

PHYTO-FABRICATION OF COPPER NANOPARTICLES WITH THE AQUEOUS EXTRACT OF AZADIRACHTIN INDICA (NEEM) LEAVES.

**Olabimtan Olabode.H¹, Ashade Noah.O², Abdulkarim Yahaya.M³, Aboki Mahmoud.A⁴,
Opeyemi Adegboyo.O⁵, Abubakar Zaid⁶, Suleman Stephen. M⁷**

¹Industrial and Environmental Pollution Department, National Research Institute for Chemical Technology, Zaria Kaduna State, Nigeria

²Research and Development, Outstation Coordination Department, National Research Institute for Chemical Technology, Zaria Kaduna State, Nigeria

³Textile Technology Department, National Research Institute for Chemical Technology, Zaria Kaduna State, Nigeria

⁴Scientific and Industrial Research Department, National Research Institute for Chemical Technology, Zaria Kaduna State, Nigeria

⁵Department of Science Laboratory Technology, Federal Polytechnic Ilaro Ogun State, Nigeria

⁶Department of Engineering Materials Research, Nigerian Building and Road Research Institute, North West Zonal Office, Kano State Nigeria

⁷Department of Chemistry, Nigerian Army University Biu Borno State, Nigeria

Abstract

Copper nanoparticles (CuNanoPs) in recent times are being considered as one of the celebrated and resourceful nanoparticles with their physico-chemical characteristics and their vast applications in science and technology. The environmentally balanced and cheap approach has been adopted in the synthesis of CuNanoPs with aqueous extract of Azadirachta indica (neem) leaves. They were characterized by FT-IR which registered the leaves aqueous extract and copper metal framework (496cm⁻¹) with the phytochemicals. As well, TEM (Transmission electron microscopy) which reflects the irregular, circular and randomly distributed particles under the setting conditions of the image processor (imageJ) as; distance in pixels (81.4222), known distance (500), pixel aspect ratio (1.0), and unit of length (nm) and scale (0.1628pixels/nm). Twelve CuNanoPs were identified and calculated (Mean area, standard deviation, and polydispersity index). Descriptive statistics for the mean area (Mean=121.98nm², standard error=2.40, range=24.883nm², maximum=131.39nm² and minimum=106.50nm²); Standard deviation (Mean=13.40, standard error=0.91, range=10.36,

maximum=20.34 and minimum=9.98) and polydispersity index (Mean=10.94%, standard error=0.62%, range=7.06%, maximum=15.48% and minimum=8.42%).

Keyword: Copper nanoparticles area, neem leaves, phytochemicals, FTIR and SEM.

1. INTRODUCTION

Nanotechnology centers mostly on the formation, preparation, and control of the structure and size of the particles with measurements below 100 nm [1]. It connects with the standards of chemical and physical techniques to produce nanosized particles with a particular capacity. It is currently making a rising sensation of interest in health, biological and chemical sciences. Nanoparticles completely display new or improved properties dependent on some explicit factors like size, shape, high surface to volume proportion and direction [2], [3]. Be that as it may, the groupings based on their sources are metal nanoparticles, their oxides, bimetallic, and other inorganic nanoparticles [4]. Faraday recognized the truth of metal nanoparticles and Mie gave a quantitative implication of their colouration [5]. Metal nanoparticles are used in catalysis [6], sensors [7], and optoelectronics [8] because of reliance on their

electrical, therapeutic, magnetic, and catalytic potentials [9]. Copper, zinc, gold, magnesium, silver, and titanium nanoparticles are specifically important due to their antibacterial properties against *Bacillus subtilis* and *Staphylococcus aureus*, medical applications, dental materials, water treatment, sunscreen moisturizers, and coatings [4]. As of late, metal, metal oxides, clay, silicate, and polymer nanoparticles have been integrated and utilized in a few applications. The magnitude of their sizes and the high surface region has improved their utilization and expansion in material science [10]. Their properties are size-related concerning the nature of their immediate environment. Hence, the necessary and desired properties can be acquired by changing the surroundings of nanoparticles [11]. Copper nanoparticles have imminent applications in medicine, optics, hardware, lubricant production, antimicrobial agents, nanofluids and conductive materials [12]. The inclination of copper nanoparticles against silver is because of the lower cost of copper, the physicochemical steadiness, and the simplicity of blending in with polymers [9]. Nanoparticles offer higher reactivity and yet may bring about bunch arrangement which will affect their fundamental properties [9]. Much work has been achieved with respect to the combination and reliability of copper nanoparticles. However, it will be necessary to generate information that will bridge the characteristics of copper nanoparticles in various media under different conditions. The term phytofabrication reveals the production of the nanoparticles with the assistance of the plant constituents to forestall likely agglomeration of the nanoparticles [1]. Plant constituents incorporate the protein and catalysts substance, for example, reductases which are engaged with the normal decrease of the substrates, for example, aurum chloride, titanium chloride, and silver nitrate, into their respective nanoparticles as gold, titanium, and silver with uses in physical, material, and biological sciences. Some selected plant extracts have successfully been employed as reducing agents for the production or synthesis of nanoparticles [13]. This approach could be profitable over the microbial method because of the maintenance and stability of the plant cells and tissues. It has been indicated that numerous plants can effectively take-up and bio-reduce metal particles from soils and matrices during detoxification procedure and treatment forming insoluble complexes with the metal particle as

nanoparticles [14]. For instance, the aqueous extract of magnolia leaf was applied for the production of copper nanoparticles as a capping and reducing agent [15]. Utilizing CuSO_4 as a substrate, copper nanoparticles were established to be produced with curd, milk, butter, soap nut, lime juice, and tamarind juice as stabilizing and capping agents under acidic system [16]. *Artabotrys odoratissimus* (Nag Champa) has been likewise adopted as a reducing and capping agents for the production of copper nanoparticles from CuSO_4 at 95°C , which brought about particles from 109 to 135 nm in size [17]. The utilization of *Nerium oleander* and L-ascorbic acid as a reducing agent and stabilizer has been accounted for in a publication [4] [18]. *Datura metel* leaf extract was used at room temperature for the production of nanoparticles [18]. Potato starch has been accounted for as a capping agent for copper nanoparticles with the influence of an anti-oxidant (L-ascorbic acid) and a catalyst (NaOH) [9]. There is tremendous enthusiasm for metal nanoparticles with regards to their unpredicted chemical and physical properties uncovered at the nanoscale level. Factors such as reaction time, pH, and the concentration of the precursor, temperature, and catalyst [19] do affect their Physico-chemical parameters eventually.

The active components of aqueous neem leaves extract as azadirachtin, nimbin and trace others could enhance the green synthesis of copper nanoparticles [20]. In this work, the synthesis, area dimension, and heterogeneity of copper nanoparticles with aqueous extract of neem leaves as natural reducing; stabilizing and capping agent was established.

2. MATERIALS AND METHODS

Copper Nitrate (Precursor), deionized water, fresh neem leaves, magnetic stirrer, blender, burette, beakers, magnetic stirrer, and heater.

2.1. *Neem leave aqueous extract*

Some fresh matured leaves of neem leaves were harvested and washed with deionized water. 25 g of the washed and fresh leaves were crushed and heated with 100 ml of deionized water in a beaker. The mixture was heated at 50°C for 3min. under a reflux system and reduced pressure in a rotary evaporator to produce a greenish color extract. Then the extract was cooled and

centrifuged at 10000 rpm and filtered. The filtrate was filled in a 50ml burette.

2.2. Synthesis, Precipitation and isolation of Cu-NanoPs

20mg of Copper nitrate was weighed into 50ml deionized water and with the temperature of the magnetic stirrer at 65oC and continuous stirring, the aqueous extract of the neem leaves in a burette was being added dropwise into the solution of copper nitrate till light green color forms. This indicates the formation of copper nanoparticles in water. The mixture after 24 h was further centrifuged at 4000 rpm for 3min. The recovered precipitate was allowed to dry for 2 h at 100oC in an oven.

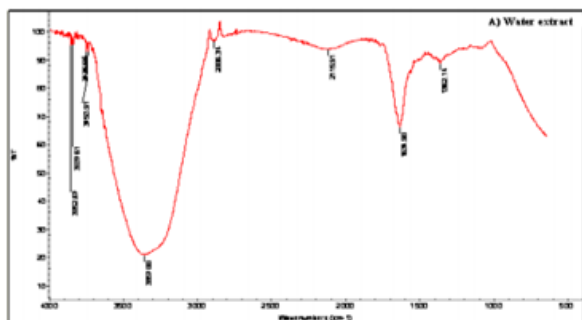


Figure 1. FTIR spectrum of aqueous extract of neem leaf.

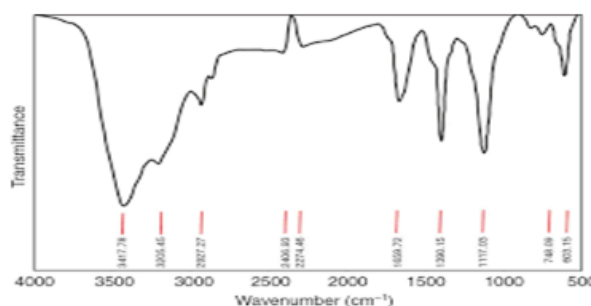


Figure II. FTIR of Precipitated CuNanoPs in aqueous neem leaf extract.

2.3. Characterization

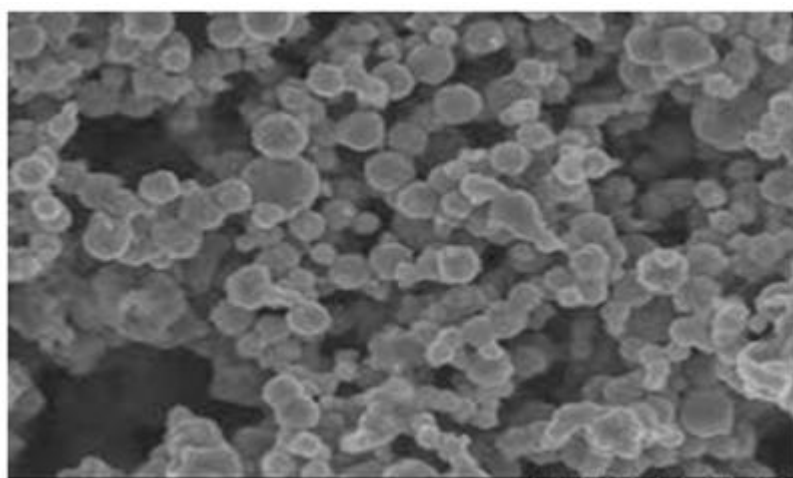
Copper nanoparticles dispersed in the aqueous extract of neem leaves were characterized with FTIR and transmission electron microscopy (TEM) [21]

2.4. Polydispersity index (PDI)

This is measure of the heterogeneity of particle sizes in a mixture [22]. It was estimated by

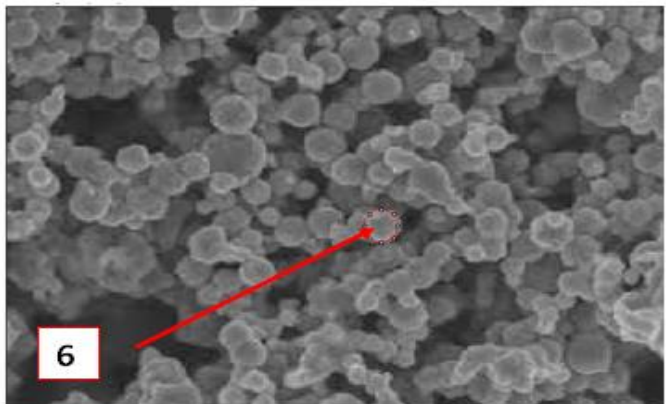
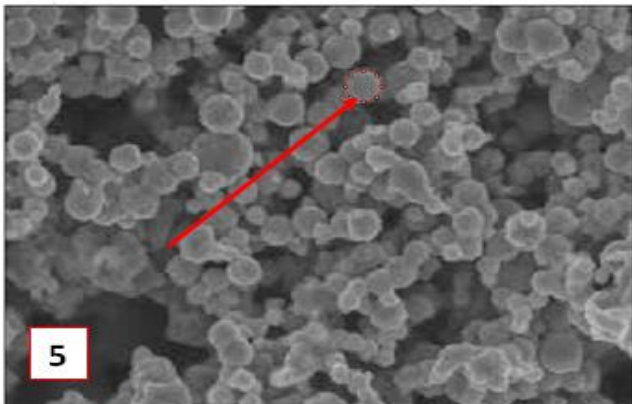
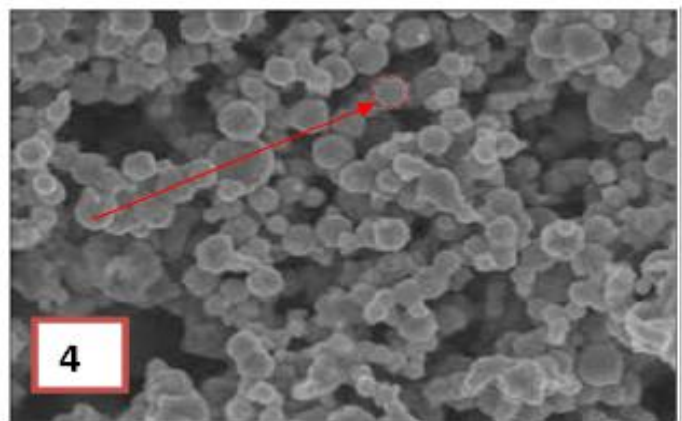
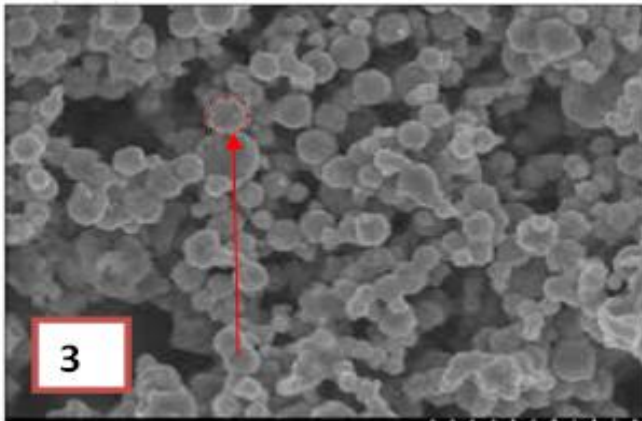
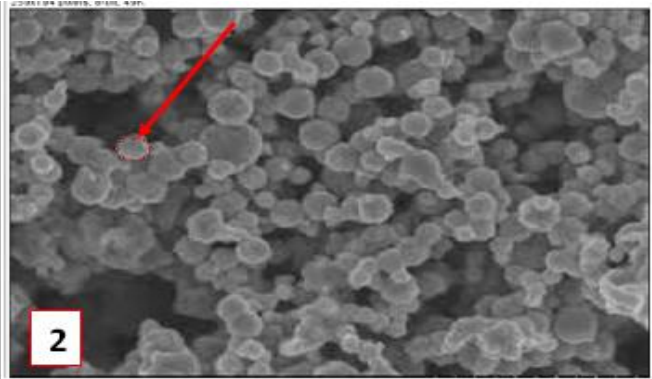
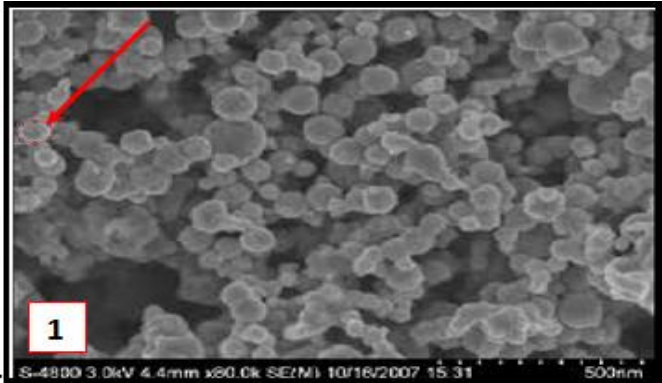
$$\text{PDI} = \frac{\text{Standard deviation} \times 100\%}{\text{Mean Area}}$$

3.RESULTS AND DISCUSSION



Distance in pixels: 81.4222
Known distance: 500
Pixel aspect ratio: 1.0
Unit of length: nm
Click to Remove Scale
 Global
Scale: 0.1628 pixels/nm
OK Cancel Help

Figure III. Transmission electron microscopy (TEM) of the precipitated CuNanoPs in aqueous extract of neem leaf with setting parameters.



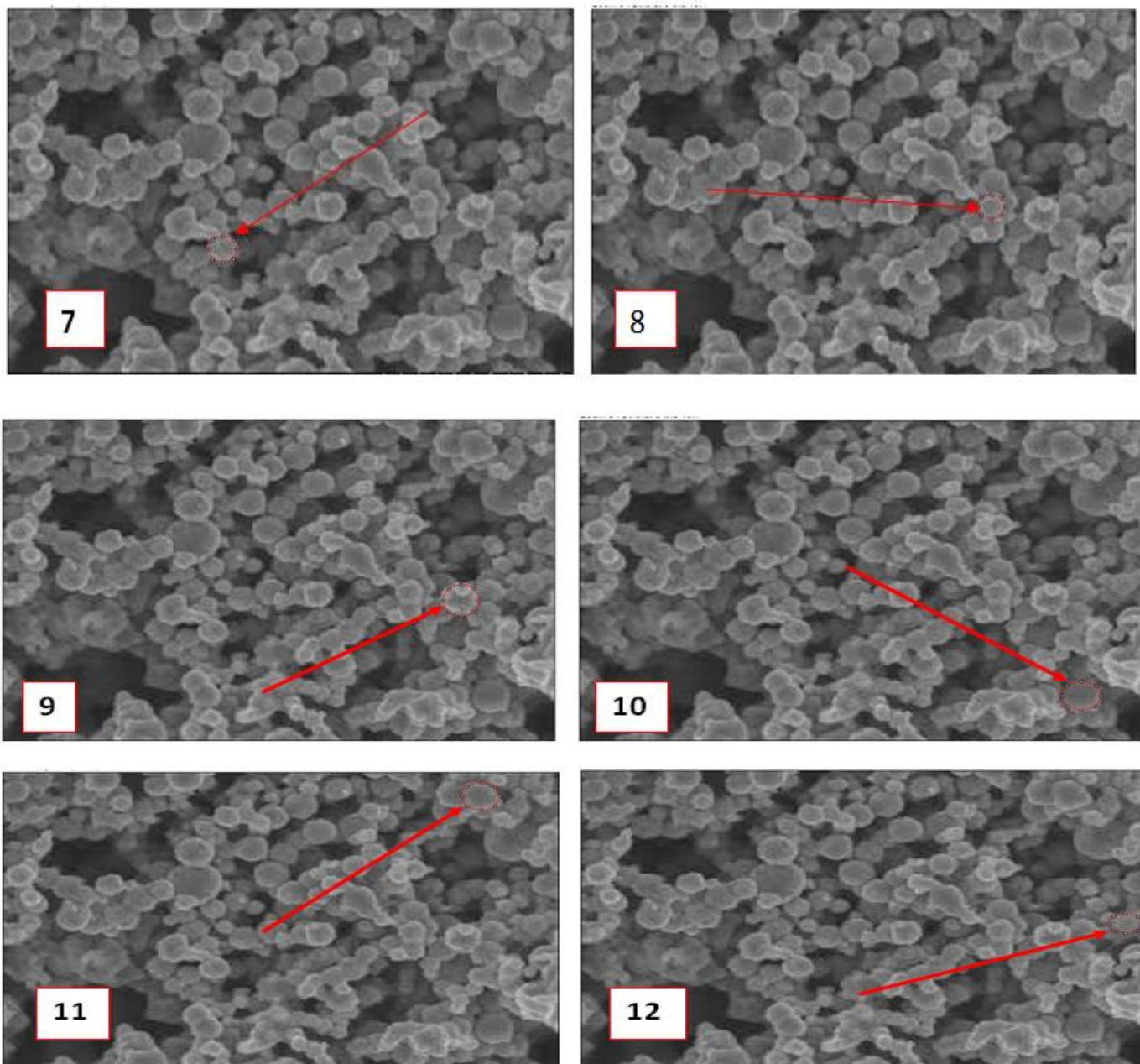


Figure.IV. Transmission electron microscopies of twelve (12) CuNanoPs embedded with neem leave aqueous extract.

Table I. Mean area, standard deviation and polydispersity indices of copper nanoparticles precipitated in neem leaf aqueous extract.

Serial Number CuNanoP	Cu NanoP Mean Area(nm ²)	Standard deviation (σ)	Cu NanoP Area size without error(nm ²)	Polydispersity index (%)
1	138.643	16.585	138.643 ± 16.585	11.962
2	125.500	14.235	125.500 ± 14.235	11.343
3	122.382	15.228	122.382 ± 15.228	12.443
4	114.080	9.989	114.080 ± 9.989	8.756
5	118.542	9.979	118.542 ± 9.979	8.418
6	130.603	15.380	130.603 ± 15.380	11.776
7	128.832	13.352	128.832 ± 13.352	10.364
8	129.282	11.884	129.282 ± 11.884	9.192
9	131.387	20.339	131.387 ± 20.339	15.480
10	106.504	11.807	106.504 ± 11.807	11.086
11	117.576	14.214	117.576 ± 14.214	12.089
12	117.088	10.975	117.088 ± 10.975	9.373

Table III. Descriptive statistics of CuNanoPs (1-12) standard deviation

Test	Value
Mean	13.40
Standard error	0.91
Range	10.36
Maximum	20.34
Minimum	9.98

Table II. Descriptive statistics of CuNanoPs (1-12) mean area

Test	Value
Mean	121.98
Standard error	2.40
Range	24.883
Maximum	131.39
Minimum	106.50

Table IV. Descriptive statistics of CuNanoPs (1-12) polydispersity index

Test	Value
Mean	10.94
Standard error	0.62
Range	7.06
Maximum	15.48
Minimum	8.42

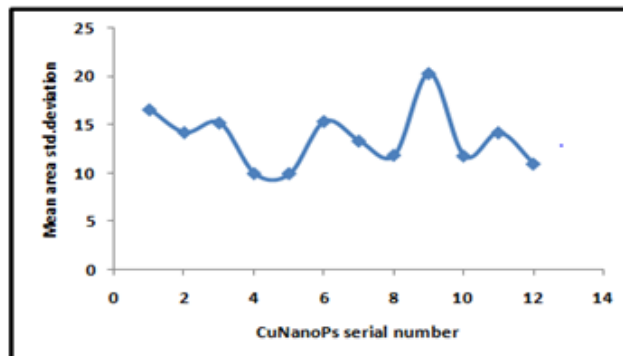
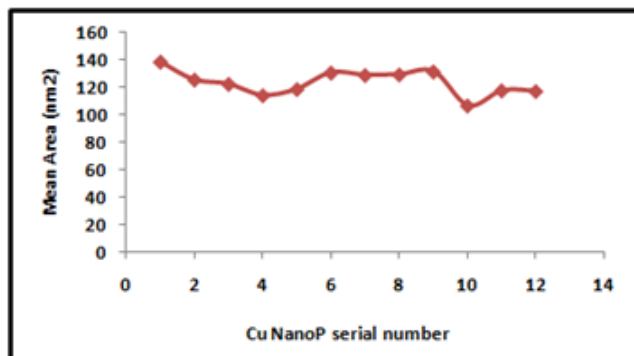


Figure V. Mean area (nm²) of the CuNanoPs (1 to 12) Figure VI. Mean area standard deviation of the CuNanoPs (1 to 12)

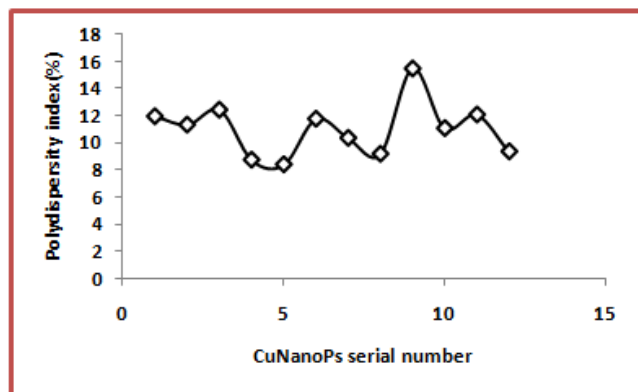


Figure VII. Polydispersity indices of CuNanoPs (1 to 12)

Table I. shows the serial CuNanoPs form number 1 to 12 with regards to their mean area, standard deviation, area sizes without error and polydispersity indices. Table II, III, and IV are the descriptive statistics of mean area, standard deviation, and polydispersity index of CuNanoPs respectively. The FT-IR spectra for the aqueous extract of *Azadirachta indica* leaf in figure I, reveals the availability of O-H stretching at 3,357 cm⁻¹, C=C bond at 1634cm⁻¹, C-H stretching at 2886cm⁻¹, and methyl substituent at 1,362 cm⁻¹. An observed band at 1716cm⁻¹ is for the stretching by ester carbonyl group

(-RCOH), at 1600cm⁻¹ for C=N and C=C stretching vibration with the aromatic conformation of the chlorophyll.

The identified bands are directly attributed to the presence of terpenes and chlorophyll in the aqueous *Azadirachta indica* leaf. Figure II. is the FT-IR spectra of phyto-synthesized copper nanoparticles embedded with the aqueous extract of *Azadirachta indica* leaves which serves a stabilizing and reducing agent. A band at 3500cm⁻¹ is of O-H stretching from alcohols and phenols. At 1599cm⁻¹ is the N-H group as the primary amine. At 1238cm⁻¹ are C-O with phenol, acid, and flavonoids. At 500cm⁻¹ is of copper metal that was bound and saturated with either or jointly with the phenolic acid, flavonoids or carboxylic acid found in the aqueous extract of the leaf. Figure III is the transmission electron microscopy (TEM) of the precipitated copper nanoparticles in the aqueous extract of *Azadirachta indica* leaves. The scanning condition which was at; distance in pixel(81.4222), known distance (500), pixel aspect ratio(1.0) and unit in length(nm) were applied in achieving nanoparticles

which are characterized with irregular circular structures which are randomly distributed against a background believing to be the leaves extracts.

Furthermore, the cross-sectional view as indicated with a red arrow on an individual (12) copper nanoparticles were estimated for their mean area (nm²), standard deviation and polydispersity indices.

Figure V is the plot of the mean areas of copper nanoparticles form number one to twelve. The highest mean area was with CuNanoP- 1 at 138.64nm² and the least with CuNanoP-10 at 106.50nm². Additionally, table II is the descriptive statistics with the mean area of 121.98nm², standard error of 2.40 and a range of 24.883. Meanwhile, the differences with this two-dimensional factor (Area) justify the irregularity and random distribution of the nanoparticles. Standard deviation (figure VI) was higher with CuNanoP-9(20.34), which by correction of the particle area without error gives 113.39±20.34 and least with CuNanoP -5 (9.98) and by correction 118.54±9.98. Moreover, table III is the descriptive statistics with the mean value of 13.40, standard error of 0.91 and range of 10.36. Figure VII, The polydispersity which is a measure of the heterogeneity of the particle sizes in a mixture retains the highest value with CuNanoP-9 (15.48%) and with CuNanoP-5 the least (8.42%). Table IV is the descriptive statistic of polydispersity indices of the CuNanoPs with the mean value of 10.94%, standard error of 0.62 and range of 7.06%

4.CONCLUSION

Copper nanoparticles are useful and valuable metal nanoparticles. They have been successfully prepared with a reduced and stabilizing potential of phytochemicals of *Azadirachta indica* aqueous leaves extract which is cheap, simple and environmentally balanced. The average mean area of 121.98nm², standard deviation between the particles 13.40 and polydispersity index of 10.94% were achieved with the conclusion that the produced CuNanoPs are polydispersed in a heterogeneous system.

REFERENCE

- [1] Prasad, R. (2020). Synthesis of Silver nanoparticles in Photosynthetic Plants.

- [2] Anon, (2020). [online] Available at: https://www.researchgate.net/publication/311660363_Synthesis_of_Nano_Particles_From_Aloe_Vera_Extract_-_Review_Paper [Accessed 8 Jan. 2020].
- [3] Mirzaei, H. and Darroudi, M. (2020). Zinc oxide nanoparticles: Biological synthesis and biomedical applications.
- [4] Tandfonline.com. (2020). [online] Available at: <http://www.tandfonline.com/doi/pdf/10.1080/0032719.2016.1172081> [Accessed 8 Jan. 2020].
- [5] Iopscience.iop.org. (2020). ShieldSquare Captcha. [online] Available at: <https://iopscience.iop.org/article/10.1088/1361-648X/aa60f3> [Accessed 8 Jan. 2020].
- [6] Anon, (2020). [online] Available at: https://www.researchgate.net/publication/303504124_Synthesis_Characterization_and_Applications_of_Copper_Nanoparticles [Accessed 8 Jan. 2020].
- [7] Anon, (2020). [online] Available at: https://www.researchgate.net/publication/303504124_Synthesis_Characterization_and_Applications_of_Copper_Nanoparticles [Accessed 8 Jan. 2020].
- [8] Jbiopest.com. (2020). [online] Available at: http://www.jbiopest.com/users/LW8/efiles/Govindajau_K.pdf [Accessed 8 Jan. 2020].
- [9] Anon, (2020). [online] Available at: https://www.researchgate.net/profile/Rida_Rehan2/publication/303504124_Synthesis_Characterization_and_Applications_of_Copper_Nanoparticles/links/57701e6708ae0b3a3b7b9020.pdf [Accessed 8 Jan. 2020].
- [10] Aitken, R., Chaudhry, M., Boxall, A. and Hull, M. (2020). Manufacture and use of nanomaterials: current status in the UK and global trends.
- [11] En.wikipedia.org. (2020). Nanocomposite hydrogels. [online] Available at: https://en.wikipedia.org/wiki/Nanocomposite_hydrogels [Accessed 8 Jan. 2020].
- [12] Anon, (2020). [online] Available at: https://www.researchgate.net/publication/303504124_Synthesis_Characterization_and_Applications_of_Copper_Nanoparticles [Accessed 8 Jan. 2020].
- [13] Mohamad, N., Arham, N., Jai, J. and Hadi, A. (2020). Plant Extract as Reducing Agent in Synthesis of Metallic Nanoparticles: A Review. Cdn.intechopen.com. (2020). [online] Available at: <http://cdn.intechopen.com/pdfs/27213.pdf> [Accessed 8 Jan. 2020].
- [14] Anon, (2020). [online] Available at: https://www.researchgate.net/publication/264465860_Biological_synthesis_of_copper_nanoparticles_using_Magnolia_kobus_leaf_extract_and_their_antibacterial_activity [Accessed 8 Jan. 2020].
- [15] Anon, (2020). [online] Available at: https://www.researchgate.net/publication/257494093_Large-scale_green_synthesis_of_Cu_nanoparticles [Accessed 8 Jan. 2020].
- [16] Anon, (2020). [online] Available at: https://www.researchgate.net/publication/268507809_Biosynthesis_of_Copper_Nanoparticles_and_Their_Antimicrobial_Activity [Accessed 8 Jan. 2020].
- [17] Anon, (2020). [online] Available at: https://www.researchgate.net/publication/267573163_Synthesis_of_copper_nanoparticles_by_two_different_methods_and_size_comparison [Accessed 8 Jan. 2020].
- [18] Anon, (2020). [online] Available at: https://www.researchgate.net/publication/236975421_Phytosynthesis_of_silver_nanoparticles_by_'Cissus_quadrangularis'_influence_of_physiochemical_factors [Accessed 8 Jan. 2020].
- [19] Anon, (2020). [online] Available at: https://www.researchgate.net/publication/236975421_Phytosynthesis_of_silver_nanoparticles_by_'Cissus_quadrangularis'_influence_of_physiochemical_factors [Accessed 8 Jan. 2020].
- [20] Handoo, S. (2020). Can You Eat Neem Leaves? What Are The Health Benefits?. [online] STYLECRAZE. Available at: <https://www.stylecraze.com/articles/amazing-benefits-of-neem-leaves-for-skin-hair-and-health/> [Accessed 8 Jan. 2020].
- [21] Hassanien, R., Husein, D. and Al-Hakkani, M. (2020). Biosynthesis of copper nanoparticles using aqueous Tilia extract: antimicrobial and anticancer activities.
- [22] En.wikipedia.org. (2020). Dispersity. [online] Available at: <https://en.wikipedia.org/wiki/Dispersity> [Accessed 13 Jan. 2020].