of the difference in the structural complexity of both the bacteria. The gram positive bacteria (*Staphylococcus aureus*) have thick peptidoglycan cell membrane which gives the gram positive bacteria resistance against the CuNPs [13].

5. Conclusion

This investigation concludes that the green synthesized *Cassia tora* copper oxide nanoparticles have strong antibacterial activity. They were able to inhibit both the gram positive and gram negative bacteria. These nanoparticles could be used as an antibacterial agent; however, further in-depth studies are required before bringing these nanoparticles from bench to bedside.

6. References

[1] Haleem A, Javaid M, Singh RP, Rab S, Suman R. Applications of nanotechnology in medical field: a brief review. Glob Heal J 2023;7:707. https://doi.org/10.1016/j.glohj.2023.02.008.

[2] Rajesh KM, Ajitha B, Reddy YAK, Suneetha Y, Reddy PS. Assisted green synthesis of copper nanoparticles using Syzygium aromaticum bud extract: Physical, optical and antimicrobial properties. Optik (Stuttg) 2018;154:593600. https://doi.org/10.1016/j.ijleo.2017.10.074.

[3] Rejiya CS, Cibin TR, Abraham A. Leaves of Cassia tora as a novel cancer therapeutic - An in vitro study. Toxicol Vitr 2009;23:10348. https://doi.org/10.1016/j.tiv.2009.06.010.

[4] Anyebe DA, Tajudeen YO, Shemishere UB, Yaro CA, Oladele EO, Maiyama MI. Methanol leaf extract of Cassia tora ameliorates dextran sulfate sodium-induced ulcerative colitis in BALB/c mice. Sci African 2021;13:e00865. https://doi.org/10.1016/j.sciaf.2021.e00865.

[5] Gopalakrishnan V, Muniraj S. Neem flower extract assisted green synthesis of copper nanoparticles - Optimisation, characterisation and anti bacterial study. Mater Today Proc 2019;36:8326. https://doi.org/10.1016/j.matpr.2020.07.013.

[6] Hasheminya SM, Dehghannya J. Green synthesis and characterization of copper nanoparticles using Eryngium caucasicum Trautv aqueous extracts and its antioxidant and antimicrobial properties. Part Sci Technol 2020;38:101926. https://doi.org/10.1080/02726351.2019.1658664.

[7] Thien.pdf n.d.

[8] Khani R, Roostaei B, Bagherzade G, Moudi M. Green synthesis of copper nanoparticles by fruit extract of Ziziphus spina-christi (L.) Willd.: Application for adsorption of triphenylmethane dye and antibacterial assay. J Mol Liq 2018;255:5419. https://doi.org/10.1016/j.molliq.2018.02.010.

[9] Wu S, Rajeshkumar S, Madasamy M, Mahendran V. Green synthesis of copper nanoparticles using Cissus vitiginea and its antioxidant and

antibacterial activity against urinary tract infection pathogens. Artif Cells, Nanomedicine Biotechnol 2020;48:11538. https://doi.org/10.1080/21691401.2020.1817053.

[10] Nawabjohn MS, Sivaprakasam P, Anandasadagopan SK, Begum AA, Pandurangan AK. Green Synthesis and Characterisation of Silver Nanoparticles Using Cassia tora Seed Extract and Investigation of Antibacterial Potential. Appl Biochem Biotechnol 2022;194:46478. https://doi.org/10.1007/s12010-021-03651-4.

[11] Thiruvengadam M, Chung IM, Gomathi T, Ansari MA, Gopiesh Khanna V, Babu V, et al. Synthesis, characterization and pharmacological potential of green synthesized copper nanoparticles. Bioprocess Biosyst Eng 2019;42:176977. https://doi.org/10.1007/s00449-019-02173-y.

[12] Amaliyah S, Pangesti DP, Masruri M, Sabarudin A, Sumitro SB. Green synthesis and characterization of copper nanoparticles using Piper retrofractum Vahl extract as bioreductor and capping agent. Heliyon 2020;6:e04636. https://doi.org/10.1016/j.heliyon.2020.e04636.

[13] Padil VVT, Černík M. Green synthesis of copper oxide nanoparticles using gum karaya as a biotemplate and their antibacterial application. Int J Nanomedicine 2013;8:88998. https://doi.org/10.2147/IJN.S40599.