Green Route Synthesis of Copper Oxide Nanoparticles Against MCF-7 Cell Line (Breast Cancer)

Abdul Rehman[1a], Awais Shahid Minhas[1b]

[1]Department of Physics, International Islamic University, Islamabad, 44000, Pakistan

Corresponding Author's email awaisminhas1995@gmail.com

ABSTRACT

Growing nanostructures by green synthesis is an attractive method with a lot of applications, such as antibacterial, anticancer, antifungal and water treatment, etc. Copper oxide nanoparticles have been fabricated using the extract of *Aloe Barbandanum*, famous with the name of *Aloe vera*. These nanoparticles are important due to their applications in medical sciences. Structural, optical, compositional, and morphological properties of synthesized nanoparticles were studied and reported here. The phases and crystallite size were determined using X-ray Diffraction and the UV-visb was used to find out the direct bandgap and the absorbance near the visible range. Fourier Transform Infrared Spectroscopy spectra confirmed the presence of a strong band of metal oxide near 606 cm^{-1}. Using Scanning Electron Microscope, the average grain size was obtained as small as 60 nm. These fabricated CuO NPs were tested for their possible uses against the MCF-7 cell line (breast cancer cells) and results are presented.

Keywords. Green Synthesis, *Aloe barbandanum*, Copper Oxide Nanoparticles, MCF-7 cell line.

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INTRODUCTION

MCF-7 is a Breast cancer line. Breast cancer is the commonest cancer in women accounting for 25% of all cancer cases and 15% of all cancer deaths worldwide (Ahmad et al., 2008; Torre at al., 2015). Treatments are highly effective in early stages but results in advanced cancer are poor (Lee et al., 2012). Surgery, radiotherapy, chemotherapy and novel targeted therapies are the main treatment methods that are being used but have many side effects (Sinha et al., 2003). The essential system has been developed for drug delivery by using nanoparticles (NP), nanocrystals, nanosuspension, solid lipid nanoparticles, and nanoemulsion, etc (De Jong et al., 2008; Marcato et al., 2008). Drugs from medicinal plants are found to be comparatively fewer side effects (Soppimath et al., 2001; Gelperina et al., 2005). The plant derived compounds, have a significant role in drug delivery for anticancer retreatment (Rajeshkumar et al., 2003).

There are more than 3000 plants that may be considered for the treatment of cancer (Alam et al., 2014; Maravajhala et al., 2012). Copper is an essential trace element of almost all living beings, acting as a catalytic cofactor in oxidation-reduction reactions (Redox). Daily dietary intake of copper is in the range of 0.6 – 1.6 mg. Beef, shellfish, and legumes are rich in copper, which is mainly absorbed in the small intestine (duodenum). The liver is the main handler of Cu after absorption, it distributes Cu to serum and tissues, and the rest is excreted in bile. Ceruloplasmin is a major Cu-carrying protein in the blood, about 95% of Cu is bound to plasma proteins, which are subsequently distributed to peripheral tissues. Though Cu is essential for life but is a very highly toxic substance, therefore requires very tight homeostatic mechanisms that regulate its absorption, excretion, and bioavailability. Cu has a unique abilitto interchange between oxidized form (Cu^{++}), and reduced state (Cu^{+}) by accepting or donating a single electron in oxidation-reduction reactions.

Any imbalance in copper homeostasis would induce oxidative stress and oxidative damage. Cu related oxidative stress/ damage induces cellular damage and impairs cellular repair processes in the long term. High levels of Cu in serum and tumors tissue are seen especially in cancers of lung, liver, breast, and gastrointestinal malignancies. This is a remarkable finding which points towards the utility of Cu for the treatment of Cancer (Cunzhi et al., 2003). The synthesis of copper oxide (CuO) nanoparticles by using the green method reduces the level of toxicity of the prepared nanoparticles. Green synthesis is a simple, low cost and environment friendly method (Kulkarni et al., 2014). By using *Aloe barbandanum* (Aloe vera) plants, copper oxide nanoparticles have been successfully extracted. The transition element such as copper oxide has monoclinic structure with the lattice constant a = 4.6, b = 3.4, c = 5.1 and $\alpha = \gamma = 90° \neq \beta$. These properties make it an excellent candidate for use as anti-cancer material. Copper oxide had been synthesized by the wet precipitate method using copper acetate monohydrate.

The effect of synthesized NPs on germination and seeding growth is well studied (Ansilin et al., 2016; Singh et al., 2017). The antimicrobial properties of CuO NPs have attracted particular attention because they are the most basic member of the family of a copper compound. Ahmed et al., have studied antimicrobial agents such as small antibiotic cations polymers metal NPs and antimicrobial peptides. They prepared the copper oxide nanoparticles from leaf extract, which were eco-friendly, inexpensive, and highly stable (Ahamed et al., 2014; Sivaraj et al., 2014). Till now copper oxide nanoparticles have been successfully synthesized by using Zingiber Officinale stem extract, from aspergillus niger fungi and by Aloe Barbadensis and several other biological sources (Ahmad et al., 2008; Torre et al., 2015; Lee et al., 2012). Green synthesis of copper oxide nanoparticles using aloe vera leaf extract, without involving any chemical reagent and its antibacterial activity against fish bacterial pathogens was studied by Kumar et al., (2007). The plant leaf extracts from Magnolia kobus, *Syzygium aromaticum*, tamarind, lemon juice, *Capparis zeylanica* Linn, *Ocimum sanctum*, *Aegle marmelos*, and Nerium oleander have been used for the green synthesis of copper nanoparticles and also reported (Sinha et al., 2003; De Jong et al., 2008; Marcato et al., 2008). A study on the synthesis of copper oxide nanoparticles for application of antimicrobial and anticancer activity is also found in the literature (Soppimath et al., 2001; Gelperina et al., 2005). Study on MCF-7 cell line by using green synthesized nanoparticles has also been reported (Rajeshkumar et al., 2003; Alam et al., 2014; Maravajhala et al., 2012).

Synthesis of CuO nanoparticles

Fifty grams of Aloe vera leaves were ground with mortar and pestle, and added to 50 ml water, and stirred at 80°C temperature for three hours. After filter, it was refrigerated to bring the solution into gel form. Then 6 g of copper nitrate solution was added to 50 mls of blue-colored gel and stirred at 120°C for about 7 hours till the color of the gel was changed into red. The red-colored gel was dried at 80°C in the oven for 2 hours to convert it into powder form. The powder was calcinated at 350°C for 3 hours and further ground with mortar and pestle. The color of the powder changed to black, confirming that the copper oxide nanoparticles were achieved.

RESULTS AND DISCUSSION

For structural analysis, an X-ray diffraction pattern was obtained, Figure 1 representing the corresponding X-ray diffraction patterns of CuO nanoparticles synthesized by a green route synthesis scheme. Diffraction peaks are observed at 35.74°, 36.18°, 43.21°, 73.20°, 74.14°, and 76.80° sharply with the corresponding miller indices (002), (101), (110), (103), (004), and (211) respectively (Cunzhi et al., 2003). The peaks were matched with JCPDS cards # ICSD ID 61323. The synthesized CuO nanoparticles show several diffraction peak values introducing the appearance of the high percentage of crystalline phases, however, the most preferred diffraction peak belongs to the plane (200). The crystallite size (D) can be calculated using the famous Debye Scherer formula, $D = k\,\lambda\,\beta\,\cos\theta$ (1) Where β is the full width at half maximum of the corresponding peak, k is a dimensionless constant and ~0.94, λ is the wavelength of Cu-Kα radiation 1.54 Å and θ is the Bragg angle. Since β has an inverse relation with D, it means that the observed broader peaks confirm the fabrication of smaller CuO nanoparticles. Using diffraction data, the inter-planar spacing (d) and Miller indices (h,k,l) were determined and presented in Table 1. The SEM micrographs of CuO NPs were obtained and presented in Figure 2, which clearly showed a well dispersed, versatile crystalline form with spherical shape distribution nanoparticles. The particle sizes were determined as 60 nm. It was observed that the addition of copper nitrate with the *Aloe vera* did not change the shape of nanoparticles (Kulkarni et al., 2014).

Energy Dispersive X-Rays were used to determine the chemical composition of fabricated nanoparticles. The plot of copper oxide nanoparticles and the percentage of chemical composition in copper oxide nanoparticles are shown in Figure 3 / Table 2. As a different concentration with different energies, the peaks show copper and oxide (Ansilin et al. 2016).

The transmittance plot from the UV-visb spectrometer provides the basic data to calculate the energy band gap of copper oxide nanoparticles by using the Kubelka-Munk function. Considering the direct interband transition between the valence and the conduction bands, the energy band gap was measured by using Tauc's formula $(\alpha\, h\, \nu)^2 = A\,(h\,\nu - E_g)$ Where A is constant, α is the absorption coefficient, $h\,\nu$ is for the photon energy, and E_g is the optical energy bandgap. The E_g of the CuO NPs was obtained by plotting $(\alpha\, h\, \nu)^2$ as a function of $h\,\nu$ and extrapolation of the linear segment of the plot to $(\alpha\, h\, \nu)^2 = 0$ provides the value of energy bandgap for CuO NPs and is shown in Figure 4. The bandgap of copper oxide nanoparticles was 1.645 eV (Singh et al., 2017). The obtained band gap seems a little higher, which is due to the quantum confinement effect. This confinement influences the quantization of the energy

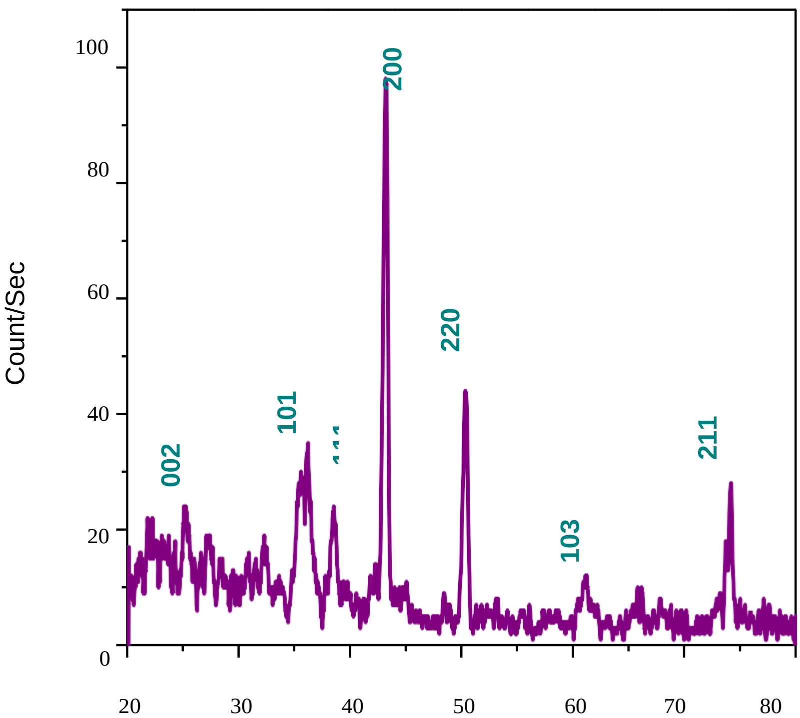

Figure 1: X-ray Diffraction Pattern of the Synthesized CuO Nanoparticles (NPS)

Table 1: Data Table of XRD Graph of Copper Oxide Nanoparticles (NPS)

Peaks	**FWHM (radian)**	**Crystallite size (nm)**	**Inter-planer Spacing (Ǻ)**	**Miller Indices (hkl)**
01	0.0073	18.87	3.34	002
02	0.0126	11.25	2.43	101
03	0.0048	29.28	2.30	111
04	0.0060	24.10	2.08	200
05	0.0078	18.99	1.80	220
06	0.0143	10.95	1.51	103
07	0.0074	22.80	1.27	211

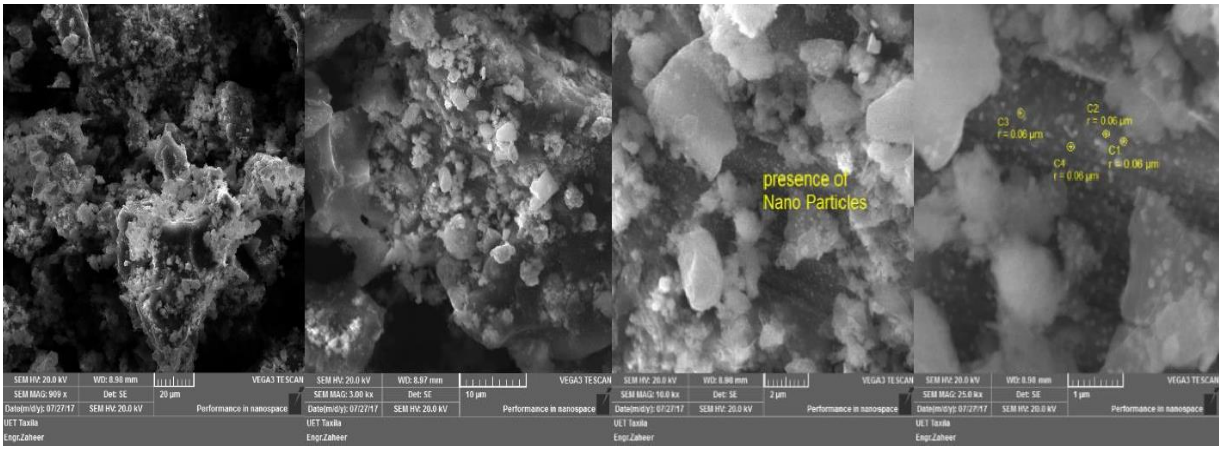

Figure 2. SEM Images of Synthesized Copper Oxide NanoparticlesPprepared by Green Route (a) 20 µm, (b) 10 µm, (c) 2 µm, and (d) 1 µm

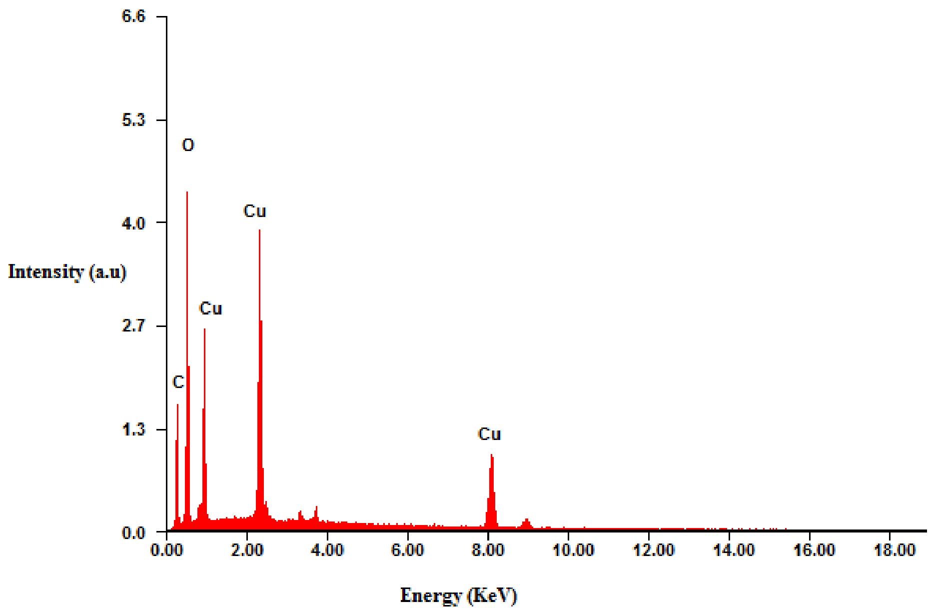

Figure 3. Energy Dispersive X-Rays (EDX) Spectra of the Synthesized CuO Nanoparticles (NPS)

Table 2: Data of Energy Dispersive X-Rays EDX Graph

Elements	Wt%	At%
CK	19.85	34.51
OK	35.21	45.95
CuK	14.81	09.65
CuK	30.13	09.90
Matrix	Correction	ZAF

and the momentum of the corresponding carriers and enhances the energy between the valence and the conduction band. The absorbance plot is shown in Figure 4, the peak was observed in the visible region near 609 nm. The decrease in peak intensity was observed, possibly due to the increasing number of nanoparticles formed as a result of copper ions reduction. The change in color from blue to red may be due to the excitation of the surface plasmon absorption of copper oxide (Ahamed, 2014; Sivaraj et al., 2014; Berra et al., 2018).

Chemical bonding and functional groups were studied using Fourier transform infrared spectroscopy (FTIR) (Shimadzu IRTracer-100 FTIR) at the Department of Physics, IIUI. All spectra were acquired in the mid-infrared range from 350 to 4000 cm^{-1} in transmittance mode. Figure 5 shows the FTIR spectra of copper oxide nanoparticles by the green route method. The peaks around 1739 cm^{-1} are due to the amide I band of proteins/enzymes. The bands observed at 1368 $cm^{-1}$are assigned to stretching vibrations of the primary amines, alcohols, and alkanes. The intense bands observed at 1123 cm^{-1} have been assigned to C-O strong stretching. FTIR spectra show a strong band near 606 cm^{-1}, relates to M–O vibrational bond frequencies that support the presence of monoclinic phases (Ahmad et al., 2012). The overall observation proves the existence of phenolic compounds, terpenoids, or proteins that are bound to the surface of CuO nanoparticles and act as reducing agents, and significantly reduces the particle sizes. The nanoparticles are affected by the interaction between the terpenoids and CuO NPs become stabilized as terpenoids are effective capping and stabilizing agents. The stability of CuO nanoparticles may be due to the free amino and carboxylic groups that have interacted with the copper surface. Moreover, the proteins present in the medium prevent agglomeration and aids in the stabilization by forming a coat, covering the metal nanoparticles (Berra et al., 2018). The anticancer activity through MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazo-lium bromide) assay. The MTT assay is a colorimetric assay for metabolic activity of assessing cells (J. Huang et al., 2007). NAD(P)H- dependent cellular oxidoreductase enzymes might, under definite conditions, reflect the possible cell number present. These enzymes are used for reducing the tetrazolium dye MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide to its insoluble formazan, which has a purple color. Other closely related tetrazolium dyes including XTT, MTS, and the WSTs, are used in conjunction with the intermediate electron acceptor, 1-methoxy phenazine methosulfate (PMS). With WST-1, which is cell-impermeable, the reduction occurs outside the cell via plasma membrane electron transport (K. Karthik et al., 2011). MCF-7 Cell line was grown and maintained in FCS + GPPS + RPMI1640 media at 37°C in presence of a 5% CO2 supply using Shellab incubator (Sheldon manufacturing inc. the USA). To determine the anticancer activity of the compounds 96 well plates comprising of 1 × 105 cells per well were used along with negative control and blank. To test the wells, a series of different concentrations were added in consecutive wells. In the negative control, only cells at the set number were added and the growth media without cells and test compounds were taken as blank. After 24 hours of incubation 10 µL of 5mg/ml, MTT (Sigma-Aldrich) was added to each well and incubated for 2 hours. Then 100 µL of the soluble solution (40% vol / voldimethyl formamide in 2% glacial acetic acid and 16% wt/vol SDS at pH 4.7) was added to each well and incubated at room temperature for one hour. The absorbance was recorded at 550 nm in the

ELISA plate reader. All the experiments were repeated three times and relative cell viability (%) as a percentage relative to the untreated control cells is shown in Table 3.

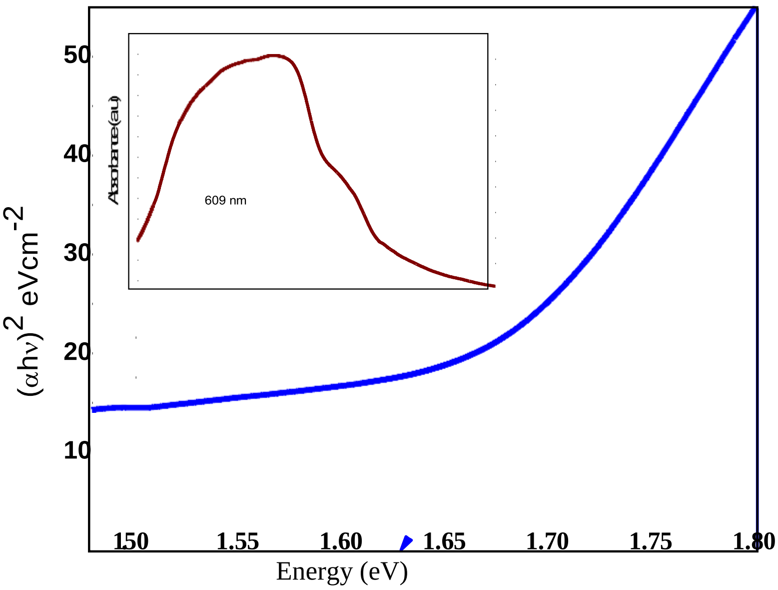

Figure 4: Tauc Plot and Absorbance Graph of Synthesized Copper Oxide Nanoparticles (NPS)

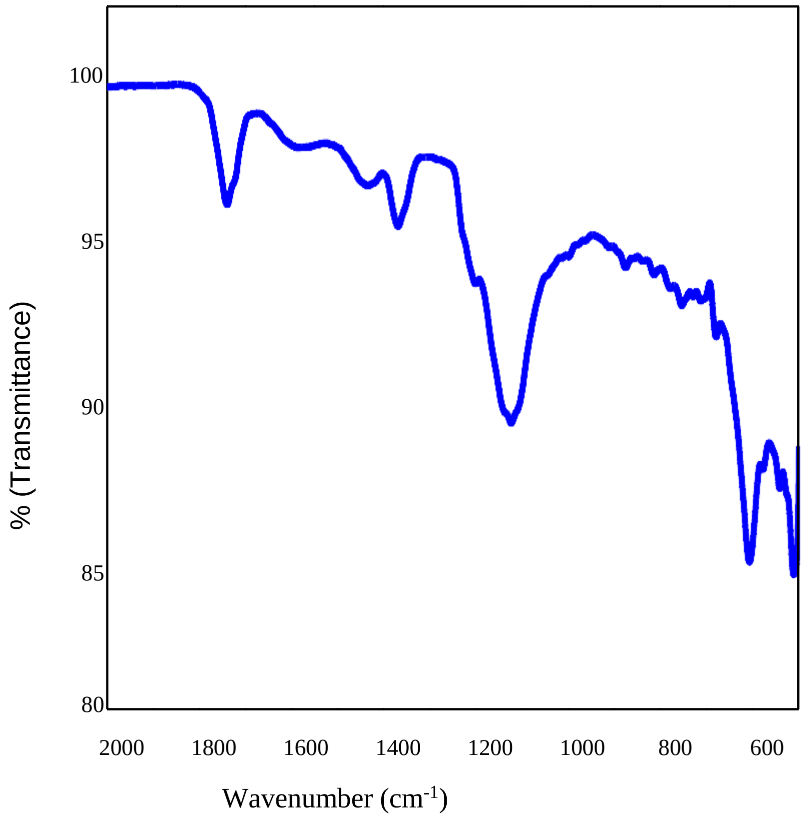

Figure 5: FTIR Spectra of Synthesized Copper Oxide Nanoparticles

Table 3. Data collection for the test of anticancer activity.

Compound	CuO	E2	E1	NPs
IC_{50} Value	6.125µg	Nil	6.655µg	2.498µg

CONCLUSION

We have successfully synthesized copper oxide nanoparticles using *Aloe vera* extract. The synthesized copper nanoparticles were analyzed using XRD, UV-visb, SEM, and EDX. The green route synthesized CuO NPs show a monoclinic phase, with an average particle size of 60 nm by using morphological investigation. There are many plants are available for cancer treatment such as *vinca*, *Taxus*, *turmeric* and *neem*, etc. These medical plants have many chemical components such as vinblastine, taxol, vincristine, and have many anticancer classes of constituents like phenolic compounds, flavonoids, saponins, and tannins. These all compounds have their important characters in the treatment of cancer. These green synthesized CuO NPs are cost-effective, eco- friendly, and easy to handle with the capability to serve as anticancer agents. We discovered the efficacy of CuO NPs in human MCF-7 (breast cancer) by MTT assay. We were able to replicate the method of producing CuO NP's which have a potential of killing breast cancer cells in vitro. Further studies are required to see if this technique can be taken forward as a clinical tool.

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