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Characterization and Antibacterial Activity of Green Synthesized Copper Oxide Nanoparticles (CuO NP's)

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Abstract

Copper oxide nanoparticles (CuO NP's) have gained attraction due to their unique properties in environment, energy storage, sensing, catalysis and biomedical applications. This article deals with the green synthesized CuO NP's, its characterization and antibacterial activity. Characterization analysis such as Field Emission – Scanning Electron Microscope (FE-SEM), Fourier Transform Infrared Spectroscopy (FTIR), Transmission Electron Microscope (TEM), High Resolution Transmission Electron Microscope (HR-TEM), X-Ray Diffraction (XRD), Energy Dispersive X-Ray Spectroscopy (EDXS), Atomic Force Microscopy (AFM) and Zeta Potential analysis were characterized on green synthesized CuO NP's. The characterized nanoparticles were screened for different techniques which helps to confirm the size, morphology, structure and its various functional groups. The synthesized nanoparticles were further subjected for antibacterial activity using MIC method. The results showed excellent inhibitory activity against *E. coli*, *S. aureus*, *P. aeroginosa* and *S. typhii*. Thus green synthesis offers advantages such as cost-effectiveness, environmental friendly and mostly suitable for biomedical areas. However, further studies are required for the act of therapeutic agents.

Keywords: Copper oxide nanoparticles, Curry leaf, characterization, antimicrobial activity, MIC.

I. INTRODUCTION

Murraya koenigii (*M. koenigii*) is generally called as "Curry leaf", belongs to Rutaceae family. These leaves are commonly available in Sri Lanka, South Asian countries and also in India. It is found everywhere in India, which grows to a height of 6 meters and its diameter is about 15-40 cm, smooth, thin, grey or brown bark and has dense shady crown (Mhaskar *et al.,* 2000). *M. koenigii* leaf extract contains bioactive components namely saponins, flavonoids, carbohydrates, amino acids and proteins that plays a significant role in the synthesis of metallic nanoparticles (Amooaghaie *et al.,* 2015).

Recently, green nanotechnologies have been found to be important in a range of biomedical fields. Natural reagents, plant extracts, reductants and capping agents can be used as effective substitutes for chemical and physical procedures in the production of nanoparticles (Lakki *et al.,* 2020). Green synthesis has replaced conventional techniques, but one major obstacle is the lack of information regarding its effects on human health and the environment. By using natural extracts instead of chemical components, expecting to contribute with less environmental impacts (Patino-Ruiz *et al.,* 2021). Many constituents and biochemical agents that can function as stabilizing and reducing agents to synthesize green nanoparticles can be found in plants. Compared with alternative biological, physical, and chemical approaches, green synthesis methods are more stable, nontoxic, economical, and environmentally benign (Mustapa *et al.,* 2021).

The creation of strong techniques with fewer harmful effects is necessary to overcome the negative effects of antibiotics and lower the incidence of bacterial infections such as those caused by the biosynthesis of nanoparticles by using plants. Recently, nanotechnology has advanced tremendously in terms of scientific study and applications (Reddy, 2017 and Sharmila *et al.,* 2018). Recent advances in nanotechnology have led to the creation of nanoscale particles with potent antibacterial activity against multidrug resistant bacterial pathogens (Aljeldah *et al.,* 2023). Metal NPs can be divided into numerous categories, such as silver, gold, copper etc.

Ananda Murthy *et al.,* 2018 reviewed various related studies on synthesize of copper and copper oxide nanoparticles and also determined its multifunctional applications of the synthesized nanoparticles especially in pharmacological fields. The synthesized copper oxide nanoparticles were characterized by using 4 different characterization analysis namely XRD, SEM, FTIR, UV-Vis Spectroscopy analysis. Marjan Asemani and Navideh Anarajan (2019) reported the synthesis of CuO NP's using the leaf extract of *Juglans regia*. The synthesized copper oxide nanoparticles were first confirmed by UV-Vis spectrophotometry followed by SEM and XRD analysis. Khushboo Singh *et al.* (2014) determined antimicrobial activity – MIC of

green synthesized silver nanoparticles using 2 different plants namely *Phyllanthus amarus* and *Tinospora cordifolia*.

The antibacterial activity of CuO NP's was investigated by Karam Khan *et al.* (2025). They analysed using two methods of determining – Minimum Inhibitory Concentration (MIC) and Well diffusion method. The MIC of CuO NP's for *Bacillus cereus* and *Pseudomonas aeruginosa* were found to be 17mm and 20mm respectively. Ziad T. Khodair *et al.* (2019) experimented the synthesis of CuO NP's and its evaluation of its antibacterial activity against *P. aeruginosa* from different diabetic foot ulcer patients in Iranian Hosptial.

Hence in this study, CuO NP's were synthesized using *Murraya koenigii* (Curry Leaf) which were evaluated for further analysis. Synthesized CuO NP's is subjected for characterization study which acts as a preliminary confirmation of the synthesized nanoparticles in different ways. Initially UV-Visible spectroscopy (UV-Vis) was determined in previous research (Deepika and Noorjahan, 2018) to confirm the wavelength of the CuO NP's. The study was further extended to characterize via FE-SEM, XRD, EDAX, TEM, HR-TEM, FTIR, AFM, Zeta Potential analysis and also its antibacterial activity using Minimum Inhibitory Concentration (MIC) method.

Materials and Methods

Preparation of Green Synthesized Copper Oxide Nanoparticles (CuO NP's)

Fresh leaves of *M. koenigii* (Curry leaf) commonly known as Karuveppelai in Tamil, were procured from the market, Chennai, Tamil Nadu, India. The procedure was carried out and published in previous article (Deepika and Noorjahan, 2018). The resultant powdered sample was subjected for various characterization.

Characterization of green synthesized CuO NP's

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was carried out to study the interaction of biomolecules present in the green synthesized CuO NP's and the changes in chemical compositions of the mixtures were determined. FTIR spectra of green synthesized CuO NP's synthesized using *M. koenigii* leaf extract was recorded in SHIMADZU-8400 spectrometer using KBR pellet method which was assessed in Ethiraj College for Women (Autonomous), Chennai, Tamil Nadu, India.

Field Emission Scanning Electron Microscope (FE-SEM)

The arrangement of green synthesized CuO NP's was determined by Field Emission Scanning Electron Microscope (FE-SEM) [JSM-7600F, JEOL. Inc. JAPAN] at SRM Institute of Science and Technology, Kattankulathur, Chennai, Tamil Nadu, India. The resultant FE-SEM images were topographically observed and analyzed based upon the outer surface examination.

Transmission Electron Microscope (TEM)

Transmission Electron Microscope (TEM) is a technique used to study the shape and size of the green synthesized CuO NP's. TEM study was carried out using Philips (Technai 10) in IIT Madras, Chennai, Tamil Nadu, India. Thin layer of the sample were processed on a copper grid by just placing a very small aliquot of the sample on the grid, extra sample solution was removed using a blotting paper and the film on TEM grid was subjected to dry by placing it under incubator. The images were obtained by Technai, twin 200 KV and a bias voltage of 200 KV was used to confirm the respective sample is nanoparticles.

High Resolution Transmission Electron Microscope (HR-TEM)

HR-TEM study is used to determine the structural changes occurs in the green synthesized CuO NP's. High Resolution Transmission Electron Microscope (HR-TEM) was carried out in IIT Madras, Chennai, Tamil Nadu, India using JEOL – 3010 instrument with ultrahigh resolution.

X-Ray Diffraction (XRD)

X-Ray Diffraction pattern is a technique used to determine the size and shape of the unit cell which depends on the peak appearance and expressed based upon the electron density. Location of the atom can be determined based on the appearance of the peak. XRD measurement of green synthesized CuO NP's was drop-coated on glass on a Brukeraxs-D8 advance instrument operated at a voltage of 40 kV and current of 20 mA with Cu Ka radiation which was carried out in IIT Madras, Chennai, Tamil Nadu, India. The size of the green synthesized CuO NP's was also reported from X-ray line broadening using the Debye – Scherrer formula.

$CS = K\lambda / \beta \cos \theta$

Where CS stands for crystalline size

Constant [K] = 0.94

$\lambda = 1.5406 \times 10\text{-}10$

β is the full Width at Half Maximum [FWHM]

Full Width at Half Maximum in radius [β] = FWHM x π / 180

Cos θ = Bragg's angle.

Energy Dispersive X-Ray Spectroscopy (EDXS) analysis

Energy Dispersive X-Ray Spectroscopy (EDXS) analysis was examined to study the crystalline nature of green synthesized CuO NP's and also to identify the chemical compounds present in the sample. EDXS analysis was carried out using AMETEK in IIT Madras, Chennai, Tamil Nadu, India.

Atomic Force Microscopy (AFM)

Atomic Force Microscopy (AFM) is a technique used to study the morphology of green synthesized CuO NP's. The study was carried out in Madras Veterinary College, Vepery, Chennai, Tamil Nadu, India. The resultant images were obtained using a Digital Instrument – WITEC Nanospace microscope at 27°C.

Zeta Potential analysis

Zeta potential is used to determine the electrokinetic potential present in the green synthesized CuO NP's in liquid medium for the surface charge of the particle. Zeta potential analysis of the sample was carried out using the instrument HORIBA Scientific SZ-100 (Version – 2.00) at Madras Veterinary College, Vepery, Chennai, Tamil Nadu, India.

Antibacterial Activity of Green Synthesized CuO NP's By MIC Method

Four isolates of bacterial species namely, *Escherichia coli, Staphylococcus aureus, Pseudomonas aeroginosa* and *Salmonella typhii w*ere obtained (Clinical samples) from Girishwari Hospital, Chennai, Tamil Nadu, India. Each isolate was sub-cultured separately and used for the determination of antimicrobial activity of green synthesized CuO NP's by Minimum Inhibitory Concentration (MIC) - Resazurin Microtitre Assay 96-well plate procedure by Anjum and Anil (2013).

Results

Green synthesis of CuO NP's

The reduction of Copper ions into CuO NP's were reported in previous article (Deepika and Noorjahan, 2018). Using the synthesized CuO NP's which were subjected for characterization using different techniques and also its antibacterial activity.

Characterization of Green synthesized CuO NP's

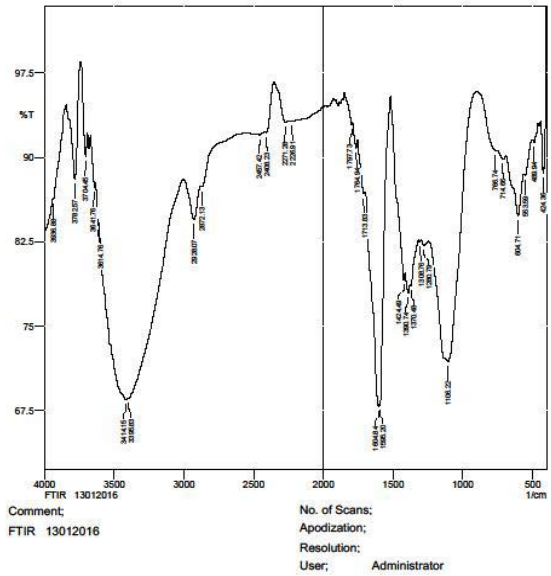
FTIR

FTIR Spectroscopy is a technique used for the determination of the interactions between various components and alterations occurred in the chemical composition present in the resultant solution. FTIR measurements of the green synthesized CuO NP's were carried out to describe the desirable biomolecules which were subjected for the capping, reduction and effective stabilization of the bio-reduced CuO NP's. The results of FTIR analysis of green synthesized CuO NP's were represented in Figure – 1. The absorption wavelength of the FTIR analysis ranges from 500-4000 cm-1. The results of the FTIR analysis revealed the presence

of functional groups present in the green synthesized CuO NP's which expressed the number of absorption peaks and each one reflecting its complex nature. The absorption of the peak at 3414 cm^{-1} is the characteristic of N-H stretching vibrations indicates strong hydrogen bonding. The peaks at 424 cm^{-1}, 489 cm^{-1}, 553 cm^{-1}, 604 cm^{-1}, 714 cm^{-1} and 766 cm^{-1}, may be due to the presence of crystal, chemical bonding and relative intense of carbonates. The peaks at 1424 cm^{-1}, 1370 cm^{-1} and 1390 cm^{-1} indicates CH_2 asymmetric bending band. The peak at 1604 cm^{-1} reveals the presence of OH group, peak at 1595 cm^{-1} indicates C=C bond group and 1106 cm^{-1} was due to C-O groups as well as the bands at 3936.88 cm^{-1}, 3782.57 cm^{-1}, 3704.45 cm^{-1} and 3641.15 cm^{-1} represents the aliphatic C-H stretching and bending lines.

Bands at 3414.15 cm^{-1} and 3395.83 cm^{-1} denotes O-H stretching, H-bonding alcohols and phenols. The bands at 2928.07 cm^{-1}, 2872.13 cm^{-1}, 2271.28 cm^{-1} and 2226.91 cm^{-1} may be due to N-H groups of amine. Thus the results of the FTIR spectrum of green synthesized CuO NP's revealed that CuO NP's was surrounded with various organic molecules such as alcohols, aldehydes, ketones, terpenoids and carbonic acid that acts as capping and reducing agents which provides strength to the nanoparticles.

Figure - 01: FTIR Analysis of green synthesized CuO NP's

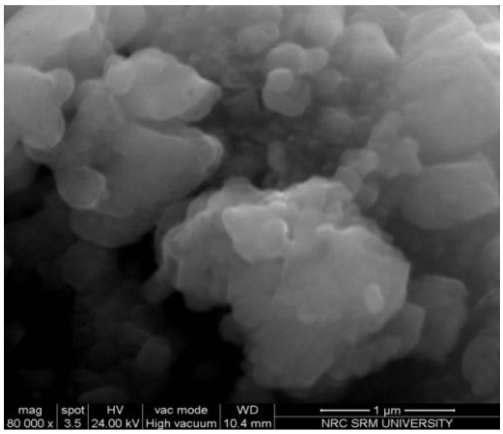


	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	424.36	88.593	1.853	441.72	417.61	1.072	0.109
2	489.94	91.338	0.588	499.58	461.01	1.413	0.074
3	553.59	88.354	0.551	561.31	500.55	2.797	0.064
4	604.71	84.836	4.217	690.55	562.27	7.601	1.307
5	714.66	89.867	0.435	759.99	691.51	3.072	0.064
6	766.74	90.617	0.223	900.8	760.95	4.285	0.172
7	1106.22	71.864	16.092	1246.07	901.76	30.758	13.4
8	1280.79	82.194	0.435	1297.18	1247.03	4.23	0.068
9	1308.76	82.469	0.19	1318.4	1298.15	1.686	0.011
10	1370.48	78.613	0.517	1376.27	1319.37	5.266	0.03
11	1390.74	77.982	1.094	1414.85	1377.23	3.945	0.135
12	1424.49	79.093	1.643	1467.89	1415.81	4.499	0.206
13	1595.2	67.983	1.38	1599.06	1518.04	6.524	0.154
14	1604.84	67.844	1.159	1696.47	1600.02	11.43	0.409
15	1713.83	86.754	0.765	1756.26	1707.08	2.549	0.097
16	1764.94	90.807	1.108	1790.98	1757.23	1.277	0.101
17	1797.73	92.91	0.435	1847.88	1791.95	1.435	0.03
18	2226.91	93.225	0.004	2227.88	2059.1	4.95	0.101
19	2271.28	93.143	0.911	2353.26	2245.24	2.637	0.207
20	2408.23	92.215	0.788	2418.84	2354.22	1.715	0.146
21	2457.42	91.991	0.254	2551.93	2419.8	4.722	0.073
22	2872.13	87.385	0.141	2879.85	2552.9	13.452	0.014
23	2928.07	84.47	3.18	3001.37	2880.81	7.763	0.899
24	3395.83	68.565	0.201	3399.68	3002.33	43.828	1.222
25	3414.15	68.457	1.127	3609.93	3400.65	28.711	3.694
26	3614.76	83.998	0.754	3634.05	3610.9	1.591	0.061
27	3641.76	87.309	1.224	3669.73	3635.01	1.744	0.116
28	3704.45	90.049	3.676	3742.06	3691.91	1.458	0.413
29	3782.57	88.033	7.892	3821.15	3743.03	2.803	1.403
30	3936.88	86.29	0.318	3939.78	3858.76	3.814	0.213

Field Emission Scanning Electron Microscope (FE-SEM) of green synthesized CuO NP's

FE-SEM is a technique used to view topographical surfaces of the given sample. The result of the Field Emission Scanning Electron Microscope (FE-SEM) of green synthesized CuO NP's was presented in Plate - 01. The result of FE-SEM analysis showed that the green synthesized CuO NP's was spherical in shape and they were found to be in small and homogenous sizes with smooth surfaces. Thus the result of the FE-SEM image was similar to SEM image indicating the spherical shape of the nanoparticles.

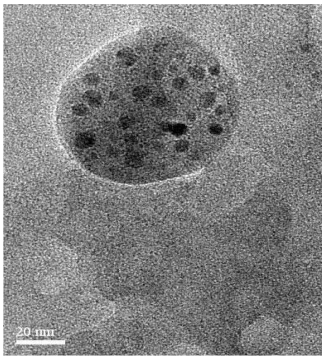
Plate – 01 : Field Emission Scanning Electron Microscopic (FE-SEM) image of green synthesized CuO NP's



Transmission Electron Microscope (TEM) of green synthesized CuO NP's

Transmission Electron Microscope (TEM) is another type of microscope which is used for the characterization of the metal nanoparticles. When a beam of electrons were exposed to an ultra-thin sample, interaction of the sample with the electrons was occurred and it passes through the sample pathway. An image of the sample were gathered from the combination of the electrons which were transmitted through the sample. The images of the samples were amplified and focused on the imaging device. The result of TEM micrographs of the green synthesized CuO NP's was depicted in the Plate - 02. TEM image provides a clear crystal structure of green synthesized CuO NP's. Nanostructure of the given sample was observed to be spherical in shape which was in diffused form with the size of 20 nm in diameter. The synthesized nanoparticles was found to be well structured without any other cluster formations in it.

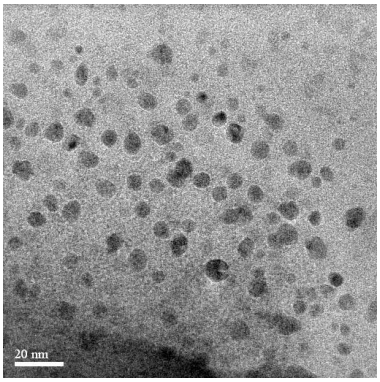
Plate - 02 : Transmission Electron Microscopic (TEM) image of green synthesized
CuO NP's



High Resolution Transmission Electron Microscope (HR-TEM) of green synthesized CuO NP's

High Resolution Transmission Electron Microscope (HR-TEM) is a technique used for the determination of morphology and the size of the synthesized nanoparticles. The result of HR-TEM of green synthesized CuO NP's was shown in the Plate – 03. HR-TEM images revealed the appearance of spherical dark spots with light coloured structures of the synthesized CuO NP's. The size of the nanoparticles ranged from 5 nm to 100 nm in diameter respectively.

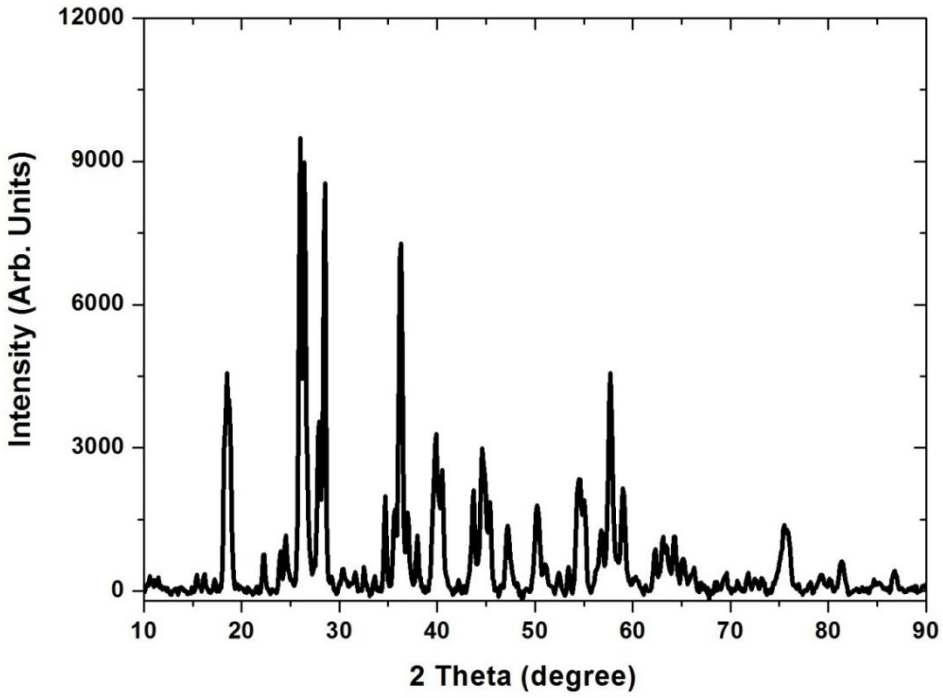
Plate - 03 : High Resolution Transmission Electron Microscopic (HR-TEM) image of green synthesized CuO NP's



X-Ray Diffraction (XRD) of green synthesized CuO NP's

The result of the X-Ray Diffraction pattern of the green synthesized CuO NP's was depicted in Figure - 02. The result of the XRD pattern depicts a high amount of crystallinity of CuO NP's with a diffraction angle ranged from 10-90° at 2θ (degree). The result of the XRD pattern of green synthesized CuO NP's revealed various significant peaks at 2 θ = 27.5°, 36° and 58°. These peaks of the sample corresponds to (113), (200) and (220), Bragg's reflections of the Face Centered Cubic (FCC) morphology of the synthesized CuO NP's respectively (Standard JCPDS No. 89-5899). The size of the nanoparticles was also obtained from X-Ray line which was calculated using the Debye-Scherrer formula. The interplanar spacing values were also listed as 1.7698, 1.7385 and 8.2615 respectively.

Figure - 02: XRD Analysis of green synthesized CuO NP's



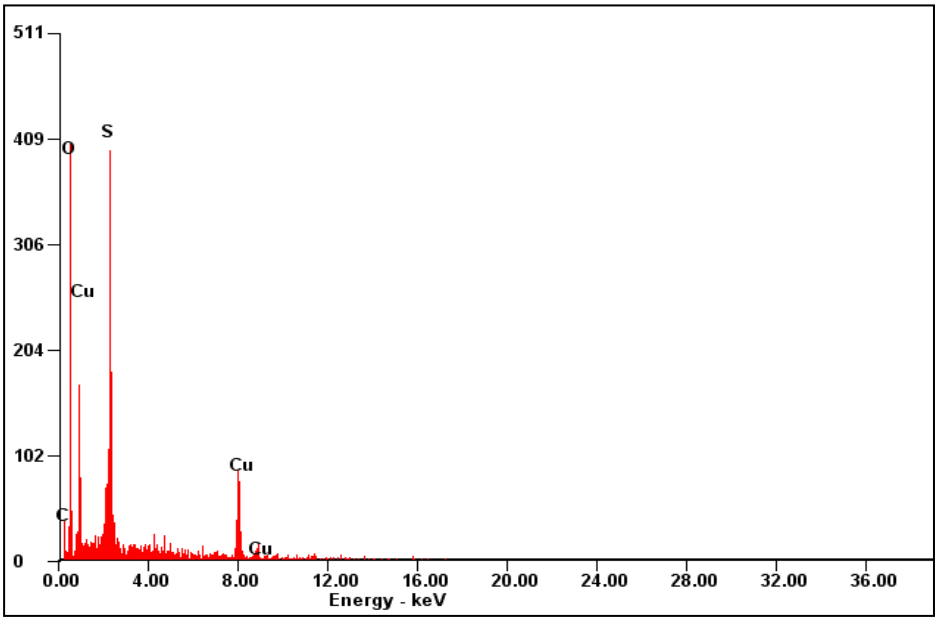
Energy Dispersive X-Ray Spectroscopy (EDXS) of green synthesized CuO NP's

The results of the Energy Dispersive X-Ray Spectroscopy (EDXS) of green synthesized CuO NP's were presented in Figure - 03. The results of EDXS study revealed that optical absorption peak were recorded at 8 Kev, which was found to

be suitable for the absorption of metallic CuO NP's. From the Energy Dispersive Detector (EDS) signals, it was clear that green synthesized CuO NP's was reduced using plant source ie., aqueous leaf extract of *M. koenigii*. Hence, the reduced CuO NP's was found to be 28.27% of weight percentage. Weak signals were also depicted in the EDXS figure which shows the presence of sulphur and carbon in the resultant peak. Thus the resultant image indicated that the phytocomponents present in the fresh aqueous leaf extract of *M. koenigii* along with their secondary metabolites would have also been involved in the reduction process. Hence the EDXS analysis showed that weight percentage of green synthesized CuO NP's was 28.27% and also indicated the presence of CuO NP's in the resultant solution which was synthesized using aqueous leaf extract of *M. koenigii* (Curry leaf).

Figure – 03:

Energy Dispersive X-Ray Spectroscopy (EDXS) Analysis of green synthesized CuO NP's

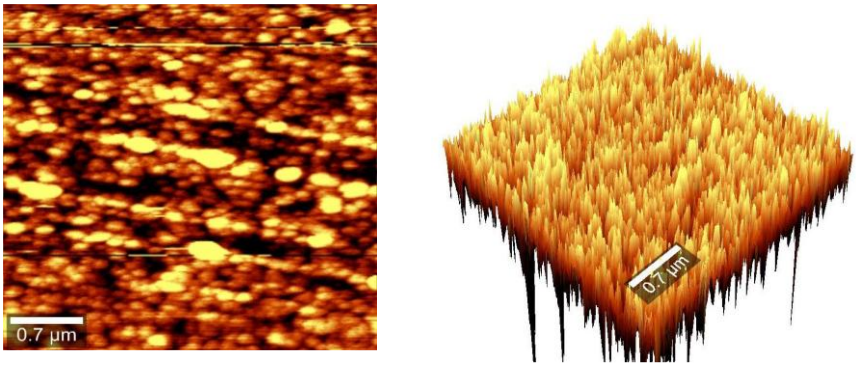


Atomic Force Microscopy (AFM) of green synthesized CuO NP's

Atomic Force Microscopy (AFM) is a technique used to produce the images in the form of 2-dimensional and 3-dimensional shapes of the sample with respect to topographical pattern of the green synthesized CuO NP's. The results of AFM images of green synthesized CuO NP's were depicted in Plate – 04 (a and b). It showed that it was almost spherical in shape as similar to the results obtained in SEM and TEM analysis of green synthesized CuO NP's. The AFM images also anticipated

three dimensional view of the sample surface over 2x2 µm scan and uniform height arrangement was also observed around 0.7 µm. Thus the results of AFM images revealed that the green synthesized CuO NP's has the characteristic features of bifunctional compounds and also represented in topographical depiction. It was found to be in various sizes ranging from 40nm to 100nm with uniform spherical structures.

Plate – 04 (a and b): Atomic Force Microscopy (AFM) analysis of green synthesized CuO NP's



Zeta Potential Analysis

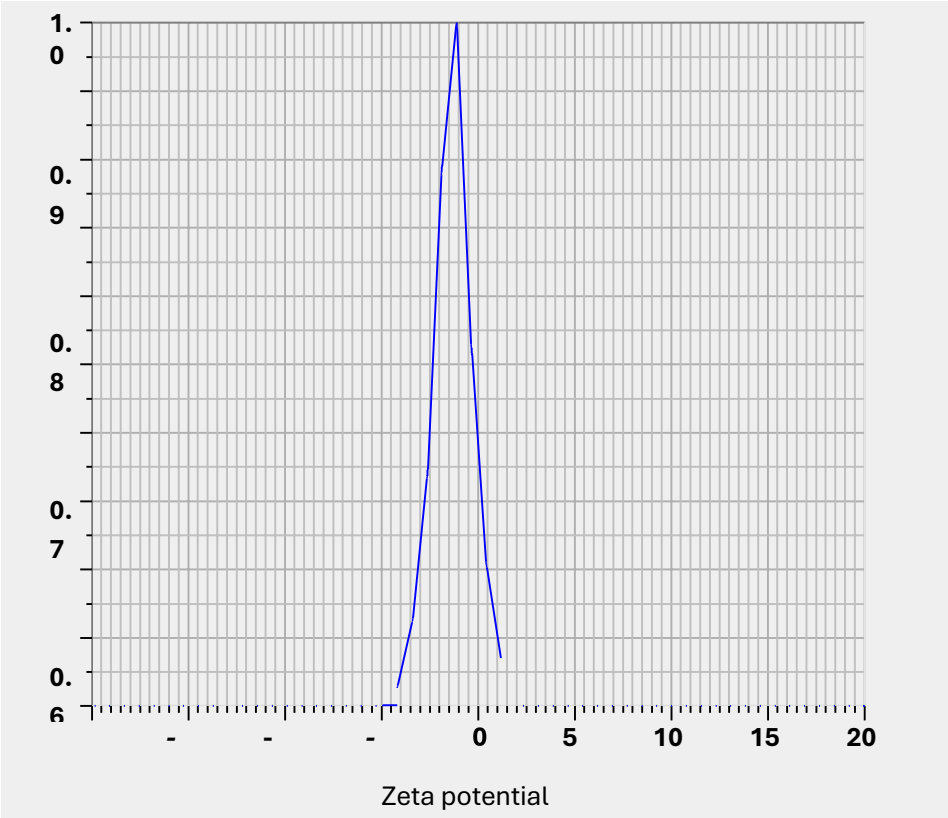
Zeta potential analysis was carried out to determine whether the synthesized nanoparticles contains positive or negative charges which helps the nanoparticles to penetrate into the membrane of the target cell. The result of zeta potential analysis of green synthesized CuO NP's was presented in Table – 01 and Figure – 04. The results obtained from the zeta potential analysis corresponds at 25°C and approximately ranged from -19.2 mV to -13.0 mV which was correlated to the elctrophoretic mobility that ranged from -0.000149 cm2/Vs to -0.000101 cm2/Vs respectively. Hence, if the zeta potential was greater than +30 mV or less than -30mV indicates that green synthesized CuO NP's has active cationic and anionic charges respectively.

Table – 01: Data of Zeta potential of green synthesized CuO NP's

Measurement type: Zeta potential
Temperature of the holder: 25.0 °C
Medium Viscosity: 0.895 mPa.S
Conductivity: 0.754 mS/cm
Electrode Voltage: 3.3 V

Peak No.	Zeta Potential	Electrophoretic Mobility
1.	-19.2 mV	-0.000149 cm^2/Vs
2.	-15.9 mV	-0.000123 cm^2/Vs
3.	-13.0 mV	-0.000101 cm^2/Vs

Figure – 04: Zeta potential of green synthesized CuO NP's



Value for Zeta potential = -16.03±3.10 mv

Antibacterial activity of green synthesized CuO NP's - Minimum Inhibitory Concentration (MIC) (Resazurin Microtitre Assay 96-well plate)

The least concentration of the antibacterial activity which was used to inhibit the growth of the bacterial isolates was determined as Minimum Inhibitory Concentration (MIC). After the incubation period of about 24 hours, 96 well plate were visualized for the antibacterial activity of green synthesized CuO NP's using Resazurin dye. Change of colour was observed as MIC value (Plates – 05 and Tables – 02). MIC of the bacterial isolates revealed the green synthesized CuO NP's, the least concentration used to inhibit the growth of bacterial isolates (Table – 02) was found to be 125 µg/ml concentration in *Pseudomonas aeruginosa* and *Salmonella typhii* followed by *Staphylococcus aureus* (250 µg/ml concentration) and *Escherichia coli* at 500 µg/ml concentration of the sample. The colour change of resazurin dye revealed the antibacterial activity of the samples. The change of colour from violet to pink showed the antibacterial activity of green synthesized CuO NPs (Plates – 05). Thus from the overall result of the antibacterial activity, green synthesized CuO NPs showed prominent inhibitory activity against all the 4 bacterial species

Plate – 05:

Antibacterial activity of green synthesized CuO NP's Minimum Inhibitory Concentration (MIC) (Resazurin Microtitre Assay 96-well plate)

E. coli

P. aeruginosa

S. typhii

S. aureus

Negative Control

Positive Control

Without Culture

With Culture



Table - 02: Minimum Inhibitory Concentration (MIC) of green synthesized CuO NP's against bacterial isolates

S. No	Microorganisms	Concentrations of green synthesized CuO NP's									
		1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	31.25 µg/ml	15.25 µg/ml	7.812 µg/ml	Positive control 10µg/ml	Bacterial Culture
1	*Escherichia coli*	-	-	+	+	+	+	+	+	-	+
2	*Pseudomonas aeruginosa*	-	-	-	-	+	+	+	+	-	+
3	*Salmonella typhii*	-	-	-	-	+	+	+	+	-	+
4	*Staphylococcus aureus*	-	-	-	+	+	+	+	+	-	+

\+ = Presence of bacteria

\- = Absence of bacteria

Discussion

Aqueous leaf extract of *M. koenigii* was further used for the synthesis of CuO NP's. The resultant metal nanoparticles was further confirmed using UV-Visible Spectrophotometer and also other characterization techniques. The absorbance of the spectral analysis was observed at 410 nm. The result of the study is in accordance with the report of Pestryakov *et al.* (2004); Zhao *et al.* (2004); Das *et al.* (2013) and Hemalatha and Makeswari (2017). FTIR analysis of green synthesized CuO NP's was carried out, which revealed the presence of various chemical compounds that are responsible for capping, reduction and effective stabilization of bio-reduced CuO NP's. The absorbance of green synthesized CuO NP's using FTIR analysis ranged from 500-4000 cm^{-1}. Nanoparticles was encircled with various biomolecules such as alcohols, aldehydes, ketones, terpenoids and carbonic acid which acts as reducing agents of green synthesized nanoparticles. The resultant biochemicals was in accordance with Caroling *et al.* (2015); Ashtaputrey *et al.* (2017), Palajonna *et al.* (2017) and Hari *et al.* (2019). FE-SEM was carried out to study the topographical surfaces of the green synthesized CuO NP's. The results of the study revealed that they were spherical in shape with homogenous sizes. Similar results were also reported by Renu Sankar *et al.* (2014). TEM analysis was studied to determine the shape and size of the green synthesized CuO NP's. The synthesized nanoparticles appeared spherical in shape with 20nm in diameter. The results of the study is in agreement with the results reported by Maqusood Ahamed *et al.* (2010), Caroling *et*

al. (2015), Jayandran *et al.* (2015); Raja Naika *et al.* (2015); Seeram Hariprasad *et al.* (2016) and Nagajyothi *et al.* (2016). HR-TEM study was carried out to determine the morphology and size of the green synthesized CuO NP's. The result of the study revealed the appearance of spherical dark spots with light coloured spherical structures of CuO NP's. Similar results were recorded by Pulicherla *et al.* (2017) and Rahul Shah *et al.* (2019). XRD was carried out to determine the size and shape of the green synthesized CuO NP's which was studied based on the electron density. Various peak formations were observed and significant peaks were recorded at 2θ = 27.5°, 36° and 58°. These peaks corresponds to (113), (200) and (220), Bragg's reflections Face Centered Cubic (FCC) respectively. The interplanar spacing values were recorded to be at 1.7698, 1.7385 and 8.2615. The results was correlated with the previous literature and were found to be closely matching with the 2θ values as reported by Amrut *et al.* (2010); Abboud *et al.* (2013); Vinod Vellora *et al.* (2013); Jayalakshmi and Yogamoorthi (2014) and Baskar *et al.* (2016). EDXS study was carried out to identify the chemical compounds present in the green synthesized CuO NP's. The synthesized CuO NP's was found to be 28.27% (weight percentage) which was represented in peak formation. Similar results were reported by Ren *et al.* (2009) and Faheem Ijaz *et al.* (2017). AFM was determined to study the 2D and 3D morphological structures of the green synthesized CuO NP's. AFM images of green synthesized CuO NP's appeared to be spherical in shape, similar to that of SEM and TEM images which were uniformly distributed. Similar spherical arrangements of green synthesized CuO NPs were observed by Zainab *et al.* (2018). Zeta potential was carried out to determine the electrokinetic potential surface charges of the green synthesized CuO NP's. The obtained zeta potential image corresponds at 25°C and approximately ranged from -0.000149 cm2/Vs to - 0.000101 cm2/Vs respectively. Hence, green synthesized CuO NP's was found to have active cationic and anionic charges respectively. The results of the study are in accordance with the results of Yendukar *et al.* (2017). According to Melendrez *et al.* (2010), synthesized nanoparticles was found to be stable in nature when they contain electric charges which helps nanoparticles to penetrate into the cell membranes of the organisms for fighting against various diseases. The study was further extended to determine the antimicrobial activity of green synthesized CuO NP's. Minimum Inhibitory Concentration (MIC) (Resazurin Microtitre Assay) was carried out in bacterial isolates to determine the lower concentration of the samples against the bacterial isolates. In green synthesized CuO NP's, the minimum concentration to inhibit the growth of bacterial isolates was recorded in *S. typhii* and *P. aeruginosa* was at 125µg/ml concentration followed by *S. aureus* (250 µg/ml concentration) and *E. coli* (500 µg/ml concentration). The change of colour of resazurin dye from violet to pink showed the antibacterial activity of green synthesized CuO NP's. Similar results of

antibacterial activity through MIC method was reported by Maqusood Ahamed *et al.* (2014).

II. CONCLUSION

To conclude from the overall results of the study, green synthesized CuO NP's was synthesized using biological source – aqueous leaf extract of *M. koenigii* (Curry leaf) which acts as an alternate method for synthesis of Copper oxide (CuO) nanoparticles due to its easily availability, time consumption and cost effectiveness. Aqueous leaf extract of *M. koenigii* acts as a best reducing agent for the synthesis of nanoparticles. The characterization - UV-Vis spectroscopy, FTIR, SEM, FE-SEM, TEM, HR-TEM, XRD, EDXS, AFM and Zeta potential confirms the synthesized CuO NP's. Further, green synthesized CuO NP's showed excellent *invitro* – antimicrobial activity using MIC method. Hence it is confirmed from the results of the study that the green synthesized CuO NP's can be used in biomedical fields and also it ensures that biological synthesis of nanoparticles can minimize the risks to human beings and also to the environment by improving the efficacy of quality, performances and also acts as an alternative method for various diseases.

III.REFERENCES

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