

# Green synthesis of Copper oxide and Cobalt oxide nanoparticles using *Spinacia Oleracea* leaf extract

Authors: Yameen Ahmed <sup>1,2\*</sup>, Jamshaid Hussain <sup>1</sup>, Sohaib Asif <sup>3</sup>

Affiliations: <sup>1</sup> Hazara University Mansehra, KPK

<sup>2</sup> School of Physical Science and Electronics, Central south university

<sup>3</sup> School of Computer Science and Engineering, Central South University

\*Corresponding Author: yameenwazir@yahoo.com

## Abstract

The investigation aims at the synthesis of copper oxide and cobalt oxide nanoparticles using *Spinacia Oleracea* leaf extract. The *Spinacia Oleracea* leaf extract behaves as a reducing agent in the nanoparticle synthesis. Plant extract was first prepared and then treated with copper and cobalt salt solutions to get the precipitate. The salt solutions used for this purpose are copper sulphate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) and cobalt chloride hexahydrate ( $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ). The UV-Vis, XRD, EDX and SEM techniques are used to find optical, structural and morphological properties of copper oxide and cobalt oxide nanoparticles. The average crystallite size of copper oxide nanoparticles is 44 nm and have a hexapod structure. The UV absorption peaks are at 326 nm and 506 nm for copper oxide and cobalt oxide nanoparticles.

## Introduction

In the nanoscience applications, nanoparticles with a size of less than 100 nm are being widely used [1]. Metal oxide and metal nanoparticles (NPs) are emerging candidates in the field of nanotechnology and nanoscience. Depending on their shape and size, these nanoparticles with optical, electrical and physical properties play a vital role. They have a large number of applications, such as antioxidants, catalysis, sensors, antibacterial, etc. Oxides of metal nanoparticles such as, CoO, ZnO, TiO<sub>2</sub>, SnO<sub>2</sub> and CuO, have been studied for their biomedical and environmental applications [2]. For the synthesis of nanoparticles, various chemical and physical methods have been used which includes; high-temp solution phase, thermal decomposition, hydro-thermal micro emulsion and reduction etc. [3]. However, green synthesis of nanoparticles is considered as a significant branch of nanotechnology [4, 5]. This method is cost effective and eco-friendly as compared to the conventional synthesis methods, where chemical additives, high temperature pressure and energy are used [6]. Therefore, it is necessary to develop and use safe methods which must be low cost, efficient, and non-hazardous and environment friendly. Due to this reason, many researchers adopted green synthesis method to fabricate nanoparticles [7]. Materials which are derived from plants are used to prepare nanoparticles and is also the best alternative to chemical and physical method. Plant extracts contain bio-active compounds like flavonoids, saponin, phenolic acid and tannins [8]. These bioactive compounds are good chelation agents and can donate hydrogen and quench singlet oxygen. Due to their redox activities green synthesis of NPs is more suitable than the physio-chemical techniques. Plant extracts can be processed via easy protocols and are easy to handle and non-toxic [9, 10].

Copper oxide nanoparticles exhibit high potentiality in the metal oxide NPs owing to its antimicrobial, catalytic, optical and low-cost properties. Copper oxide NPs have a bandgap ranging from 1.35-3.5 eV. Due to their suitable bandgap, easy availability, low toxicity and surface synthesis among different semiconductor photocatalysts [11]. Copper oxide NPs gain attention due to its good oxygen adsorption capability and large surface area and considered a promising candidate with good photocatalytic activity [12]. Copper oxide is a widely studied semiconductor of II-VI group and is a p type semiconductor. Because to its good optical properties which allows stable emission at room temperature, it is used in various applications such as photonic and field emission devices and sensors [13]. Copper oxide NPs can

be used as magnetic storage media, optical switch and gas switch due to its photochemical and photoconductive properties [14].

Cobalt oxide NPs are used in wide range of applications owing to their oxidation as well as high resistance to corrosion [15]. It is a p-type anti-ferromagnetic semiconductor. It is a multifunctional material with many applications such as energy storage materials, anode in Li-ion batteries, dyes and pigments, sensors and heterogeneous catalysis [16]. Apart from the green synthesis, different chemical and physical techniques were used to synthesis cobalt oxide nanoparticles such as solution combustion method, microwave process, thermal decomposition of sol gel, hydrothermal reaction etc. [17].

Earlier reports of green-synthesized CuO-NPs are investigated using plant extract such as Albizia lebbek [18], Euphorbiaesula [19], Bifurcaria bifurcate [20] and Nerium oleander [21], Aspergillus fumigates [22], Madhuca longifolia [23] and Lemongrass [24]. Arunkumar et al. [11] have reported that the Lanatana camara leaf extract-mediated synthesized CuO-NPs degraded the MB dye at 94% on irradiation with sunlight. Sathiyavimal et al. [25] have reported that the Sidaacuta leaf extract-mediated synthesized CuO-NPs degraded 93% MB dye in bout 100 min. The biosynthesized CuO-NPs using Gloriosa superba leaf extract were examined under Gram-negative and Gram-positive bacteria and relatively more susceptible to the CuO-NPs than E. coli bacteria [26].

Plant extract mediated synthesis of  $\text{Co}_3\text{O}_4$  NPs has been investigated in the following research works. Dubey et al. [27] reported the synthesis of cobalt oxide NPs using latex of Calotropis procera. Bibi et al. [28] used Punica granatum peel extract for the fabrication of cobalt oxide NPs from cobalt nitrate hexahydrate. Diallo et al. [29] discovered the potential of leaves of Aspalathus linearis extract as bioreduction agent for synthesizing of  $\text{Co}_3\text{O}_4$  NPs.

## Materials and Methods

### Preparation of Leaf Extract

For the preparation of leaf extract, fresh leaves of Spinacia Oleracea (Spinach) were collected from the local field of Dhodial, Mansehra by wearing gloves. These leaves were then washed 3 times with tap water to remove dust from it, washed it again 3 times with distilled water and placed them on a clean aluminum foil to dry for about 3 hours. After that, the leaves were cut into small pieces with the help of scissors. Then weighed 10 g of cut leaves with the help of electric weight balance, put these 10gms leaves in 100 ml deionized water and placed a magnetic bar into it and sealed the opening of the beaker with aluminum foil to protect it from the dust present in the surroundings, then placed the beaker on magnetic stirrer for 50 minutes at 70°C. We saw that the color of the water changed to green. The beaker is removed from the magnetic stirrer and placed it on a table for about 20 min and let it cooled. When the water got cooled, it is filtered with the help of Whatmann filter paper in a conical flask. The flask is then sealed with aluminum foil.

Table 1 Details of salt concentrations

Salts	Distilled water (ml)	Salt Concentration (M)	Salt concentration (g)
Copper Sulphate Pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ )	100	0.12	3
Cobalt Chloride Hexahydrate ( $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ )	100	0.106	2.5

### Synthesis of Copper Oxide Nanoparticles

Placed the copper sulphate pentahydrate solution on the magnetic stirrer and put the magnetic bead in the beaker. After about 20 minutes of heating, 10 ml of spinacia oleracea leaf extract was added drop wise in the solution using a burette. About 10 minutes after addition of leaf extract, the color the solution changed from light blue to green. The temperature at that time was noted to be 70°C. Then, removed the solution

from the magnetic stirrer and placed it on a safe place to let it cool, also covered the beaker with aluminum foil to protect it from dust particles. Next day the cooled solution then put into three test tubes, these test tubes were then placed into the centrifuge for 20 minutes and set the centrifuge to 4500 rotations per minute. One test tube is then used for UV and the other two were saved for further characterization.

### Synthesis of Cobalt Oxide Nanoparticles

Placed the cobalt chloride hexahydrate solution on the magnetic stirrer and put the magnetic bead in the beaker. After about 15 minutes of heating, 15 ml of spinacia oleracea leaf extract was added drop wise in the solution using a burette. About 10 minutes after the addition of leaf extract the color the solution changed from light pink to reddish, the temperature at that time was noted to be 70°C. Then, removed the solution from the magnetic stirrer and placed it on a safe place to let it cool, also covered the beaker with aluminum foil to protect it from dust particles. Next day the cooled solution then put into three test tubes, these test tubes were then placed into the centrifuge for 20 minutes and set the centrifuge to 4500 rotations per minute. One test tube is then used for UV and the other two were saved for further characterization.

Table 2 Details about the synthesis of nanoparticles

Salt	Salt concentrations (ml)	Leaf extract concentrations (ml)	Time taken for heating (min)	Temperature (C)	Time taken for centrifuge (min)
(CuSO <sub>4</sub> .5H <sub>2</sub> O)	0.12	10	30	70	20
(CoCl <sub>2</sub> .6H <sub>2</sub> O)	0.106	15	30	70	20

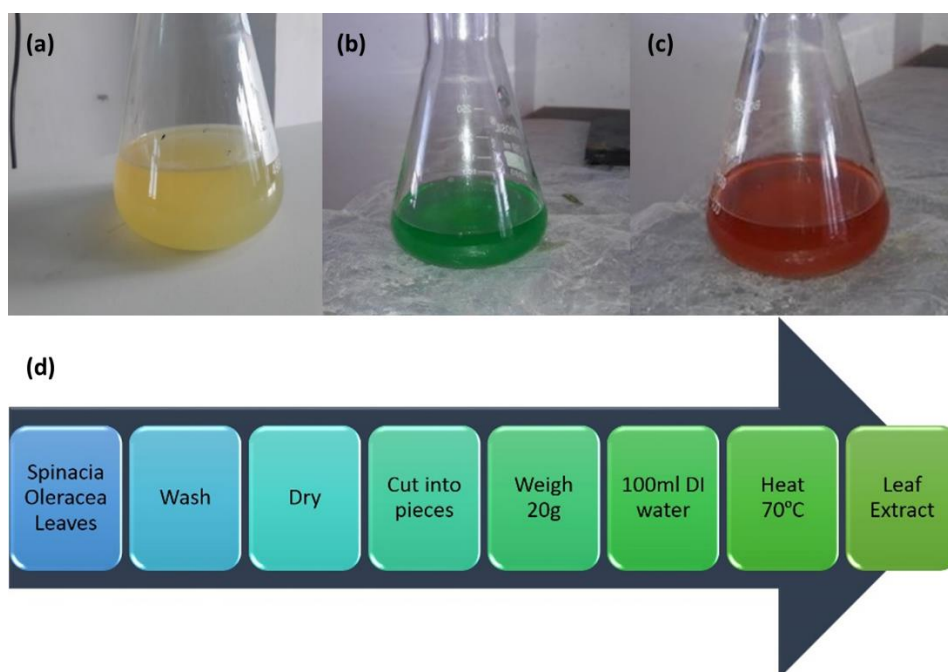


Figure 1 (a) leaf extract, (b) Copper oxide nanoparticles solution, (c) Cobalt oxide nanoparticles solution, (d) Flow chart for the preparation of leaf extract

## RESULT AND DISCUSSIONS

The absorbance peak for biosynthesized Copper oxide nanoparticles from *Spinacia Oleracea* extract was at 326 nm indicating the formation of copper oxide nanoparticles from Copper Sulphate Pentahydrate. To analyze the optical properties of extracted copper oxide NPs, the optical absorption spectra was used. From this spectrum the type of electronic transition and the band gap of the material were determined [30]. When a material absorbs photon of energy greater than the energy band gap, an electron is transported to the conduction band (CB) from the valence band (VB), an immediate rise in the absorbance of the semiconductor to the wavelength occurs corresponding to the energy of the band gap. The relationship of the incident photon to the absorption coefficient depends on the electronic transition type. The transition is direct if the momentum is conserved and it is indirect if the momentum is not conserved. The figure shows the absorption spectrum of copper oxide nanoparticles. The UV-Vis spectrum showed the absorption peak at 326 nm and the cut off wavelength at 400 nm. The reported material is a direct band gap semiconductor material. According to the literature, the absorption peak of copper oxide nanoparticles ranges from 250-300 nm, but in our work the absorption peak is at 326 nm. The higher value of absorption peak is due to the formation of some other metastable and stable copper oxide NPs. The energy band gap was calculated from the equation  $E_g = hc/\lambda$ . Where  $\lambda$  is the cut-off wavelength,  $c = 3 \times 10^8$  m/s is the speed of the light and  $h = 4.13566 \times 10^{15}$  eV is the Plank's constant. The energy bandgap comes out to be 3.1 eV. The cut-off wavelength was obtained by extrapolating the line to the base, where  $\alpha$  is zero. As compared to the bulk value (3.25 eV), there is a slight blue shift in the band gap (3.1 eV) [30] which is due to the quantum confinement effect [31].

The absorbance peak for biosynthesized Cobalt oxide NPs from *Spinacia Oleracea* extract was at 509 nm indicating the formation of cobalt oxide nanoparticles from cobalt chloride hexahydrate. The UV-Vis spectrum of cobalt oxide NPs showed the absorption peak at 509 nm. This absorption band can be attributed to the plasma resonance absorption of the cobalt oxide nanoparticles. Cobalt oxide nanoparticles show a plasmon absorption band in the range of 350-550 nm which is its distinguishing feature [32]. The formation of non-oxide cobalt nanoparticles might be the reason of the strong surface plasmon. The peel extracts of *Spinacia Oleracea* acts as a reducing-cum-surface capping agent that can be credited to the fabrication of nanoscale cobalt oxide. Cobalt oxide NPs were formed from the reduction of cobalt chloride hexahydrate in the presence of *Spinacia Oleracea* leaf extract, which act as a reducing, stabilizing and capping agent [33]. The energy band gap was calculated from the equation:  $E_g = hc/\lambda$ , where  $h = 4.13566 \times 10^{15}$  eV is the Plank's constant,  $c = 3 \times 10^8$  m/s is the light velocity, and  $\lambda$  is the cut-off wavelength. The energy bandgap comes out to be 2.15 eV. Extrapolation of the line to the base gives the cut-off wavelength 575 nm.

The XRD spectrum (Fig. 4.3) reveals the diffraction peaks at different angles. XRD diffraction peaks at  $24.406^\circ$  and  $25.978^\circ$  corresponds to the planes (202) and (101) of Monoclinic CuO. The peak at  $29.775^\circ$  corresponds to the plane (110) of Cubic Cu<sub>2</sub>O. The peak at  $36.197^\circ$  corresponds to the plane (004) of Tetragonal Cu<sub>4</sub>O<sub>3</sub>. The peaks at  $18.283^\circ$ ,  $18.553^\circ$ ,  $20.03^\circ$ ,  $27.93^\circ$ ,  $29.543^\circ$ ,  $31.653^\circ$ ,  $44.656^\circ$ ,  $55.071^\circ$ ,  $57.823^\circ$  corresponds to the planes (200), (103), (022), (301), (204), (153), (008), (056), (525) of Orthorhombic Cu<sub>64</sub>O. The most intense peak is observed at  $2\theta = 25.978^\circ$  that corresponds to the diffraction of the CuO nanoparticles having monoclinic structure. In our work, in addition to the stable oxides, i.e., CuO and Cu<sub>2</sub>O, copper with incorporated oxygen phases Cu<sub>4</sub>O<sub>3</sub>, Cu<sub>8</sub>O, and Cu<sub>64</sub>O have also been reported. These oxides of copper are also found in pure commercial copper powder. These are metastable phases formed in the early oxidation stage of copper. The suboxide Cu<sub>64</sub>O is formed as the interstitial mixture of very low content of O atoms in the Cu lattice with a ratio 1:64 of O to Cu.

The size of the nanoparticles was calculated by Debye-Scherrer equation.

$$D = (0.9\lambda) / \beta \cos\theta$$

Where  $\lambda$  is the wavelength of X-rays ( $\text{Cu } K\alpha = 1.54604 \text{ \AA}$ ),  $\beta$  is the full width half maximum value in terms of radians,  $\theta$  refers to diffraction angle. The average size of copper oxide nanoparticles was calculated using Scherer equation is 44nm as shown in table 4.1

Table 3 Details of size estimation, Bragg's angle and miller indices obtained from XRD

Degree $^{\circ}$	(hkl)	FWHM	Size (nm)	Average Size (nm)
24.406	202	0.236	34	44
25.978	101	0.177	46	
29.778	110	0.472	17	
36.197	004	0.197	42	
18.283	200	0.098	82	
18.553	103	0.098	82	
20.03	022	0.118	68	
27.93	301	0.197	42	
29.543	204	0.157	52	
31.65	153	0.197	45	
44.65	008	0.394	22	
55.071	056	0.472	19	
57.823	525	0.394	23	

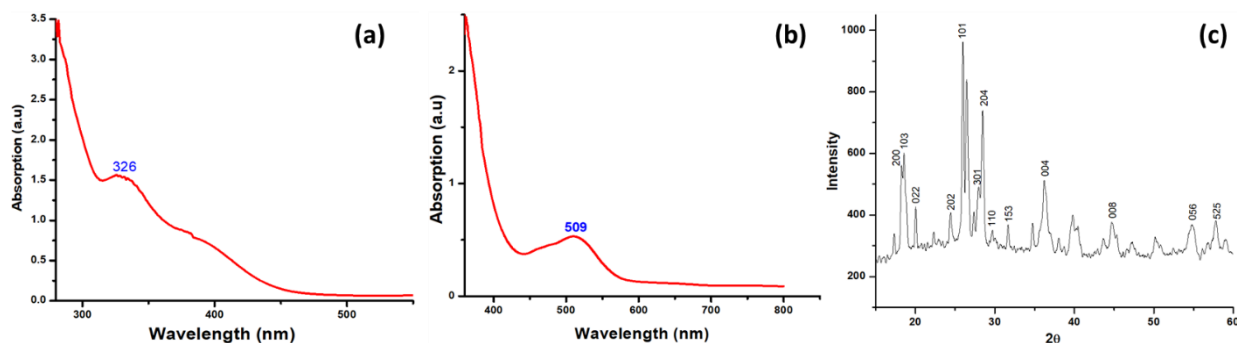


Figure 2 UV-Vis spectroscopy of (a) Copper oxide nanoparticles, and (b) Cobalt oxide nanoparticles, (c) XRD graph of copper oxide nanoparticles

EDX is used to investigate the sample composition and the X-rays emitted from the sample by stimulating the electrons present in the inner shell of atoms of the sample. Every single constituent has its own characteristic X-rays. Elements present in a sample can be distinguished by identifying the released X-rays. Using the EDX spectroscopy, Fig confirmed the presence of copper oxide and cobalt oxide nanoparticles. Generally typical absorption peaks of copper oxide nanoparticles are nearly at 0.5 keV and 8 keV. Generally typical absorption peaks of cobalt oxide nanoparticles are nearly at 0.8 keV, 6.9 keV and 7.6 keV.

SEM is another characterization tool which provided the advance understanding of the size, shape and morphology details of nanoparticles. The copper oxide nanoparticles may have different shapes such as cubic, octahedral and hexapod. (New download) Figure 4.6 shows SEM image of synthesized hexapod Copper Oxide ( $\text{Cu}_2\text{O}$ ) particles. The surface morphology of cobalt oxide NPs was investigated by SEM and response is shown in Fig 4.7, it is obvious from the SEM image that particles were spherical in shape,

agglomerated with average size of less than 80 nm. Agglomerations in the particles depend upon the nature of the extract and the compounds present in the extract [34] because bimolecular cap and stabilize the individual particle. Reactivity and attraction of the functional groups results in the formation of larger size particles. These particles have coatings of the different biological compounds which have surface hydroxyl groups. Due to intermolecular hydrogen bonding among these agents, the particles appear to be agglomerated.

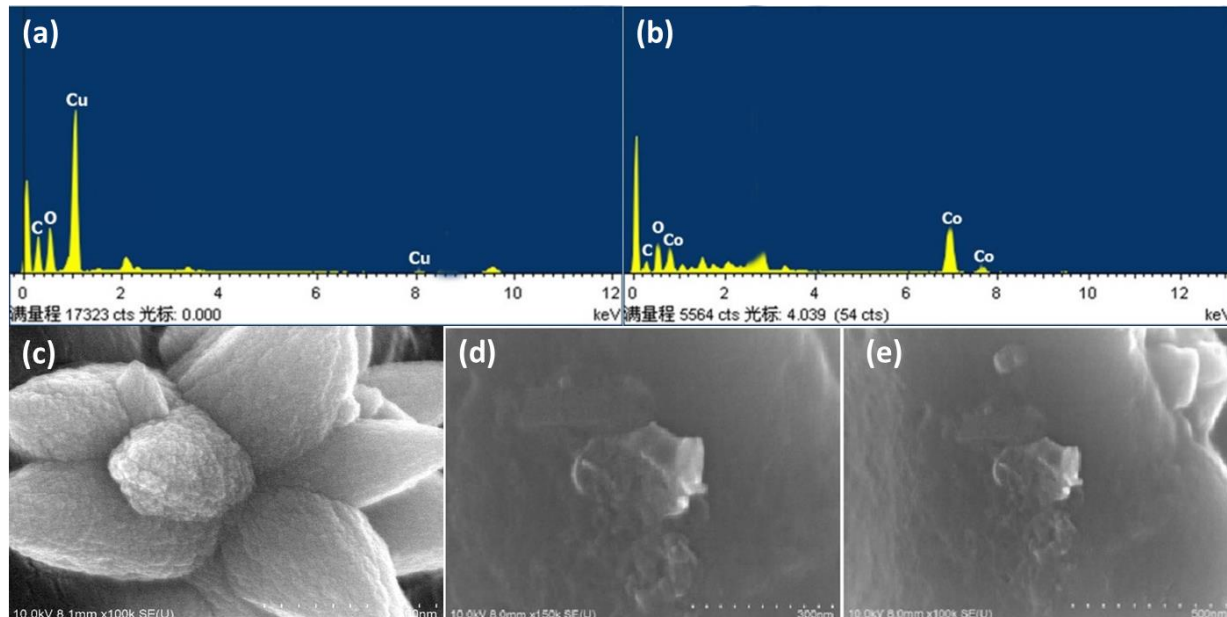


Figure 3 (a, b) EDX images of copper oxide nanoparticles and cobalt oxide nanoparticles. (c, d, e) SEM images of copper oxide nanoparticles and cobalt oxide nanoparticles

## CONCLUSION

Copper oxide and cobalt oxide nanoparticles can be prepared from reduction method which is greener and environmentally suitable, cheap and best as compared to conventional methods. In summary, we have demonstrated a facile green method to synthesize low-cost copper oxide and cobalt oxide nanoparticles by employing *Spinacia Oleracea* leaf extract as both the reducing and capping agent. The prepared nanoparticles were analyzed by various techniques such as UV-Vis, XRD, EDX and SEM. These techniques revealed the successful synthesis of copper oxide and cobalt oxide nanoparticles. Since the reagents used in the reaction medium are completely non-toxic and environmentally friendly, this green method can be readily used for biomedical applications.

## References

1. Linic, S., et al., *Photochemical transformations on plasmonic metal nanoparticles*. Nature materials, 2015. **14**(6): p. 567-576.
2. Muthuvel, A., M. Jothibas, and C. Manoharan, *Effect of chemically synthesis compared to biosynthesized ZnO-NPs using Solanum nigrum leaf extract and their photocatalytic, antibacterial and in-vitro antioxidant activity*. Journal of Environmental Chemical Engineering, 2020. **8**(2): p. 103705.
3. Remya, V., et al., *Silver nanoparticles green synthesis: a mini review*. Chemistry International, 2017. **3**(2): p. 165-171.
4. Patra, J.K., Y. Kwon, and K.-H. Baek, *Green biosynthesis of gold nanoparticles by onion peel extract: Synthesis, characterization and biological activities*. Advanced Powder Technology, 2016. **27**(5): p. 2204-2213.

5. Phukan, A., R.P. Bhattacharjee, and D.K. Dutta, *Stabilization of SnO<sub>2</sub> nanoparticles into the nanopores of modified Montmorillonite and their antibacterial activity*. *Advanced Powder Technology*, 2017. **28**(1): p. 139-145.
6. Raveendran, P., J. Fu, and S.L. Wallen, *Completely "green" synthesis and stabilization of metal nanoparticles*. *Journal of the American Chemical Society*, 2003. **125**(46): p. 13940-13941.
7. Fazlzadeh, M., et al., *A novel green synthesis of zero valent iron nanoparticles (NZVI) using three plant extracts and their efficient application for removal of Cr (VI) from aqueous solutions*. *Advanced Powder Technology*, 2017. **28**(1): p. 122-130.
8. Asif, M., *Antiviral and antiparasitic activities of various substituted triazole derivatives: A mini review*. *Chemistry International*, 2015. **1**(2): p. 71-80.
9. Benouis, K., *Phytochemicals and bioactive compounds of pulses and their impact on health*. *Chemistry International*, 2017. **3**(3): p. 224-229.
10. Hailu, Y.M., et al., *Composition of essential oil and antioxidant activity of Khat (Catha edulis Forsk), Ethiopia*. *Chem. Int*, 2017. **3**: p. 25.
11. Arunkumar, B., S.J. Jeyakumar, and M. Jothibas, *A sol-gel approach to the synthesis of CuO nanoparticles using Lantana camara leaf extract and their photo catalytic activity*. *Optik*, 2019. **183**: p. 698-705.
12. Vinothkumar, P., et al., *Effect of reaction time on structural, morphological, optical and photocatalytic properties of copper oxide (CuO) nanostructures*. *Journal of Materials Science: Materials in Electronics*, 2019. **30**(6): p. 6249-6262.
13. Debbichi, L., et al., *Vibrational properties of CuO and Cu<sub>4</sub>O<sub>3</sub> from first-principles calculations, and Raman and infrared spectroscopy*. *The Journal of Physical Chemistry C*, 2012. **116**(18): p. 10232-10237.
14. Chiang, C.-Y., et al., *Copper oxide nanoparticle made by flame spray pyrolysis for photoelectrochemical water splitting—Part II. Photoelectrochemical study*. *International journal of hydrogen energy*, 2011. **36**(24): p. 15519-15526.
15. Chen, M., J. Liu, and S. Sun, *One-step synthesis of FePt nanoparticles with tunable size*. *Journal of the American Chemical Society*, 2004. **126**(27): p. 8394-8395.
16. Kaviyarasu, K., et al., *Synthesis and characterization studies of NiO nanorods for enhancing solar cell efficiency using photon upconversion materials*. *Ceramics International*, 2016. **42**(7): p. 8385-8394.
17. Dong, C., et al., *Hydrothermal synthesis of Co<sub>3</sub>O<sub>4</sub> nanorods on nickel foil*. *Materials Letters*, 2014. **123**: p. 187-190.
18. Jayakumarai, G., et al., *Phytofabrication and characterization of monodisperse copper oxide nanoparticles using Albizia lebbeck leaf extract*. *Applied Nanoscience*, 2015. **5**(8): p. 1017-1021.
19. Nasrollahzadeh, M., S.M. Sajadi, and M. Khalaj, *Green synthesis of copper nanoparticles using aqueous extract of the leaves of Euphorbia esula L and their catalytic activity for ligand-free Ullmann-coupling reaction and reduction of 4-nitrophenol*. *RSC Advances*, 2014. **4**(88): p. 47313-47318.
20. Abboud, Y., et al., *Biosynthesis, characterization and antimicrobial activity of copper oxide nanoparticles (CONPs) produced using brown alga extract (Bifurcaria bifurcata)*. *Applied Nanoscience*, 2014. **4**(5): p. 571-576.
21. Sebeia, N., M. Jabli, and A. Ghith, *Biological synthesis of copper nanoparticles, using Nerium oleander leaves extract: characterization and study of their interaction with organic dyes*. *Inorganic Chemistry Communications*, 2019. **105**: p. 36-46.
22. Ghareib, M., et al., *BIOSYNTHESIS OF COPPER OXIDE NANOPARTICLES USING THE PREFORMED BIOMASS OF ASPERGILLUS FUMIGATUS AND THEIR ANTIBACTERIAL AND PHOTOCATALYTIC ACTIVITIES*. *Digest Journal of Nanomaterials & Biostructures (DJNB)*, 2019. **14**(2).

23. Das, P., et al., *Madhuca longifolia* plant mediated green synthesis of cupric oxide nanoparticles: a promising environmentally sustainable material for waste water treatment and efficient antibacterial agent. *Journal of Photochemistry and Photobiology B: Biology*, 2018. **189**: p. 66-73.
24. Le Tu, H., *Biosynthesis, characterization and photocatalytic activity of copper/copper oxide nanoparticles produced using aqueous extract of lemongrass leaf*. *Composite Materials*, 2019. **3**(1): p. 30-35.
25. Sathiyavimal, S., et al., *Biogenesis of copper oxide nanoparticles (CuONPs) using Sida acuta and their incorporation over cotton fabrics to prevent the pathogenicity of Gram negative and Gram positive bacteria*. *Journal of Photochemistry and Photobiology B: Biology*, 2018. **188**: p. 126-134.
26. Naika, H.R., et al., *Green synthesis of CuO nanoparticles using Gloriosa superba L. extract and their antibacterial activity*. *Journal of Taibah University for Science*, 2015. **9**(1): p. 7-12.
27. Lee, R.B., et al., *The relationship between iron and Ilmenite for photocatalyst degradation*. *Advanced Powder Technology*, 2018. **29**(8): p. 1779-1786.
28. Bibi, I., et al., *Corrigendum to 'green and eco-friendly synthesis of cobalt-oxide nanoparticle: characterization and photo-catalytic activity'*[*Adv. Powder Technol.* **28** (2017) 2035–2043]. *Advanced Powder Technology*, 2017. **10**(28): p. 2796.
29. Diallo, A., et al., *Green synthesis of Co<sub>3</sub>O<sub>4</sub> nanoparticles via Aspalathus linearis: physical properties*. *Green Chemistry Letters and Reviews*, 2015. **8**(3-4): p. 30-36.
30. Rehman, S., A. Mumtaz, and S. Hasanain, *Size effects on the magnetic and optical properties of CuO nanoparticles*. *Journal of Nanoparticle Research*, 2011. **13**(6): p. 2497-2507.
31. Neeleshwar, S., et al., *Size-dependent properties of CdSe quantum dots*. *Physical Review B*, 2005. **71**(20): p. 201307.
32. Huang, X., et al., *Plasmonic photothermal therapy (PPTT) using gold nanoparticles*. *Lasers in medical science*, 2008. **23**(3): p. 217.
33. Vilchis-Nestor, A.R., et al., *Solventless synthesis and optical properties of Au and Ag nanoparticles using Camellia sinensis extract*. *Materials letters*, 2008. **62**(17-18): p. 3103-3105.
34. Nazeruddin, G., et al., *In-vitro bio-fabrication of silver nanoparticle using Trigonella foenum seed extract*. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 2014. **5**(2): p. 167-175.