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GREEN SYNTHESIS OF CU(OH)$_2$ NANOMATERIALS USING *NYMPHAEA RUBRA* LEAVES EXTRACT AND THEIR ANTIBACTERIAL ACTIVITY

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Abstract

The present study gives the eco-friendly green synthesis for the preparation of copper hydroxide nanomaterials [CuHNMs] using Nymphaea Rubra leaves extract. Bio-molecules were responsible for the formation of CuHNMs and they found to play dual role of both reducing as

well as capping agents. The synthesized CuHNMs were characterized by Fourier Transform Infrared Spectroscopy (FTIR), Ultraviolet-Visible spectrometer (UV-Vis), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Electron Diffraction Spectrum (EDS) and Dynamic Light Scattering (DLS).

Characterization data reveals that the CuHNMs were crystalline in nature, orthorhombic in shape with an average size of 19.4 nm and Zeta Potential (Mean) was -10.0mV. The green synthesized CuHNMs were examined for its antibacterial activity and the results shows that these materials exhibit effective anti-bacterial activity against Bacillus subtilis when compared to Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli.

Keywords

Green Synthesis, CuHNMs, Nymphaea Rubra Leaves Extract, Characterization and Anti-Bacterial Activity

1. Introduction

Nanomaterials (nanopowder or nanocluster or nanocrystal) are nano sized particle with at least one dimension less than 100 nm. Recently it has broadened the scope of elevating research in various scientific disciplines due to their unique properties compared with bulk counterparts. These nanomaterials shows potential applications like catalysts, drug delivery materials, photonic materials, battery materials and biomedical applications (By Jin Ho Bang & Kenneth S. Suslick, 2010;Y. Chen et al, 2017; Xiaojing Wang, Ji Feng, Yaocai Bai, Qiao Zhang & Yadong Yin, 2016; Vadia et al., 2011).

Size of nonmaterial is similar to that of most biological molecules and structures; therefore, nanomaterials can be useful for both in *vivo* and in *vitro* biomedical research and applications. Nanomedicine has generated great enthusiasm in recent years due to important discoveries, especially in cancer therapy (Gandhali A Deshpande, 2016). The synthesis, characterization and application of biologically synthesized nonmaterial have become an imperative branch of nanotechnology. Therefore researchers in this field have been excitedly looking at biological systems as marginal eco-friendly or nontoxic systems (C.S. Espenti et al., 2016).

$Cu(OH)_2$ nanomaterials are used as broad spectrum foliar fungicide on fruits and vegetables, in the industry of rayon, good precursors for the synthesis of copper oxides (Cu_2O

and CuO), in kill mold in paints and in ceramics as colorant. Using physical and chemical methods metallic nanoparticles of specific sizes and morphologies can be synthesis (K. Madhusudhana Reddy *et al, 2016;* Monaliben Shah, Derek Fawcett, Shashi Sharma, Suraj Kumar Tripathy & Gérrard Eddy Jai Poinern, 2015; Iravani. S, H. Korbekandi, S.V. Mirmohammadi & ZolfaghariB,2014; Kalaiyarasi .R,& Rajathi .K.2016;MalikParth, Ravi Shankar, Vibhuti Malik, Nitin Sharma, & Tapan Kumar Mukherjee,2014.;Sapna Thakur, Radheshyam Rai & Seema Sharma,2014). However; these methods employ toxic chemicals as reducing agents or on biodegradable stabilizing agents and are therefore potentially dangerous to the environment and biological systems(Das.J, &Velusamy.P.2013;Nadagouda et al.,2009).

The biological methods of nanoparticles synthesis using biological entities; including bacteria, yeast, fungi (Monaliben Shah, Derek Fawcett, Shashi Sharma, Suraj Kumar Tripathy & Gérrard Eddy Jai Poinern, 2015;Keat et al., 2015;Mousa A. Alghuthaymi, Hassan Almoammar, Mahindra Rai, Ernest Said-Galiev, KamelA. Abd-Elsalam,2015;Chandra Sekhar et al.,2016) and plants were already reported as clean, non-toxic,eco-friendly and environmentally acceptable routes (C.S. Espenti et al., 2016; K. Madhusudhana Reddy *et al, 2016;*Chandra Sekhar et al.,2016). The use of plant extracts for synthesis of nanoparticles is potentially advantageous over microorganisms due to the ease of scale up, the less biohazard and elaborate process of maintaining cell culture. The present research designed with a novel, rapid, clean, non-toxic and environmentally acceptable green route for the synthesis of copper hydroxide nanomaterials by co-precipitation method using copper chloride and sodium hydroxide in the presence of *Nymphaea Rubra* leaf extract.

2. Experimental Analysis

2.1 Materials and Methods

Nymphaea Rubra fresh leaves collected from Sri Venkateswara University, Tirupati, Chittoor District, Andhra Pradesh, India. Dihydrated Cupric chloride ($CuCl_2.2H_2O$) and Sodium Hydroxide purchased from Sigma-Aldrich. Mullar Hinton Agar (MHA), Nutrient broth purchased from Hi-media laboratories Pvt. Ltd., Mumbai, India. The bacterial strains of gram-negative cultures *Escherichia coli*, *Pseudomonas aeruginosa* and gram-positive cultures *Bacillus Substilis*, *Staphylococcus aureus* were obtained from Microbial type Culture Collection, Bangalore, India.

2.1.1 Preparation of *Nymphaea Rubra*leaf extract

The *Nymphaea Rubra* leaves washed at first with running tap water followed by milli-Q water then dried in shade for 10 days to completely detract the moisture from leaves. The dried leaves grounded several times to get fine powder and the aqueous leaf extract was prepared by using 10gm of fine powder of *Nymphaea Rubra leaves* mixed with 100ml of milli-Q water and boiled at room temperature for 15 min and filtered through Whatmann No.1 filter paper. The filtered extract was preserved at 4 ^{o}C for further experimental process.

2.1.2 Synthesis of Cu $(OH)_2$ nanomaterials (CuHNMs)

For the synthesis of CuHNMs, 20 ml of aqueous *Nymphaea Rubra* leaves extract was added drop by drop to the 80 ml of standard $CuCl_2.2H_2O$ (0.01M) solution at constant stirring at room temperature for 30 min. The color change was observed from brown to light yellow. To this yellow color mixture of colloidal suspension 0.1M NaOH was added until light yellow was turned to light green. Light green color indicates the formation of CuHNMs. The path way of the extraction of leaf extract and fabrication of CuHNMs shown in the Fig 1 as Graphical Abstract.

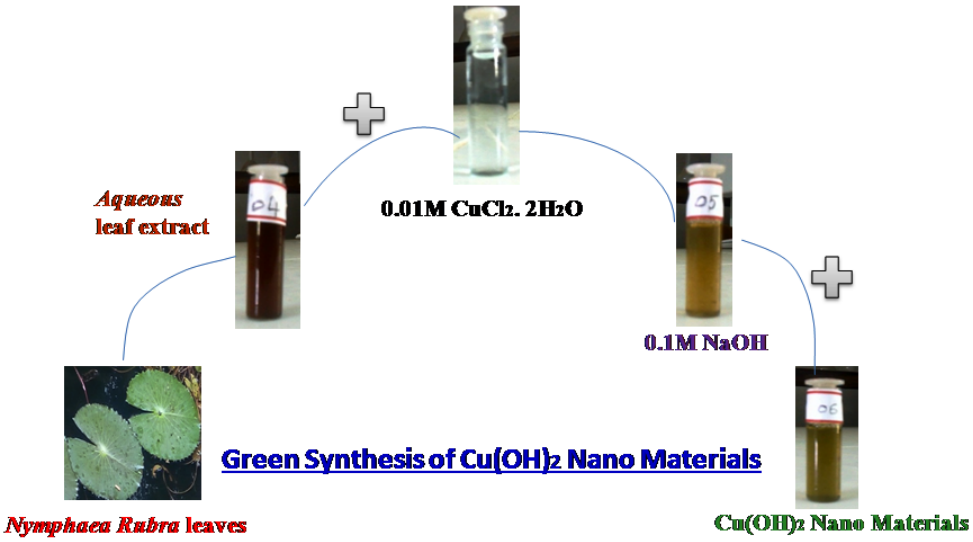


Figure 1: Graphical Abstract: *Schematic representation of formation CuHNMs by green synthesis*

2.1.3 Characterization of synthesized CuHNMs

The UV-Visible spectra recorded with Shimedzu 2400 UV-Visible double beam spectrophotometer from 200 to 800 nm range at room temperature. The dried CuHNMs were endangered to FTIR-ALPHA interferometer (ECO-ATR), Bruker, Ettlingen, Karlsruhe,

Germany, in the range of 400–4000 cm^{-1}. Mean diameter and size circulation of the nano-materials determined by DLS method using Oxford Inca Penta FET×3EDAX instrument.X-ray diffraction analysis of the green-synthesized CuHNMs cast onto glass slides are recorded using a Bruker-Binary V2 (Raw) (Cu radiation, λ = 0.1546nm) running at 40kV and 40mA, and were recorded with 2θ angle from the angle of 10-90°. SEM (Carl Zeiss EVO ma 15) experiments were performed to characterize the size and shape of the CuHNMs.

2.1.4 Anti-bacterial activity

Anti-microbial activity of CuHNMs performed by disc method of Bauer-Kirby (Kirby,W,M.M., Yoshihara, G.M., Sundsted, K, S., & Warren, J .H). The test organisms such as Pseudomonas aeruginosa, Staphylococcus aureus, Esherichia coli and Bacillus Subtilis grown in nutrient broth for 24 hrs. Nutrient agar plates were prepared, sterilized and solidified. After solidification, 100 µl of overnight culture of each organism poured in petriplates using sterile glass rod to prepare bacterial lawns. Sterile discs placed on these plates and CuHNMs were loaded at required volumes on discs and incubated at 37˚C for 24 hours. Distilled water used as a positive control for the anti-bacterial assay. Maximum zone inhibitions observed around the discs. The diameters of all zones measured by meter ruler and mean values for each organism recorded in millimeters.

3. Results and Discussion

3.1 UV-Visible Studies

Fig 2 explains UV-Visible spectral bands of CuHNMs fabricated by mixing of Nymphea Rubra leaf extract with Cu^{+} ion solution at laboratory conditions.UV-Visible spectral bands of formation of CuHNMs at various time intervals are predicted in Fig 2. Initially copper chloride solution turned into light yellow to light green.This color change confirmed the formation of CuHNMs. 5 minutes later around 360-370 nm a strong SPR band observed.

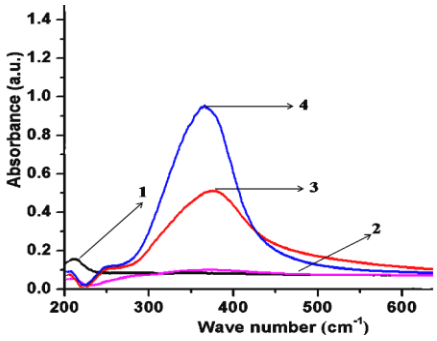


Figure 2: *UV-Visible spectra of CuHNMs obtained using Nymphaea Rubra leave extract [1- plain extract, 2- at 1min, 3- at 5min & 4- at 10 min].*

3.2 FTIR studies

Fig 3 confirms the formation of CuHNMs by green synthesis and eco-friendly method using *Nymphaea Rubra* leaves extract. Fig3A represents the possible functional groups present in the *Nymphaea Rubra* leaves extract and two absorption peaks at 3741 cm^{-1} and 3846 cm^{-1} is due to O-H stretching of hydroxyl group in phytochemicals present in the aqueous extract, 3289 cm^{-1} is due to N-H stretching in amides and 2359 cm^{-1}indicates the R-C=N, A weak signal appeared at 1641cm^{-1} is due to C=O asymmetric vibrations (Sorna Prema Rajendran & Kandasamy Sengodan,2017), and peak at 1018$cm^{-1}$is due to O-H bending vibrations in acids. Fig 3B represents the formation of CuHNMs and most of peaks present in the Fig 3A is disappears due to the formation of CuHNMs by reduction occurred by phytochemicals present in the *Nymphaea Rubra* leaf extract. These phytochemicals are acts as reducing agents as well as capping agents to the CuHNMs (Manish Mathur, 2014).Two peaks at 3362 cm^{-1} and1545 cm^{-1} indicates the hydrogen bonding in hydroxyl groups, bending mode of hydroxyl groups respectively.3740-3844 cm^{-1}peak indicates the O-H stretching vibrations in phytochemicals. Two Peaks at 1375 cm^{-1} and 851 cm^{-1} indicates Cu-OH bond and Cu-OH vibrations. A peak at 518 cm^{-1}indicating the Infra Red active modes of $Cu(OH)_2$. Cu-OH vibrations can indentified using Peak at 1010 cm^{-1} (Awwad and Albiss, 2015)..

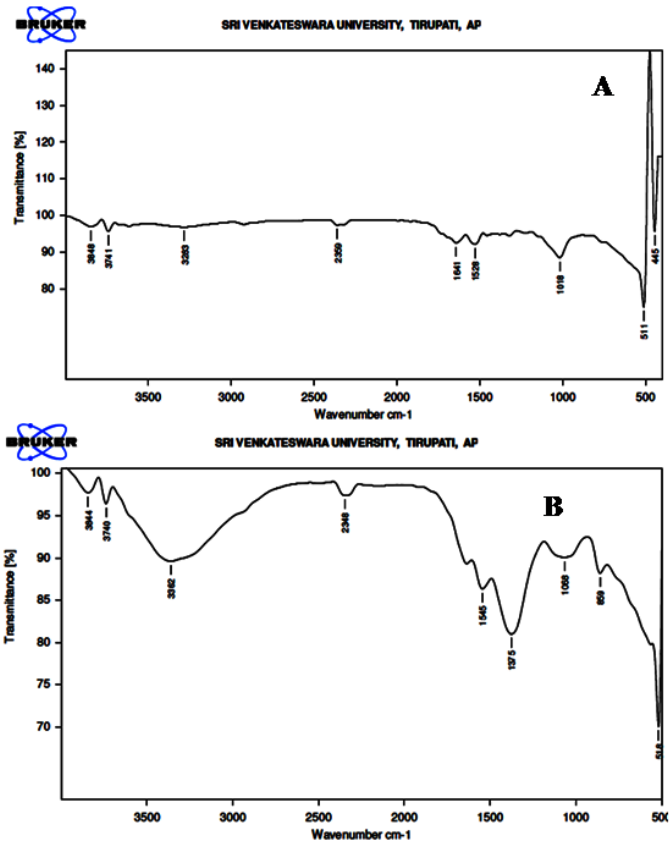


Figure 3: *FTIR spectra of Nymphaea Rubra leaf extract (A) and CuHNMs (B).*

3.3 EDS studies

Figure 4 confirms the formation of CuHNMs and also participated elements and their atomic percentage (%) present in the production of nanomaterials. EDS spectrum shows the signal peak of Cu(4.66%) and indicates the presence of CuHNMs and also the signals shows the elements of C(52.89%), O(39.43%), Mg(0.84%), Cl(1.41%),Ca(1.38%), and K(0.39%) and their percentages were shown in Fig 4.

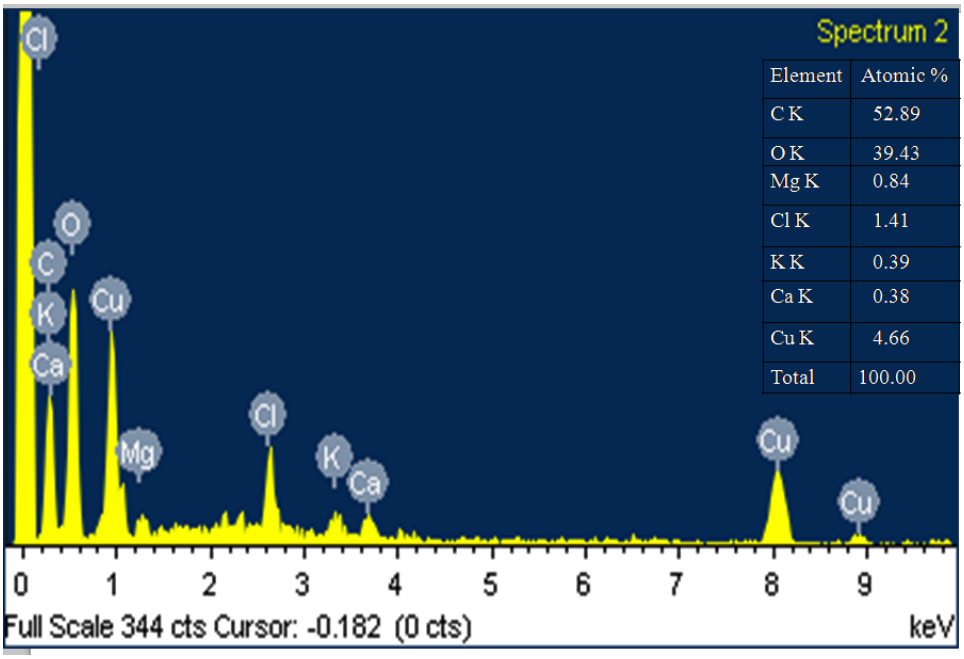


Figure 4: *EDS Spectrum of CuHNMs and Elemental analysis (Insert)*

3.4 FESEM Studies

FESEM micrograph (Fig 5) shows that CuHNMs are orthorhombic shape at different magnifications and further confirmed by XRD analysis and uniformly monodispersed with average size of 19.4nm.This is further proved by DLS

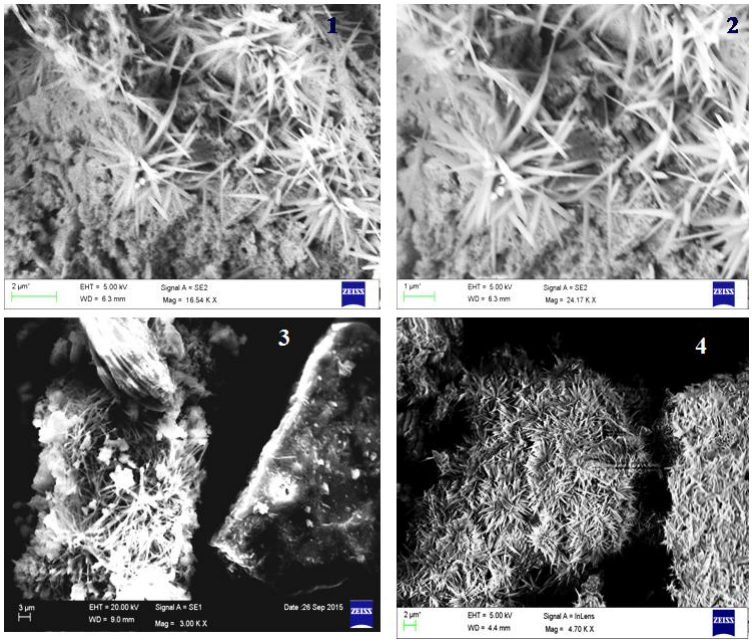
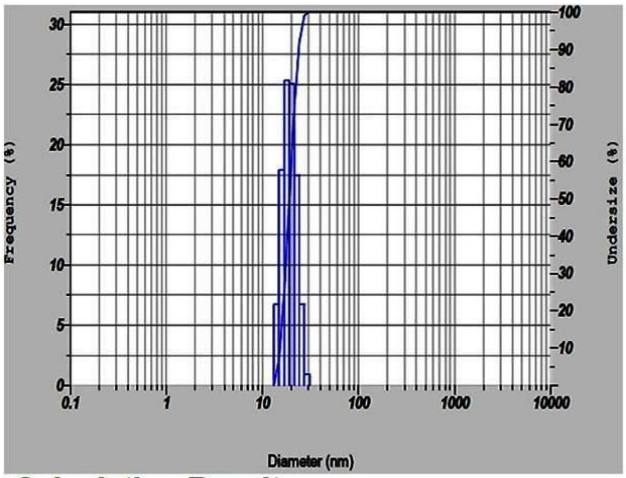


Figure 5: *FESEM micrograph of CuHNMs at different magnifications*

3.5 Particle size measurement

Figure 6 predicts the exact size of the CuHNMs and display the size of the nanomaterials with mean diameter was 19.4 nm and standard deviation at 3.3 nm (Scattering angle is 173^{o}).



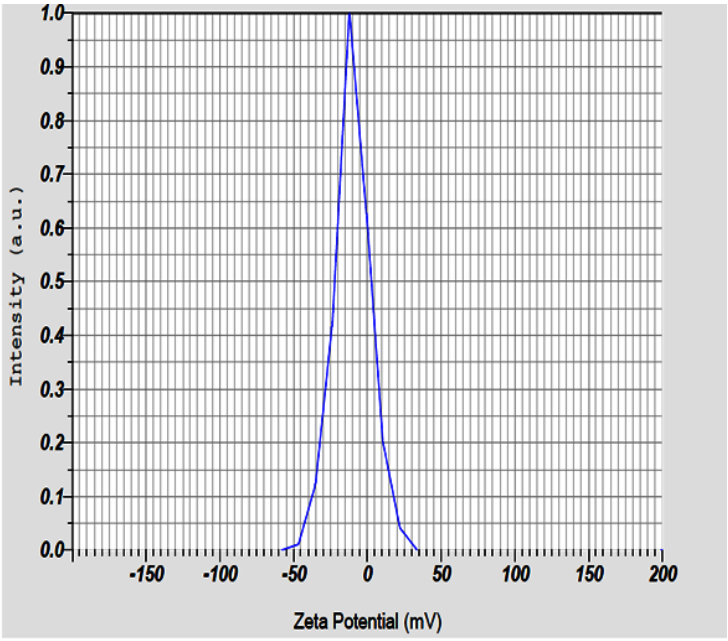
Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	19.4 nm	3.3 nm	18.2 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	19.4 nm	3.3 nm	18.2 nm

Figure 6: *Size distribution analysis of CuHNMs*

3.6 Zeta Potential Determination

The Zeta potential of the fabricated CuHNMs determined in water as a dispersant. The zeta potential (Mean) was found to be −10 mV. The negative value indicates the stability of nanomaterials due to repulsions existing between the particles. Fig7 shows the electrophoretic mobility of the CuHNMs was -0.000078cm^2/Vs.



Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-10.0 mV	-0.000078 cm2/Vs
2	— mV	— cm2/Vs
3	— mV	— cm2/Vs

Zeta Potential (Mean) : -10.0 mV
Electrophoretic Mobility Mean : -0.000078 cm²/Vs

Figure 7: *Zeta potential analysis of CuHNMs*

3.7 X-Ray Diffraction Analysis

Fig8 reveals the XRD pattern of CuHNMs obtained from *Nymphaea Rubra* leaf extract. It is found that all the reflections at different 2θ angles (16.73, 23.83, 38.20, 48.79., 56.22, and77.74) can be well indexed to the inverse spinal orthorhombic structure of CuHNMs (**PCPDFcardno.72-0140)** according to the reflection peak positions and relative intensities which confirm that then a nanomaterials synthesized in this study has high crystallinity of the CuHNMs (R. Mehdizadeh et al, 2014).

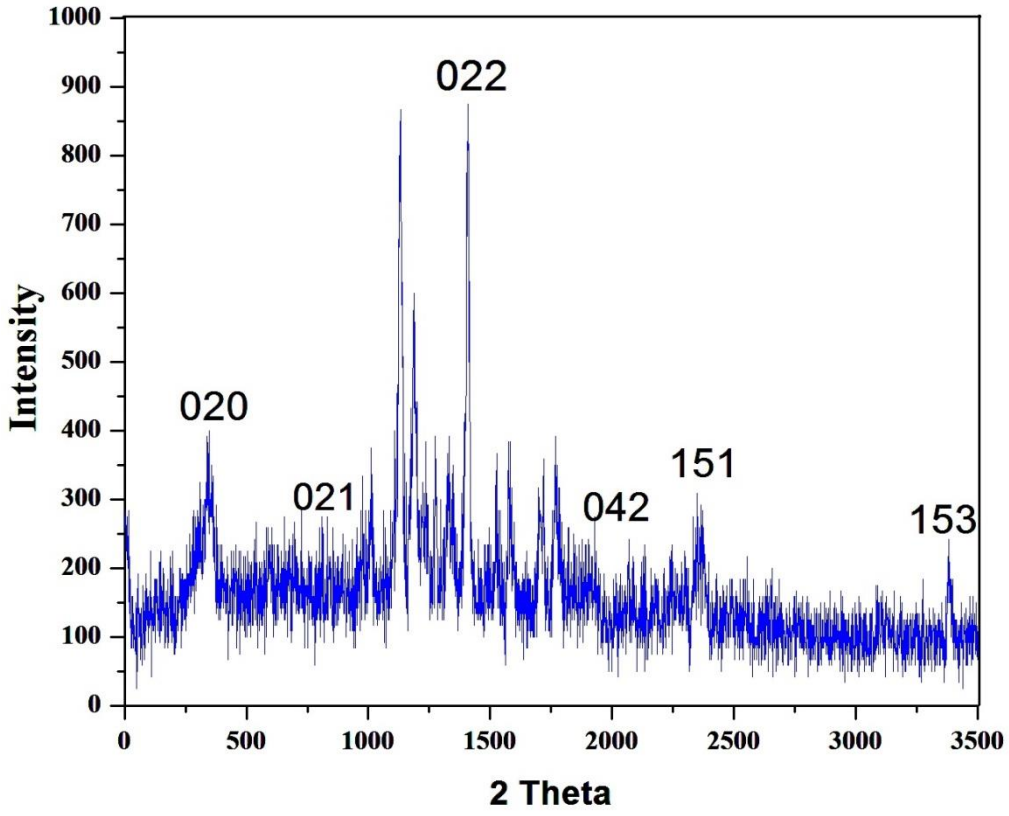


Figure 8: *X-ray diffraction pattern of CuHNMs*

3.8 Anti-Bacterial Activity

Anti-bacterial activity of CuHNMs studied using zone inhibition method. The pathogenic bacterial strains such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus Subtilis* causes spreadable diseases in human beings (Sawant.A. R.,. Raut.R. R,. Patil.T. D, Ruchi R.& Malwade,2014).The ability of nanomaterials can be estimate using anti-bacterial activity by agar well diffusion method. *Bacillus Subtilis* depicted highest sensitivity to nanoparticles compared to other tested bacterial pathogens, and these nanomaterials are controlling, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli.* The zone inhibition clearly visible was surrounding the CuHNMs. The results of all zone inhibitions predicted in Table 1.

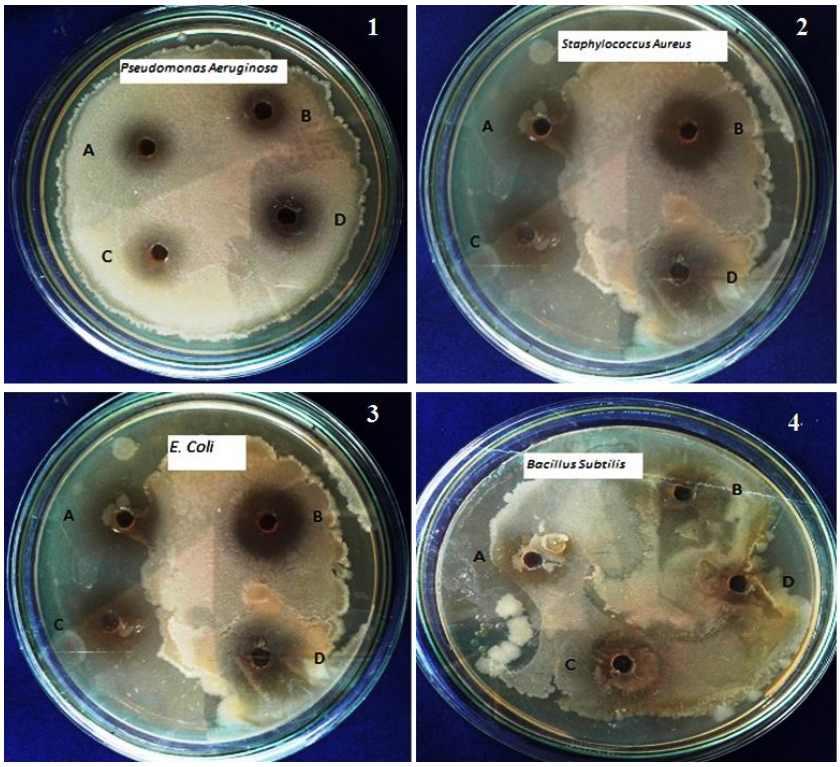


Figure 9: *Anti-bacterial activity of CuHNMs*

Table 1: *Anti-bacterial activity of CuHNMs Pseudomonas aeruginosa, Staphylococcus aureus, E.coli and Bacillus Subtilis strains and their average Diameter of zone inhibition (mm)*

S.No.	Name of the tested Organism	Diameter of zone inhibition(mm)				Average (mm)
		A	B	C	D	
1.	*Pseudomonas aeruginosa*	19	20	19	16	18.5
2.	*Staphylococcus aureus*	18	19	17	23	19.25
3.	*Escherichia coli*	21	20	20	21	20.5
4.	*Bacillus Subtilis*	23	21	22	23	22.25

4. Conclusion

In this research, a novel CuHNMs prepared by green method using *Nymphaea Rubra* leaf extract. The CuHNMs properties were characterized using several techniques. Biomolecules of aqueous leaf extract acts as reducing and capping agent. The green synthesized CuHNMs has displayed high antibacterial efficacy against *Bacillus Subtilis when compared to Pseudomonas*

aeruginosa, Staphylococcus aureus and Escherichia coli. This Green synthesis method gives promise for future eco-friendly production technologies, low cost, chemical free and also provides an interesting tool for materials science

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