GREEN SYNTHESIS OF COPPER NANOPARTICLES FROM AN EXTRACT OF *AZADIRACHTA INDICA* LEAVES AND CHARACTERIZATION

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Abstract
Copper is a metal which is cheap and more abundant in nature can be harnessed for many applications. The present work reports the green synthesis of copper nanoparticles (Cu NPs) from an extract of *Azadirachta indica* (Neem) leaves as reducing/stabilizing agent. The morphology and stability of the green synthesised Cu NPs were investigated by FESEM, FTIR and UV-Visible spectroscopy. FESEM images confirmed the formation of monodisperse and highly crystalline Cu NPs which are nonhomogeneous in particle size and shape. The formation of copper nanoparticles was confirmed by UV-Vis absorption spectroscopy showing maximum absorption peaks between 615-630nm and FTIR showed the presence of biological molecules responsible for reducing Cu^{+} ions. Different concentrations of aqueous copper sulphate salt were taken maintaining the concentration of the leaf extract and concluded that the concentration of copper sulphate salt does not play a major role in the formation of the nanoparticles. The green synthesised Cu NPs were stable for a long time.

Keywords: Copper nanoparticles, Green synthesis, Leaf extract, Chemical reduction

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DOI: 10.53555/ecb/2022.11.11.248

1. Introduction

In recent years there has been a rigorous research in the field of nanomaterials synthesis and their properties as the nanomaterials show exclusive properties at sizes below 100nm that vary from their bulk counterpart [1, 2]. For bulk materials larger than one micrometre the percentage of atoms at the surface is less compared to the total number of atoms of the material. Whereas in the nanomaterials large number of atoms are present on the surface because of the large surface area due to which they show very interesting and sometimes unexpected properties. Owing to their outstanding properties the nanomaterials are being utilised in the fields of energy conversion, chemical manufacturing, sensors, environmental aspects, biotechnology etc. [3, 4]. For all these applications, the synthesized nanoparticles should possess adequate size distribution, crystallinity and morphology. For this purpose, interest in the synthesis of noble metallic nanoparticles (MNPs) like silver (Ag), gold (Au) and copper (Cu) has increased recently which exhibit novel chemical and physical properties due to small physical dimensions [5]. The metal nanoparticles have unique electronic, magnetic, chemical and mechanical properties due to their small size and large surface area. The MNPs show an interesting optical behaviour known as surface plasmon resonance (SPR) as the nanoparticle diameter becomes comparable with, or smaller than the wavelength of incident light. This behaviour depends upon the size, shape as well as dielectric environment of metal nanoparticles [6, 7]. Among the noble metals, the copper nanoparticle (Cu NP) synthesis has attracted more attention due to its catalytic property, high stability, excellent electrical conductivity and low cost compared to Au and Ag [8-10]. Copper metallic NPs also support Plasmon resonance in visible region of the solar spectrum. Cu NPs have many applications as sensors [11-13], antimicrobial systems [14, 15], heat transfer systems [16], catalyst [17-19] and super strong materials [20, 21]. Cu NPs can be synthesised using chemical, physical and biological methods. The physical and chemical methods are complicated, require specific equipment, produces toxic residues and relatively high material cost. In the last few years the biological methods of NP synthesis have attracted great attention due to its simple, direct, nontoxicity and eco-friendly characteristics. The biological method of NPs synthesis carried out by various sources actinomycetes, fungal, bacterial, yeast, viruses, algal [22–27] and plant extracts. Plants are reservoir of phytochemicals such as polyphenols, flavonoids, terpenoids, alkaloids, vitamins, polysaccharides, saponins and proteins which act as reducing, capping and stabilizing agents for the biosynthesis of NPs and have the property to minimize the agglomeration of NPs [28]. Green synthesis of Cu NPs using various plants including, Jatropha curcas (JC), Celastrus paniculatus, Allium sativum, *Azadirachta indica* have been reported [29-33]. In fact, pure metallic Cu NPs in an aqueous phase is still a challenge for the researcher. Furthermore, it is of interest to obtain monodispersed Cu NPs by a simple and green rout. This study reports a green route for the synthesis of Cu NPs using *Azadirachta indica* leaf extract in aqueous medium which is simple, nontoxic and eco-friendly. The green synthesized Cu NPs were characterised using UV-Visible spectrophotometry, Fourier transform infrared (FT-IR) spectroscopy and Field emission scanning electron microscopy (FESEM). The surface plasmon resonance of the synthesized Cu NPs are studied in detail which helps to foresee its energy harvesting property.

2. Experimental

2.1Preparation of Plant Extract

Azadirachta indica (Neem) plant extract was used for the synthesis of copper nanoparticles. Fresh leaves of Azadirachta indica were collected, they were surface cleaned with running tap water to remove debris and other contaminated organic contents, followed by double distilled water and air dried at room temperature. About 15gm of finely cut leaves were taken in a beaker containing 200 ml double distilled water (DDW) and boiled at 70^0C for 40 minutes using magnetic stirrer until the colour of the leaf extract changes to green. Furthermore, the extract of the leaves was kept at room temperature for cooling, filtered using whatman filter paper no.1 covered with aluminium foil and stored at room temperature for the synthesis of Cu NPs.

2.2Synthesis of Copper Nanoparticles

For the synthesis of Copper Nanoparticles copper sulphate ($CuSO_4.5H_2O$) salt of GR grade is taken. Two samples having different concentrations of copper sulphate salt were prepared, sample A having 35mg copper sulphate salt and sample B having 2.5gm copper sulphate salt dissolved in 100ml double distilled water at room temperature. The two samples were thoroughly mixed using magnetic stirrer at 70^0C. While stirring in sample A 10ml (Sample C) and 20ml (Sample D) of green plant extract was added drop wise using burette very slowly until the blue colour of copper sulphate solution changes completely to white colour indicating the formation of Cu NPs. Similarly using the same procedure 10ml (Sample E) and 20ml (Sample F) of green plant extract was added to Sample B until the colour changes from dark blue to light blue colour indicating the formation of Cu NPs which was confirmed by UV-Vis spectroscopy measurements. All the prepared samples were characterised using UV-VIS spectrophotometry in the visible range 200-800nm. The chemical binding of the synthesised Cu NPs were analysed using FT-IR spectrometer. The reduction of Cu ions to Cu NPs and the uniformity of the synthesised Cu NPs were confirmed by FESEM images. All the characterizations were performed at Central facilities for research and development, Osmania University, Hyderabad.

3. Results an Discussion

3.1 SEM Analysis

The FESEM analysis was carried out to evaluate the surface morphology and uniformity of the green synthesised Cu NPs. The FESEM images shown in Fig.1 with different magnifications confirmed the formation of Cu NPs. Mono-dispersive and highly crystalline copper nanoparticles are obtained. The FESEM images also demonstrated the nonhomogeneity of the particles in terms of their size and shape. All the possible spherical and irregular shapes such as truncated hexagonal, cylindrical, and triangular shapes of Cu NPs with varying particle sizes were found in the micrographs. Due to high surface area Particles were found to moderately agglomerate resulting in the formation of medium-sized particles. From geometry, it is clear that the sizes of small individual particles are less than 100 nm in diameter, while the composite particles in lower resolution would appear higher in particle. Copper has a closed packed structure and they have a tendency to agglomerate due to high surface energy and high surface tension of the ultrafine nanoparticles.

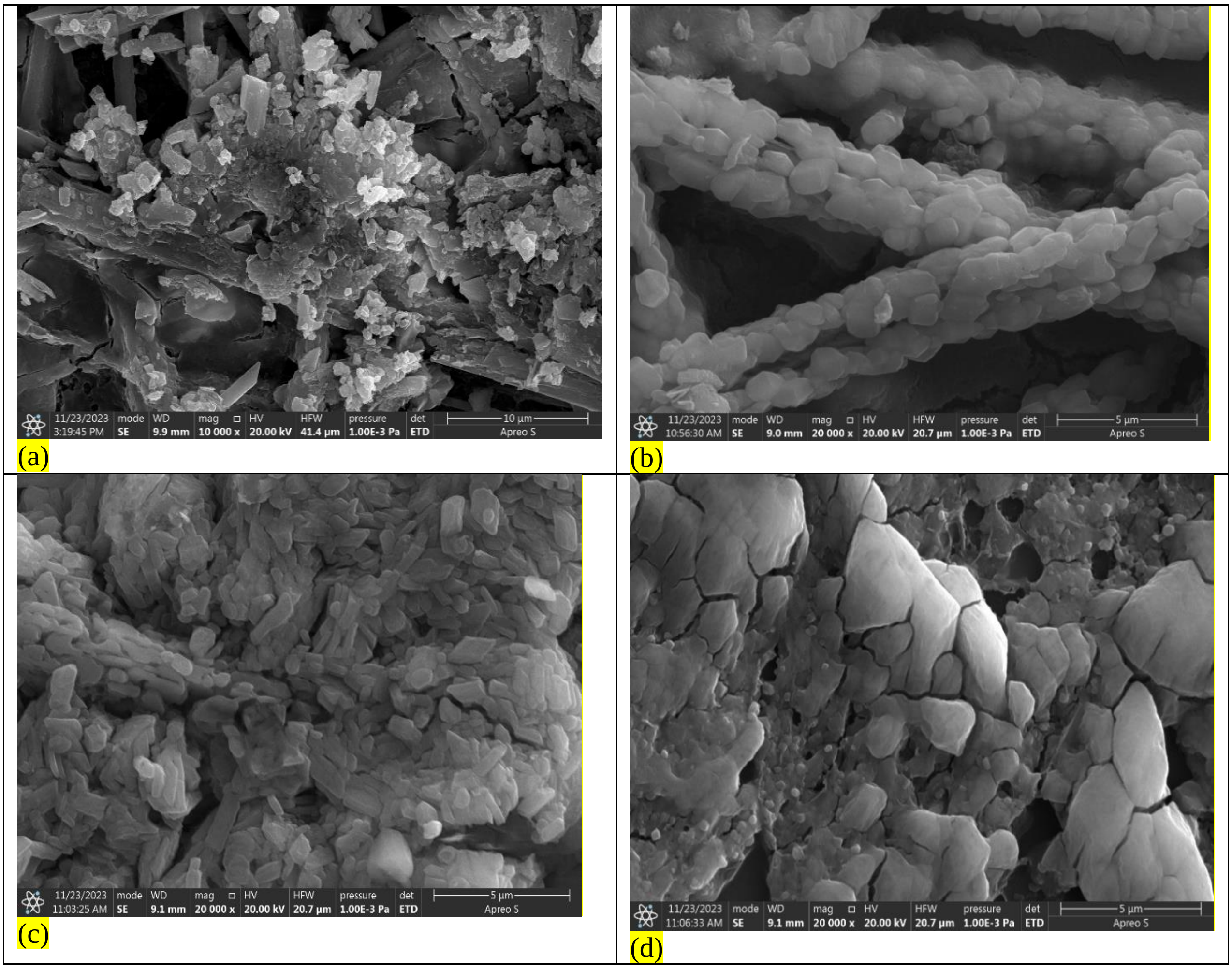


Fig. 1 FESEM images of Cu NPs prepared with 35mg of $CuSO_4$ salt and 10ml (a), 20ml (b) leaf extract and (c), (d) with 2.5gm of CuSO4 salt and 10ml, 20ml leaf extract at different magnifications.

3.2 UV-Visible Analysis

Fig. 2 shows the UV-spectra of the green synthesised Cu NPs. In all experiments, addition of plant extract of *Azadirachta indica* into the conical flask containing aqueous solution of copper sulphate led to the change in the colour of the solution. This change in colour is due to the interaction between conduction electrons of metal NPs and incident photons [34]. In sample A containing 35mg of aqueous copper salt when 10ml and 20ml of green plant extract was added the colour changed from blue to white indicating the formation of Cu NPs which was confirmed by the UV-Vis spectroscopy absorption peak at 630nm for 10ml of leaf extract and an absorption peak at 615nm for 20ml leaf extract and for both the samples the absorption has increased in visible region [35, 36]. Similarly, for the sample B containing 2.5gm aqueous copper salt the colour changed from blue to light blue after adding 10ml and 20ml green plant extract indicating the reduction of copper ions to Cu NPs which was confirmed by the UV-Visible absorption peaks at 615nm for 10ml and 800nm for 20ml leaf extract. Further an increase in the absorption is observed for 20ml green plant extract. The electronic spectra of the Cu NPs arises

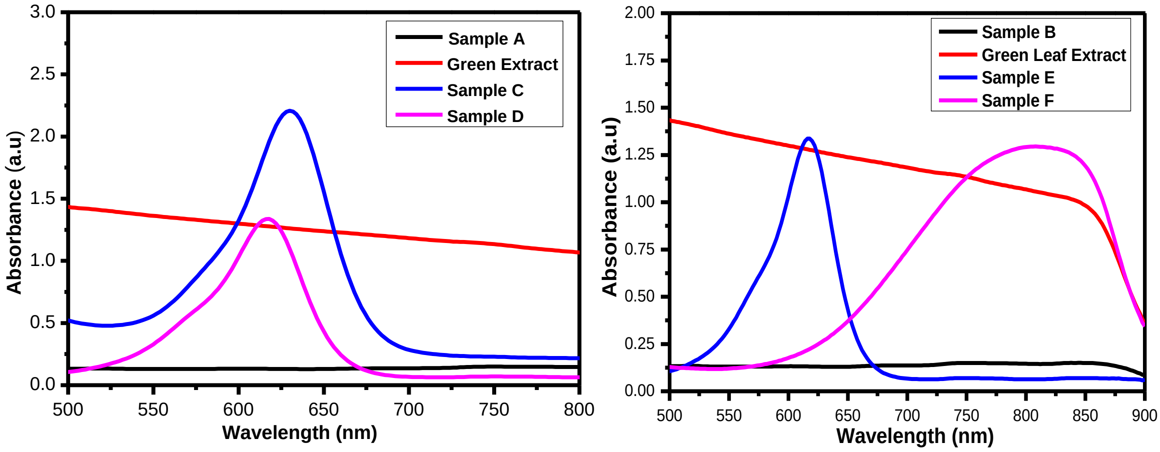
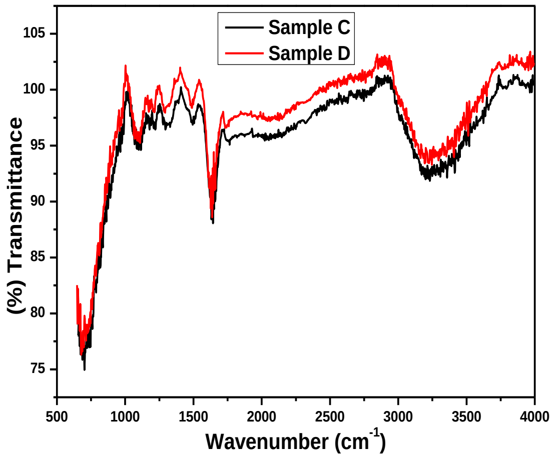


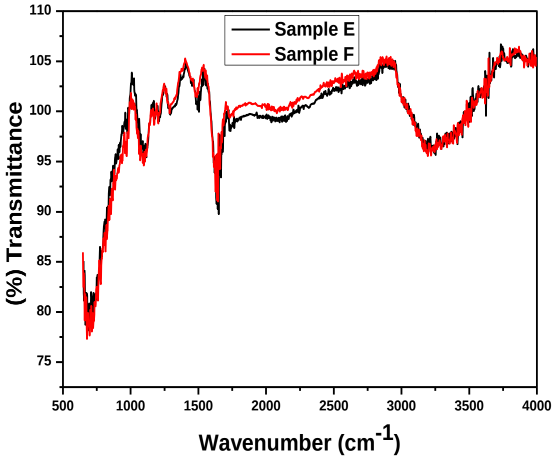
Fig. 2 UV-Visible Spectra of Synthesized Cu NPs collected for different concentrations of the plant extract.

due to the excitation of surface plasmon resonance phenomena [37]. The characteristic surface plasmon resonance peak of Cu NPs is around 560nm [38] for particle diameters ranging between 10 and 40nm. Whereas the smaller particles of sizes less than 20nm show a resonance peak at lower wavelengths with increased absorbance indicating the onset of nanoparticle formation. The variation in its bandwidth and the shift in the resonance are thus exciting parameters to characterize the metal nanoparticles. Small metal nanoparticles exhibit the absorption of visible electromagnetic waves by the collective oscillation of conduction electrons at the surface [38]. This is known as the surface plasmon resonance effect. The interest in this effect is the possibility of using it as a tracer for the presence of metal nanoparticles with a simple UV-visible spectrometer. The size dependence of the plasmon resonance for particles smaller than 20 nm [38] is a complex phenomenon.

3.3 FT-IR Spectroscopy

FT-IR Spectroscopy analysis was carried out to identify the possible biomolecules in green plant extract which are responsible for the reduction and stabilisation of Cu NPs. The FTIR spectra of the green synthesised Cu NPs are shown in Fig. 3 for all the samples. The broad peak at 3260 cm^{-1} corresponds to the hydroxyl (-OH) functional groups in alcohols and phenolic compounds, 1640 cm-1 represents the aromating bending of alkene group (C=C), 1500 cm^{-1} corresponds to (C-H) bending of alkane, 1320 cm^{-1} (C-O-C) stretching of ether group, 1100 cm^{-1} (C-O) stretching, 700 cm^{-1} represents the deformation vibration of (C-H) bonds in phenolic rings respectively. These results of FTIR analysis of the green synthesised Cu NPs indicated a unique set of the biochemical markers such as phenols, aromatic rings, aldehydes, alkenes and alkanes [39]. The flavonoid biomolecules transformed enol-form to the keto-form by releasing a reactive hydrogen atom and that can reduce Cu^{2+} ions to form Cu NPs. These biomolecules stabilize NPs by chelating with metal ions with their carbonyl groups or π-electrons [40]. Thus, results conclude that the surface of green synthesized Cu NPs were capped and stabilized by flavonoid and other phenolic compounds in the Azadirachta indica leaf extract.





4. Conclusion

In summary, this study presented a simple, cost-effective and eco-friendly green synthesis of Cu NPs using *Azadirachta indica* (Neem) leaf extract which acts as both reducing/stabilising and capping agent. Though the change in the colour of the aqueous $CuSO_4$ solution after the addition of the leaf extract confirmed the formation of Cu NPs, the same was confirmed by the UV-Vis spectroscopy absorption peaks between 610-630nm for all the samples. FTIR results confirmed the presence of functional groups responsible for the reduction of Cu^+ ions to Cu NPs. The green synthesised Cu NPs were stable for a very long time in the aqueous medium. Finally, we conclude that there was no effect of the concentration of $CuSO_4$ salt in the solution, the quantity of the plant extract in the solution plays a major role for the formation of nanoparticles.

REFERENCES

1) M. Salavati-Niasari, F. Davar, N. Mir, "Synthesis and characterization of metallic copper nanoparticles via thermal decomposition", *Polyhedron*, **27** (2008) 3514–3518.
2) J. Jang, "Conducting polymer nanomaterials and their applications", *Emissive Mater. Nanomater.*, (2006) 189–260.
3) B. Luo, S. Liu, L. Zhi, "Chemical approaches toward graphene-based nanomaterials and their applications in energy-related areas", *Small*, **8** (2012) 630–646.
4) S. Zinjarde, "Bio-inspired nanomaterials and their applications as antimicrobial agents", *Chronicles of Young Scientists*, **3** (2012) 74–74.
5) M. Grzelczak, J. P. Juste, P. Mulvaney, L. M. L. Marzan, "Shape control in gold nanoparticle synthesis", *Chem. Soc. Rev*. **37** (2008) 1783–1791.
6) K. L. Kelly, E. Coronado, L. L. Zhao, G. C. Schatz, "The optical properties of metal nanoparticles: the influence of size, shape, and dielectric environment", *J. Phys. Chem. B*, **107** (2003) 668–677.
7) K. R. Catchpole, A. Polman, "Shape control in gold nanoparticle synthesis", *Appl. Phys. Lett*. **93** (2008) 191113.
8) Y. Sui, W. Fu, H. Yang, Y. Zeng, Y. Zhang, Q. Zhao, Y. Li, X. Zhou, Y. Leng, M. Li, G. Zou, "Low temperature synthesis of Cu2O crystals: shape evolution and growth mechanism", *Cryst. Growth Des*. **10** (2010) 99–108.
9) J. Xu, K. Yu, Z. Zhu, "Synthesis and field emission properties of Cu dendritic nanostructures", *Physica E*, **42** (2010) 1451–1455.
10) Y. Chang, M. L. Lye, H. C. Zeng, "Large-scale synthesis of high-quality ultralong copper nanowires", *Langmuir*, **21** (2005) 3746–3748.
11) X. Kang, Z. Mai, X. Zou, P. Cai, J. Mo, "A sensitive nonenzymatic glucose sensor in alkaline media with a copper nanocluster/multiwall carbon nanotube-modified glassy carbon electrode", *Anal. Biochem*. **363** (2007) 143–150.
12) K. B. Male, S. Hrapovic, Y. Liu, D. Wang, J. H. T. Luong, "Electrochemical detection of carbohydrates using copper nanoparticles and carbon nanotubes", *Anal. Chim. Acta*, **516** (2004) 35–41.
13) Q. Xu, Y. Zhao, J. Z. Xu, J. J. Zhu, "Preparation of functionalized copper nano-particles and fabrication of a glucose sensor", *Sens. Actuators B*, **114** (2006) 379–386.
14) A. Esteban-Cubillo, C. Pecharroman, E. Agilar, J. Santaren, J. Moya, "Antibacterial activity of copper monodispersed

nanoparticles into sepiolite", *J. Mater. Sci*. **41** (2006) 5208–5212.

15) N. Cioffi, N. Ditaranto, L. Torsi, R. A. Picca, E. D. Giglio, L. Sabbatini, L. Novello, G. Tantillo, T. Bleve-Zacheo, P. G. Zambon, "Synthesis, analytical characterization and bioactivity of Ag and Cu nanoparticles embedded in poly-vinyl-methyl-ketone films", *Anal. Bioanal. Chem*. **382** (2005) 1912–1918.
16) K. B. Male, S. Hrapovic, Y. Liu, D. Wang, J. H. T. Luong, "Electrochemical detection of carbohydrates using copper nanoparticles and carbon nanotubes", *Anal. Chim. Acta*, **516** (2004) 35–41.
17) M. L. Kantam, V. S. Jaya, M. J. Lakshmi, B. R. Reddy, B. M. Choudary, S. K. Bhargava, "Alumina supported copper nanoparticles for aziridination and cyclopropanation reactions", *Catal. Commun*. **8** (2007) 1963–1968.
18) S. Vukojevic, O. Trapp, J.D. Grunwaldt, C. Kiener, F. Schuth, "Quasihomogeneous methanol synthesis over highly active copper nanoparticles", *Angew. Chem. Int. Ed*. **44** (2005) 7978–7981.
19) M. I. Awad, T. Ohsaka, "An electrocatalytic oxygen reduction by copper nanoparticles-modified Au(100)-rich polycrystalline gold electrode in 0.5 M KOHJ", *J. Power Sources,* **226** (2013) 306–312.
20) Y. Wang, M. Chen, F. Zhou, E. Ma, "High tensile ductility in a nanostructured metal", *Nature*, **419** (2002) 912–915.
21) R. K. Guduru, K. L. Murty, K. M. Youssef, R. O. Scattergood, C. C. Koch, "Mechanical behavior of nanocrystalline copper", *Mater. Sci. Eng. A*, **463** (2007) 14–21.
22) P. Prema, P.A. Iniya, G. Immanuel, "Microbial mediated synthesis, characterization, antibacterial and synergistic effect of gold nanoparticles using Klebsiella pneumoniae (MTCC-4030)", *RSC Adv*. **6** (2016) 4601–4607.
23) A. I. El-Batal, G. S. El-Sayyad, F. M. Mosallam, R. M. Fathy, "Penicillium chrysogenum-mediatedmycogenic synthesisof copperoxidenanoparticlesusing gamma rays for in vitro antimicrobial activity against some plant pathogens", *J. Clust. Sci*. **31** (2020) 79–90.
24) S. E. D. Hassan, A. Fouda, A. A. Radwan, S. S. Salem, M. G. Barghoth, M. A. Awad, A. M. Abdo, M. S. El-Gamal, "Endophytic actinomycetes Streptomyces spp mediated biosynthesis of copper oxide nanoparticles as a promising tool for biotechnological applications", *J. Biol. Inorg. Chem*. **24** (2019) 377–393.
25) A. Sivaraj, V. Kumar, R. Sunder, K. Parthasarathy, G. Kasivelu, "Commercial yeast extracts mediated green synthesis of silver chloride nanoparticles and their anti-mycobacterial activity", *J. Clust. Sci*. **31** (2020) 287–291.
26) A. Rahman, S. Kumar, T. Nawaz, "Biosynthesis of Nanomaterials Using Algae", *Microalgae Cultivation for Biofuels Production*, Chapter 17 (2020) 265–279.
27) A. M. Wen, N. F. Steinmetz, "Design of virus-based nanomaterials for medicine, biotechnology, and energy", *Chem. Soc. Rev*. **45** (2016) 4074–4126.
28) A. Pugazhendhi, R. Prabhu, K. Muruganantham, R. Shanmuganathan, S. Natarajan, "Anticancer, antimicrobial and photocatalytic activities of green synthesized magnesium oxide nanoparticles (MgONPs) using aqueous extract of Sargassum wightii", *J. Photochem. Photobiol. B Biol*. **190** (2019) 86–97.
29) M. K. Ghosh, S. Sahu, I. Gupta, T. K. Ghorai, "Green synthesis of copper nanoparticles from extract of Jatropha Curcas leaves: characterization, optical properties, CT-DNA binding and photocatalytic activity", *RSC Adv*. **10** (2020) 22027-22035.
30) S. C. Mali, A. Dhaka, C. K. Githala, R. Trivedi, "Green synthesis of copper nanoparticles using Celastrus paniculatus Willd. leaf extract and their photocatalytic and antifungal properties", Biotech. Reports, 27 (2020) e00518.
31) A. Yaqub, N. Malkani, A. Shabbir, S. A. Ditta, F. Tanvir, S. Ali, M. Naz, S. A. R. Kazmi and Rehan Ullah, "Novel Bio-synthesis of copper nanoparticles using Zingiber and Allium sp. With synergic effect of doxycycline for anticancer and bactericidal activity", *Current Microbiology*, **77** (2020) 2287-2299.
32) N. Nagar, V. Devra, "Green synthesis and characterization of copper nanoparticles using Azadirachta indica leaves", *Mater. Chem. Phys*. **213** (2018) 44-51.
33) I. L. Ikhioya, A. C. Nkele, F. U. Ochai-Ejeh, "Green synthesis of copper oxide nanoparticles using neem leaf extract (Azadirachta indica) for energy storage applications", *Mater. Res. Innovations*, (2023).
34) J. Jana, M. Ganguly, T. Pal, "Enlightening surface plasmon resonance effect of metal nanoparticles for practical spectroscopic

application", *RSC Adv*. **6** (2016) 86174–86211.

35) Y. Abboud, T. Saffaj, A. Chagraoui, A. El Bouari, K. Brouzi, and O. Tanane, B. Ihssane, "Biosynthesis, characterisation and antimicrobial activity of copper oxide nanoparticles (CONPs) produced using brown alga extract (Bifurcaria bifurcate)", *Appl. Nanosci*., **4(5)** (2014) 571–576.
36) K. Borgohain, N. Murase, and S. Mahamuni, "Synthesis and properties of Cu_2Oquantum particles", *J Appl. Phys*., **92(3)** (2002) 1292–1297.
37) M. K. Ghosh, S. Pathak, T. K. Ghorai, "Synthesis of two mononuclear Schiff base metal (M=Fe, Cu) complexes: MOF structure, Dye degradation, H_2O_2 sensing and DNA binding property", *ACS Omega*, **4** (2019) 16068–16079.
38) S. Link and M. A. El-Sayed, "Shape and size dependence of radiative, non-radiative and photothermal properties of gold nanocrystals" *Int. Rev. Phys. Chem*., **19(3)** (2000) 409-453.
39) R. N. Oliveira, M. C. Msncini, F. C. S. Oliveira, T. M. Passos, B. Quility, R. M. S. Thire, G. B. Mcguiness, "FTIR analysis and quantification of phenols and flavonoids of five commercially available plants extracts used in wound healing", *Rev. Mater*. **21** (2016) 767-779.
40) A. Akturk, F.K. Güler, M.E. Taygun, G. Goller, S. Küçükbayrak, "Synthesis and antifungal activity of soluble starch and sodium alginate capped copper nanoparticles", *Mater. Res. Express*, **6** (2019) 1250g3.