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Facile synthesis of Copper Oxide Nanoparticles from *Aeschynomene indica* leaf extract and its characterisation

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ABSTRACT
Nowadays, green synthesis of metal based nanoparticles from leaves extract have created wide attention among the researchers due to utilization in various fields such as photocatalytic degradation, pharmaceutical industries, etc. The present study deals with the green synthesis of Copper oxide (CuO) nanoparticles by the ethanolic extract of leaves of Aeschynomene Indica. The chemical components identified in the ethanolic extract of the leaves were identified by Gas Chromatography and Mass spectroscopy (GC-MS). Synthesized CuO nanoparticles shows a peak at 366nm in UV-visible spectroscopy (UV–Vis) and the presence of functional groups depicted by Fourier transform infrared spectroscopy (FT-IR). The crystalline nature, composition and the surface morphology of the nanoparticles were studied by X-ray diffraction (XRD), Energy Dispersive X-ray spectroscopy (EDX) and scanning electron microscopy (SEM). Dynamic Light Scattering (DLS) techniques reveals that the average size of the Green Nanoparticles of CuO was about 150 nm. The synthesized nanoparticle can be used for various microbial applications.
Key words: *Nano particles, ethanol, extract, Copper oxide*

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INTRODUCTION

Nanotechnology is a quickly growing area of importance and interest [1, 2] including a wide range of research fields. It contracts with materials or structures in nanometer scale, typically ranging from sub nanometers to numerous hundred nanometers. Nano materials are classified into compacted materials and nano dispersions [3,4]. Nanoparticles are of excessive scientific interest because they epitomize a bridge between bulk materials, molecules and structures at an atomic level [5, 6]. Magnetic poly nuclear coordination compounds (magnetic molecular clusters) goes to the special type of magnetic materials often with matchless magnetic characteristics unlike nanoparticles, which always have the supplies in sizes, molecular magnetic collections are the fully identical small magnetic nanoparticles. Nano medicine is a application of nano science and nanotechnology techniques in the field of medicine. Parts such as disease diagnosis, drug delivery and molecular imaging are being intensively researched. Medical related products holding nanoparticles are currently produced. *Aeschynomene Indica* (Fabaaceae) commonly known as 'Netti' is distributed throughout India from the Himalaya region. The plant is used traditionally as an antifungal and anti-rheumatic [7] Leaves are useful in chronic rheumatism and wound. The plant has many medicinal uses, including as a spermicidal.[8].Blood can carry foreign chemicals to cells, tissues, and organs when it comes into direct or indirect contact with any danger nanoparticles. Studying the effects of nano toxicity on blood, especially erythrocytes, is crucial in this scenario. CuO-NPs may enter the bloodstream accidentally if they are inhaled or ingested and then pass through the skin or mucosa. Few toxicity investigations revealed that erythrocytes exposed to CuO-NPs at high concentrations exhibited considerable hem agglutination, lysis, membrane injury, harmful morphological difference, and cytoskeletal abnormalities. Because of their larger surface area and possibility for more silver ions to be released, nano scale particles have a higher potential for hemolysis than micro scale particles. It was suggested that the toxicity was caused by together the silver ions removed from the nano particles and the through contact of CuO-NPs with RBCs. The size of CuO-NPs has a significant impact on how well they interact with living cells, affecting cytotoxicity, reactive oxygen species generation, and absorption

efficiency. The interaction between CuO-NPs, and membranes, internalization, cellular acceptance, and oxide stress replies are all part of the mechanism causing membrane injury, however the implications of Nano particle size on the harmfulness of CuO- NPs to RBCs remain unclear. The purpose of this learning is to consider the, hemolysis, and toxicological effects of CuO-NPs on Human RBCs. The conclusions infer that a key factor in CuO- NPs interaction with RBCs is concentration effect.

MATERIAL AND METHODS

Analytical grade Copper Chloride ($CuCl_2$) are used for the nanoparticles synthesis. Laboratory grade ethanol was used for extraction. The plant material *Aeschynomene Indica* was used for study.

Preparation of Bio-reductant (*Aeschynomene indica* Leaf extract)

The leaves of *Aeschynomene indica* were collected from Devakottai region and authentication done in Department of Botany, St. Joseph's college, Trichy, Tamil Nadu, India. The collected plant leaves were washed thoroughly several times and dried under shade. Then the dried leaves, crushed and taken for extraction. The ethanolic leaf extract of *Aeschynomene Indica* is prepared by cold percolation method [9]. About 100g was weighed and taken in a 250 ml beaker and 100 ml of ethanol was added to it. The solution was kept undisturbed for about 3 to 5 days. The green extract was filtered iteratively three times using Whatman No.1 filter paper to get a clear green solution.

Synthesis of Copper Oxide Nanoparticles

Accurately weighed quantity of metal chloride $CuCl_2.2H_2O$ was dissolved in (100 ml) water in standard flask. The solution was transferred to a beaker (250 mL) and it was stirred for 2 hours using magnetic stirrer at a constant temperature (80^oC). While the stirring was on the ethanolic plant extract (10 ml) was mixed completely with the metal chloride solution in small increments. The solution was allowed for evaporation to get the dry salt. The dry salt was collected in a silica crucible kept in muffle furnace for incineration at (450^oC) for about 6 hours. The metal oxide salt thus, formed was ground well to make it into nanoparticles [12].

Characterization of Metal Oxide Nanoparticles

The synthesized CuO nanoparticles are characterized by spectral techniques (UV- visible and FTIR), Surface morphological analysis (SEM and DLS), elemental analysis (EDAX) and X- ray analysis (XRD).

Preliminary Qualitative Phytochemical screening analysis:

Phytochemical screening is a process of considering the plant constituents with proper reagents. Based on the studies, the plant constituents are confirmed. The ethanolic extract was further diluted with ethanol and from this small amount of the extract is taken for various tests to check for the presence of active metabolites. The altered qualitative chemical test can be performed for creating a profile of given extract for its chemical composition. The following tests may be performed on extract to identify various Phyto constituents [10]

GC-MS Analysis

Interpretation of mass spectrum (GC-MS) was conducted using database of National Institute of Standards and Technology (NIST) having more than 62,000 patterns. The spectrum of the unidentified constituent was related with the spectrum of identified constituents stored in the NIST library. The retention time, molecular weight, molecular formula and composition percentage of the sample material was recorded.

RESULTS AND DISCUSSIONS

Qualitative Preliminary Phytochemical Screening

The result of qualitative analysis was showed in Table. 1. It illustrates that phytoconstituens such as alkaloids, carbohydrate, tannins, terpenoids, saponins, proteins, amino acids, quinones, steroids and Flavonoids. The following metabolites were analyzed to be absent in the ethanolic extracts -glycosides, phenols, coumarines, cholorogenic acid. Thus the phytochemical screening of the ethanolic extract is found to contain important secondary metabolites.

Table.1 Phytochemical Screening of the ethanolic extract of *Aeschynomene indica*

Types of Metabolites	Ethanolic Extract	Types of Metabolites	Ethanolic Extract
Alkaloids	+	Terpenoids	+
Flavonoids	+	Saponins	+
Phenols	_	Glycosides	_
Quinones	+	Coumarins	_
Steroids	+	Carbohydrates	+
Tannins	+	Chlorogenic acid	_
Proteins	+	Amino acids	+

(+) Present, (-) absent

Gas chromatography-Mass spectrum study (GC-MS)

On linking with the mass spectra from the record of National Institute of Standard Technology (NIST) library, the recorded mass spectra of the GC divided composites are identified. The GC-MS chromatogram of the ethanolic leaves extract of *Aeschynomene Indica* (AI) presented twenty peaks which indicate the presence of twenty chemical constituents is shown in Fig. 1. The twenty active constituents with their retention time (RT), molecular formula, molecular weight (MW) and Peak area (%) in the ethanolic leaves extract of *Aeschynomene indica* (AI) are shown in Table. 3. On comparing of the mass spectra of the parts with NIST library the five predominant (Mome Inositol, Phytol, n-Hexadecanoic acid, Stigmasterol, Neophytadiene) components were characterized and known is shown in Fig. 2.

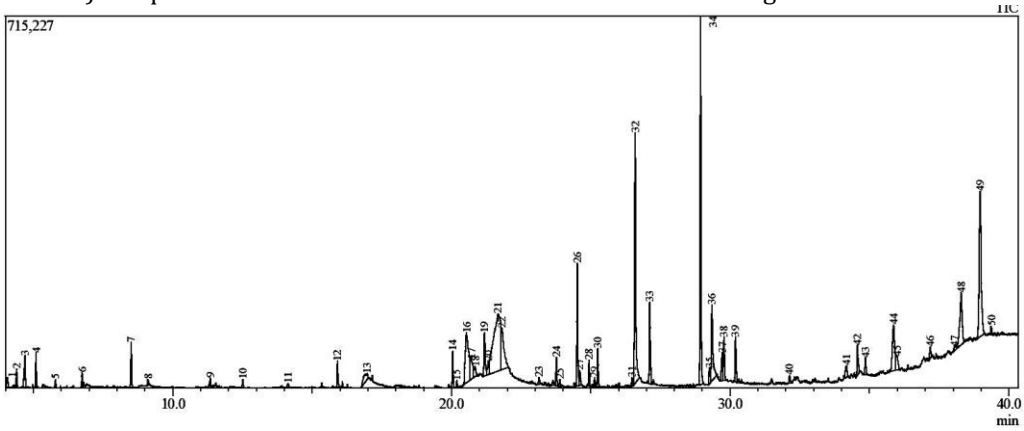
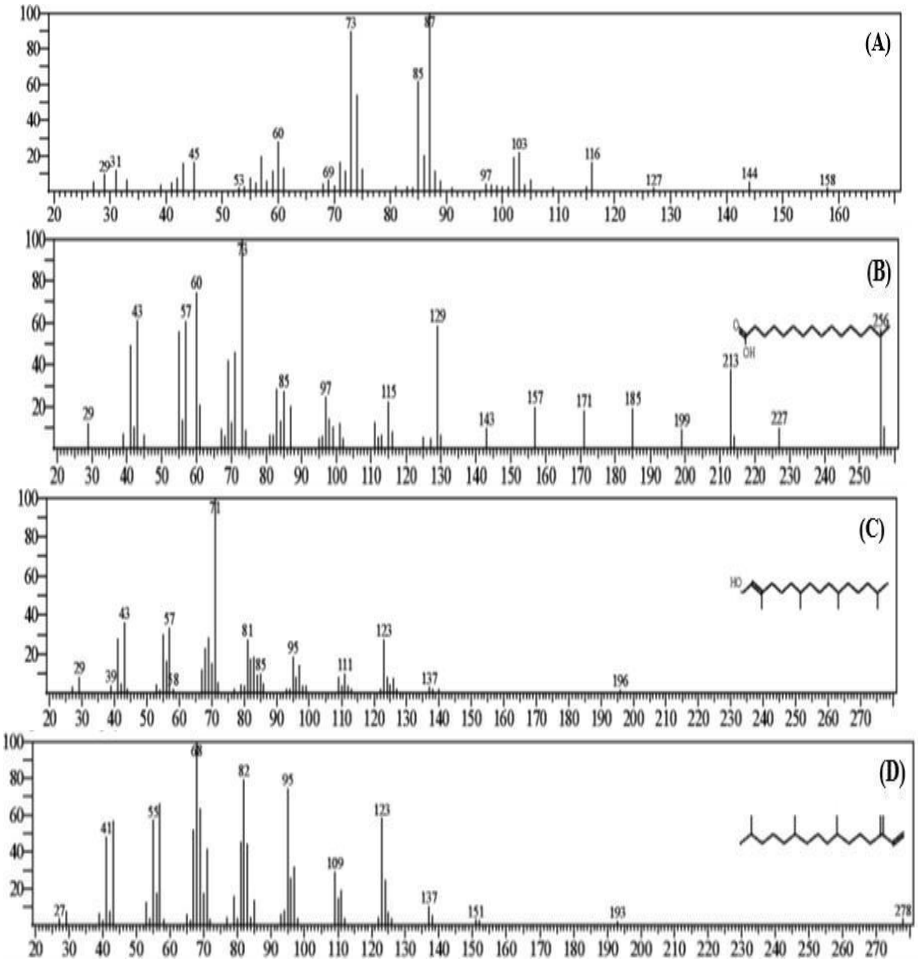


Fig. 1. GC-MS chromatogram of the ethanolic extract of the leaves of ***Aescynomene indica.***



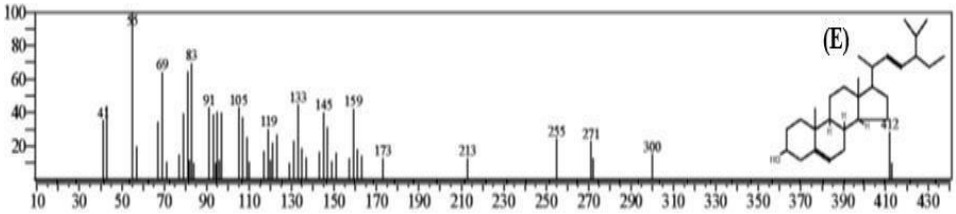


Fig. 2 Mass Spectrum of predominant phytoconstituents (A) Mome Inositol, (B) Phytol,

(C) n-Hexadecanoic acid, (D) Stigmasterol, (E) Neophytadiene present in *Aeschynomene indica*

Table. 3 Chemical components identified in the ethanolic extract of the leaves of *Aeschynomene indica* by GC-MS

S. No.	RT	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area (%)
1	4.695	Benzene, ethenyl-	C8H8	104	1.29
2	8.508	Propane, 1,1,3-triethoxy-	C9H20O3	176	1.17
3	16.978	(1sr,2sr,3sr,5sr)-5-azido- 2,3-epoxycyclopentan-1- ol	C5H7N3O2	141	1.08
4	20.537	.beta.-d-glucopyranoside, methyl	C7H14O6	194	5.5
5	20.715	Butanoic acid, 2-ethyl-	C6H12O2	116	1.85
6	21.185	Ethoxy(dimethyl)[3-(2- oxiranylmethoxy) propyl]silane	C10H22O3	218	1.78
7	21.668	Mome inositol	C7H14O6	194	13.69
8	21.805	1,2,3,4,5-Cyclopentanepentol	C5H10O5	150	3.65
9	24.516	Neophytadiene	C20H38	278	3.87
10	26.592	n-hexadecanoic acid	C16H32O2	256	10.14
11	27.114	Hexadecanoic acid, ethyl Ester	C18H36O2	284	2.95
12	28.937	Phytol	C20H40O	296	11.48
13	29.346	9,12,15-octadecatrienoic acid,(z,z,z)-	C18H30O2	278	3.9
14	29.713	Octadecanoic acid	C18H36O2	284	1.16
15	29.779	Ethyl(9z,12z)-9,12- octadecadienoate	C20H36O24	308	1.43
16	30.193	Octadecanoic acid, ethyl ester	C20H40O2	312	1.57
17	34.568	Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl Ester	C19H38O4	330	1.23
18	35.861	dl-.alpha.-tocopherol	C29H50O2	430	3.58
19	38.29	Ergost-5-en-3-ol,(3.beta.)-	C28H48O	400	4.0
20	38.97	Stigmasterol	C29H48O	412	9.52

Analysis of UV-Visible absorption spectra

The synthesized metal nanoparticles CuO-NPs from the ethanolic extract of *Aeschynomene indica* is monitored by UV-visible spectrometer [13]. For copper nanoparticles, the absorbance peak appears between the ranges of 350-850 nm. Fig. 3 indicates that the peak is observed at 366.60 nm (3.2201 AU) suggests the reduction of copper and the formation of copper oxide nanoparticles.

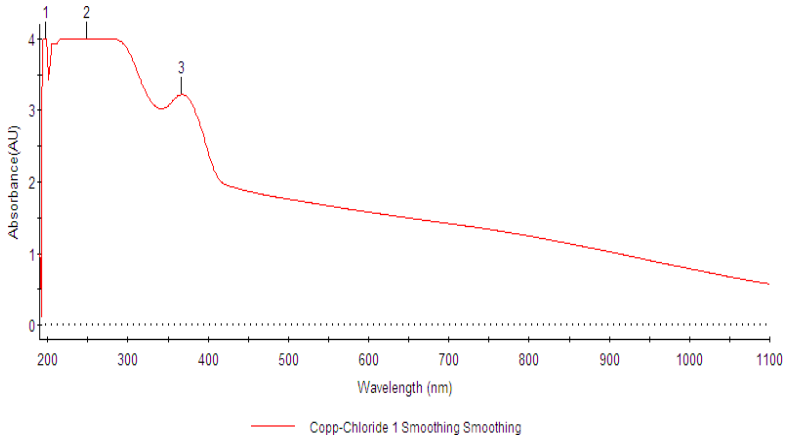
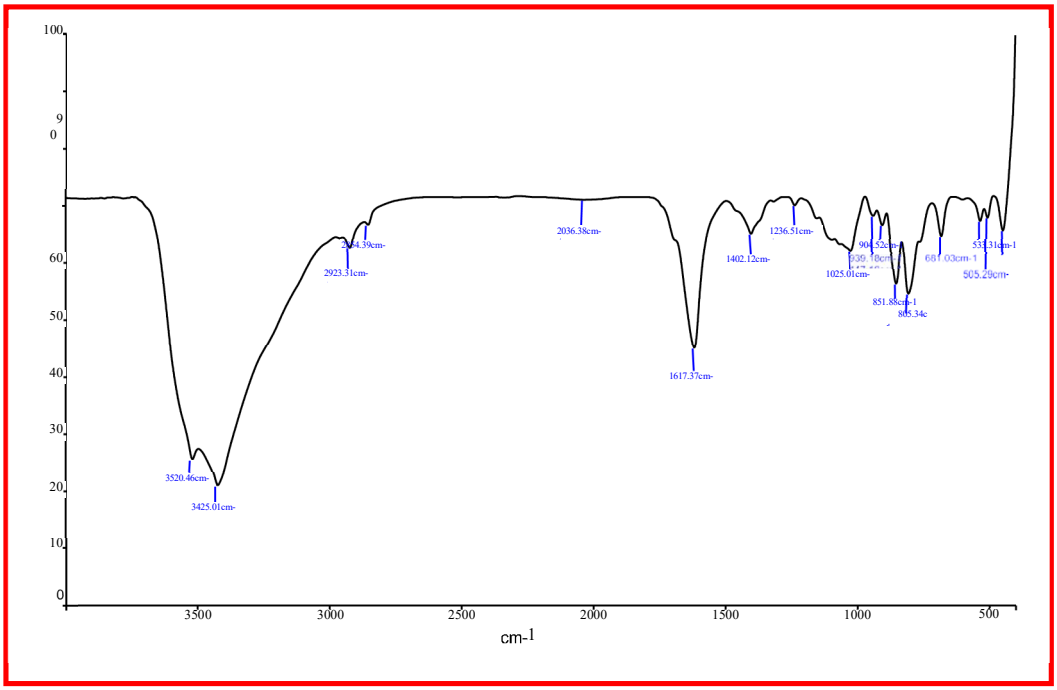


Fig.3 UV-Visible Spectra of Copper Oxide Nanoparticles

Analysis of FT-IR

The FT-IR analysis is a technical tool familiar analyses the functional groups present in the synthesized nanoparticles. The FTIR-spectrum of the synthesized Copper Oxide nanoparticle is shown in Fig. 4. We got several appearances peaks which are assigned to different stretching and bending frequencies of molecular functional groups attached to Cu Oxide surface of band 3425.01 at cm^{-1} [15], these are broad and strong band which credited to O-H (hydroxyl) stretching vibrations on the surface of copper NPs. They are the maximum strong and easily recognizable bands in the spectra, and their precise position depends on the strength of the hydrogen bonds 2923.31 cm^{-1}, it makes from C-H(Hydrocarbon) stretching vibrations in the molecules. The peak at 1617.37cm^{-1} is the characteristic of bending vibration of O-H (hydroxyl) bonds in OH groups. The peak at 1025.01cm^{-1} shows that presence of C-O stretching. The peak positions at 681.03 cm^{-1} and 533.31 cm^{-1} indicates that the presence Copper and Oxygen bond. All these peaks indicate that the formation of Copper oxide nanoparticles.

Fig. 4. FT- IR spectrum of Copper Oxide nanoparticles



X-ray Diffraction Pattern

Figure 5 shows the XRD pattern of synthesized CuO nanoparticles made from natural plant. Powder x-ray diffraction was used to confirm the effective production of copper oxide nanoparticles, with diffraction angles, the sample showed a high level of crystallinity. However, there are a few peaks at 2θ values of 32.54, 48.74, and 58.37, which correspond to the Cu planes (110), (202), and (220),

The particle size was calculated using the Scherrer formula and found to be in the 40–50 nm range. The peaks and hkl values corresponded to those reported in JCPDS card 01–074–1657 (17). The confirmation of successful synthesis of copper oxide nanoparticles was done by powder x-ray diffraction (XRD).

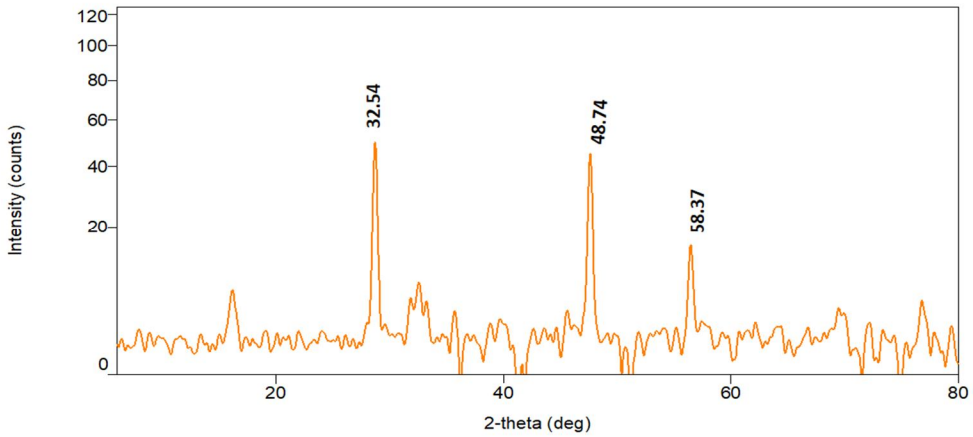


Fig.5 X-ray Diffraction of CuO-NPs

SEM Analysis

SEM micrographs of the Copper oxide nanoparticles synthesized by the reduction of copper chloride the leaves extract of *Aeschynomene indica* revealed spherical, hexagonal and cubical shapes are shown in Fig.6. It was shown that spherical and relatively uniform shape of the copper *oxide* nanoparticles was confirmed in the range of 213 nm. Whereas the ethanol extract is appearing shows that spherical and relatively uniform shape of the copper nanoparticles was confirmed in the range of 181-302 nm.

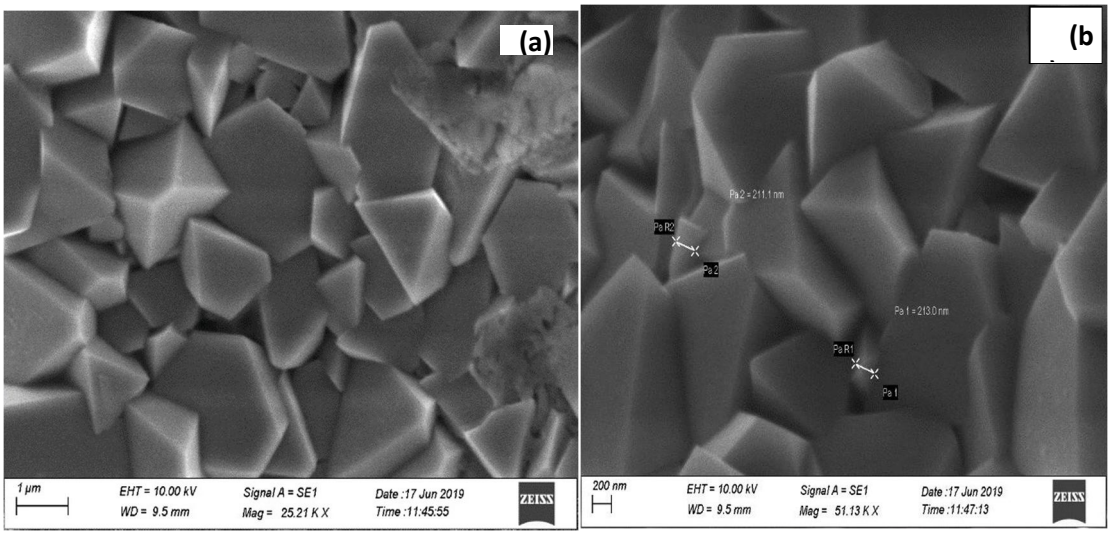


Fig. 6. SEM micrographs of CuO-NPs with different magnification (a) 1 µm (b) 200

Energy Dispersive X-Ray Analysis (EDAX)

EDAX results in (Fig.7) further confirmed the formation of the CuO nanoparticles, indicating the appropriate signals for Copper (%) and oxygen (%) in the respective images.

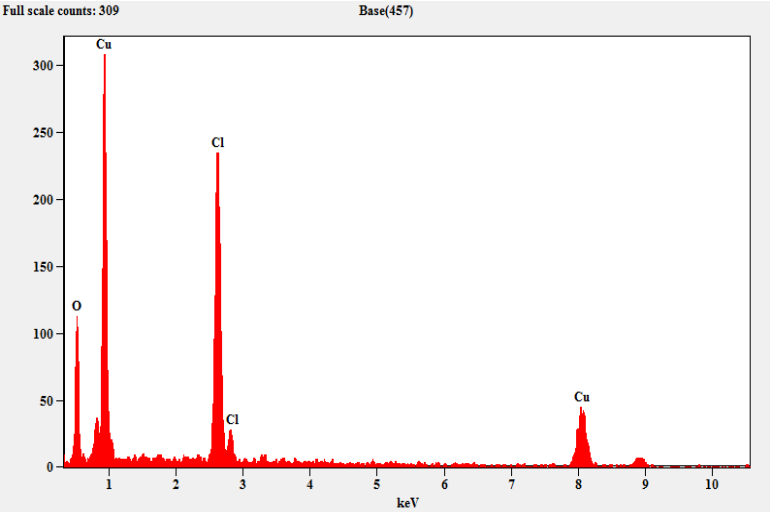


Figure. 7. EDAX Images of CuO-NPs

Particle Size Analysis (DLS)

Particle size and polydispersity key of Copper Nanoparticles were dignified by Photon Correlation Spectroscopy (PCS) Delsa Nano C (Beckman Coulter Counter, USA) Particle Size Analyzer. The usual particle size of the copper nanoparticle was recruit to be the particle size range of 50 to 1000 nm is usually measured as nanoparticles. The particle size of Copper oxide nanoparticle has to be s of 69.69 nm of width12.24 nm is confirmed by the Fig. 8

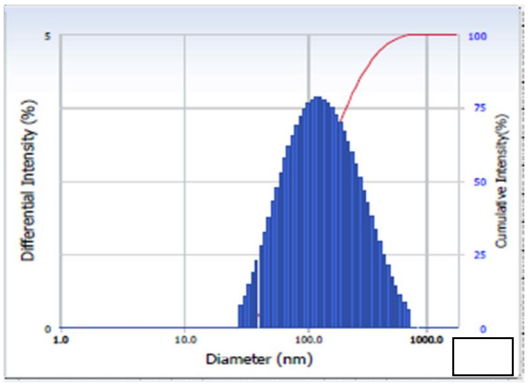


Fig. 8 DLS Images of CuO-NPs

CONCLUSION

In the current work, Green chemistry approach was adopted to synthesize the CuO -NPs using *Aeschynomene indica.* Preliminary phytochemical screening confirmed alkaloids, flavonoids, phenolic compounds are present in the ethanol extract of *Aeschynomene indica.* FT-IR results confirmed the occurrence of active ancillary metabolites that are answerable for the CuO -NPs formation. The UV peaks observed between the ranges of 350 - 850 nm confirms the formation of the CuO nanoparticles. The SEM images clearly confirm the size and the shape of the nanoparticles formed. The elemental analysis (EDAX) confirms the presence of Cu, and O atoms their corresponding synthesized nanoparticles. The experimentally calculated 20 and d value agrees with the theoretical value further confirms the formation of np by XRD.

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