

Investigation of activation energy and antibacterial activity of CuO nano-rods prepared by Tilia Tomentosa (Ihlamur) leaves

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Abstract

In recent years, fabrication of metal oxide nanoparticles is intensively gained the interest of various chemists as well as biochemist due to their wide range of applications in different fields. Therefore, we reported an easy and eco-friendly synthesis of CuO nano-rods by using Tilia Tomentosa (Ihlamur) leaves extract. Producing CuO nanoparticles exhibiting rod-like shape is rare and difficult using plant leaves, but it is possible using tilia leaves. The green synthesized CuO nano-rods were calcined at 400°C for 15 minutes and were characterized by UV-Visible spectroscopy, XRD, SEM and particle size analyzer to study their bandgap, phases, shape and sizes respectively. SEM studies reveal the formation of rod-shaped CuO nanoparticles with an average length of 95nm and that of breadth is 32nm. Further, the thermal properties of CuO nano-rods were studied by using TG and DTA analysis. Thermal studies were carried out at 6, 8 and 10°/min heating rates to study the enthalpy and activation energy in detail. The activation energy of CuO nano-rods was calculated by the Kissinger method and was found to be 12.23 kJ/mol. Antibacterial activity of CuO nano-rods was carried out using *S. aureus*, *P. fluorescens*, *E. faecalis*, *B. subtilis*, *S. enteridis* and *E. coli*.

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1.Introduction

Now-a-days, research has mainly riveted on nanotechnology due to its wide applications in different sectors such as medical, energy, textiles, agriculture, etc. Most of the researchers from different research backgrounds such as physics, chemistry, metallurgy, materials science, bioengineering are involved in the research related to nanomaterials [1-4]. In recent years, the synthesis of metal oxide nanoparticles is advancing with great speed due to its ability to produce materials in nanoscale. One of the primary advantages of fabricating metal oxide nanoparticles lies in their small size and maximum surface area. Because of the extremely refined size; the metal oxide nanoparticles exhibit unparalleled physical and chemical properties as compared with nano metals. Various fabrication methods such as chemical and physical vapor deposition, ball milling [5-10], precipitation, sol-gel, electrodeposition [11] methods are reported elsewhere. But these reported methods are sometimes tedious, slow, may require special expensive equipment, capping agents, high temperature and templates (result in impurities). On the other hand, chemical methods of preparation results in toxic by-products due to the use of strong reducing agents which are dangerous to the environment. Therefore, preparing metal oxide nanoparticles by using plant extract is preferred over the above methods due to their ease of handling, simple, environmental friendly and inexpensive method. Metal oxide nanoparticles are continuously used in different fields such as gas sensors [12], electron transistor devices, solar cells, waste water treatment, electro-catalyst, electrochemical sensors, pigments, dispersing agents in alloys, etc [13-14]. The biological synthesis of metal oxide nanoparticles by plant leaves mainly depends upon the solvent used, pH, pressure and experimental temperature [15]. The main advantage of this method lies in the huge availability of biodiversity of plants. The phytochemicals like aldehydes, ketones, flavonoids, and phenols are present in the plants and they act as reducing agents and converts metal salts into metal oxide nanoparticles [16]. The metal oxide nanoparticles prepared by various plant extracts was proved to be much faster than the other biological methods. Therefore, we synthesized CuO nanoparticles by using *Tilia Tomentosa* (Ihlamur) leaves extract. In recent years, copper oxide (CuO) nanoparticles have earned substantial importance in different sectors due to their unique properties. Most of the CuO and its complexes can be used in water purifiers, fungicides, antibacterial, antifouling agents, coatings, plastics, and textiles. Due to the narrow bandgap, CuO nanoparticles can be used as a catalyst, magneto-resistance materials, superconductors, solar cells, battery and gas sensors [17-21]. Many researchers reported the preparation of CuO nanoparticles by using different plant leaves but no one reported the preparation of CuO nano-rods by using Ihlamur plant leaves. Ihlamur or Linden belongs to the family of genus *Tilia*, generally found in the form of trees or shrubs [22, 23] in temperate and semi-tropical regions of the northern hemisphere. These plant leaves and flowers are used to prepare herbal tea for centuries in Turkey. It is light yellowish color with soothing features and proved to be very effective against the common cold, fever, headache, sore throat, high blood pressure, etc. Raja Naika et al. (2015) reported the green synthesis of copper oxide nanoparticles using *Gloriosa superba* L. plant extract and studied the antibacterial activities against pathogenic bacterial strains namely Gram -ve *Klebsiella aerogenes*, *Pseudomonas desmolyticum*, *Escherichia coli*, and Gram +ve bacteria *Staphylococcus aureus*. The synthesized CuO nanoparticles were spherical in shape, and the size was found to be in the range 5–10 nm [17]. Faheem Ijaz et al. (2017) prepared copper oxide nanoparticles by using *Abutilon Indicum* leaf extract and studied the antimicrobial, antioxidant and photocatalytic dye degradation properties. They reported that CuO nanoparticles exhibit antibacterial and antioxidant potential, indicating that they are good candidates for future therapeutic applications and concluded that the nanoparticles are good catalysts for effective degradation of Acid Black 210 [18]. P. Narasaiah et al. (2017) reported the biosynthesis of copper oxide nanoparticles from *drypetes sepiaria* leaf extract and investigated their catalytic activity to dye degradation. They performed photocatalytic activity to examine the photocatalytic degradation efficiency of CuO nanoparticles to congo red [19]. Vijay Kumar et al. (2015) synthesized copper oxide nanoparticles

by using aloe vera leaf extract and studied their antibacterial activity against fish bacterial pathogens. Their X-ray diffraction (XRD) and transmission electron microscope (TEM) analysis depicted only the monoclinic phase of prepared CuO nanoparticles with an average grain size of 20 nm. They concluded that the green synthesized CuO nanoparticles are cost-effective, biogenic molecules with the capability to serve as antimicrobial agents against fish bacterial pathogens [20]. D. Gültekin et al. (2017) reported the green synthesis of copper oxide nanoparticles using Cimin Grape (*Vitis vinifera* cv.) extract. Authors concluded that the prepared CuO nanoparticles may be a potential candidate for widespread use in industry for dye removal, catalyst, biosensor development and antimicrobial agent synthesis [24]. M. Altikatoglu et al. (2017) synthesized copper oxide nanoparticles using *Ocimum basilicum* extract and investigated their antibacterial activity against pathogenic bacterial strains *Escherichia coli* and *Staphylococcus aureus* [25]. F. Duman et al. (2016) prepared CuO nanoparticles by using Chamomile flower extract and successfully studied their antioxidant and DNA cleavage properties. They reported that their results may be useful for preventing apoptotic cell proliferation and for cancer studies [26]. Most of the physical and chemical methods require capping agents to avoid particle agglomeration and they are neither cost-effective nor eco-friendly. Therefore, environmental contamination is an important concern in the physical and chemical synthesis of metal and metal oxide nanoparticles. To obviate the environmental contaminations, there is a significant need to develop low cost, non-toxic and eco-friendly processes to synthesis nanoparticles. Eco-friendly synthesis of nanoparticles by using plant or leaves extract as reducing agents and capping agents are considered as robust, cost-effective and environmental friendly methods. Many of the researchers are already published the green synthesis of CuO nanoparticles, but very limited or no literature available on the green synthesis of CuO nano-rods using Ihlamur plant leaves according to the author's knowledge. Therefore we have prepared CuO nano-rods by using Ihlamur leaves and studied their antibacterial activities and thermal properties.

2. Material and Methods

2.1. Chemicals and reagents required

Copper (II) acetate [$\text{Cu}(\text{CH}_3\text{COO})_2$], Sodium hydroxide (NaOH) are purchased from Sigma-Aldrich. Plant extract was prepared in the lab and all the solutions were prepared by using double distilled water.

2.2. Preparation of plant extract

The dried leaves of *Tilia tomentosa* (Ihlamur) were collected from Bartın, Turkey. The plant leaves were uniformly powdered by using a blender. Ten grams of powdered leaves were mixed with 200mL of deionized water and the solution was boiled around 80°C for 15 minutes until we get a strong yellow colored solution.



Figure 1: (a) *Tilia tomentosa* (Ihlamur) leaves and (b) Ihlamur leaves extract

Then the solution was cooled to room temperature and filtered to get clean yellow colored solution of plant extract. This plant extract acts as both reducing agents as well as capping agents in preparing CuO nano-rods. Every time a small amount of the extract will be used as a reducing agent to prepare CuO nano-rods and remaining will be stored at 5°C temperature for further use. Figure 1 depicts the *Tilia Tomentosa* (Ihlamur) leaves and its extract.

2.3. Preparation of CuO nano-rods

Weighed exactly 4 grams of copper acetate powder and dissolved in 200mL of deionized water to get a 2% copper acetate solution. The mixture was stirred on a magnetic stirrer for 15 minutes at room temperature to get a homogeneous dark green solution. Then added 15mL of Ihlamur plant extract dropwise with continuous stirring at 80°C for 10 minutes on a magnetic stirrer. Add 1M NaOH dropwise till green color solution turns to black color precipitate. For complete precipitation, stir the black precipitate for 10 more minutes at 80°C. The obtained solution was centrifuged at room temperature for 10 minutes at rpm of 7500. Remove the supernatant solution and wash with water and again centrifuge it. After centrifugation for 3 times, collect the CuO nano-rods on a watch glass and dry at 70°C in an oven overnight. The obtained black colored CuO nano-rods were further calcined in a furnace at 400°C with a holding time of 15 minutes to remove any evaporable impurities. The calcined samples were cooled to room temperature and store it for further characterization. Figure 2 shows the graphical representation of preparing CuO nano-rods.

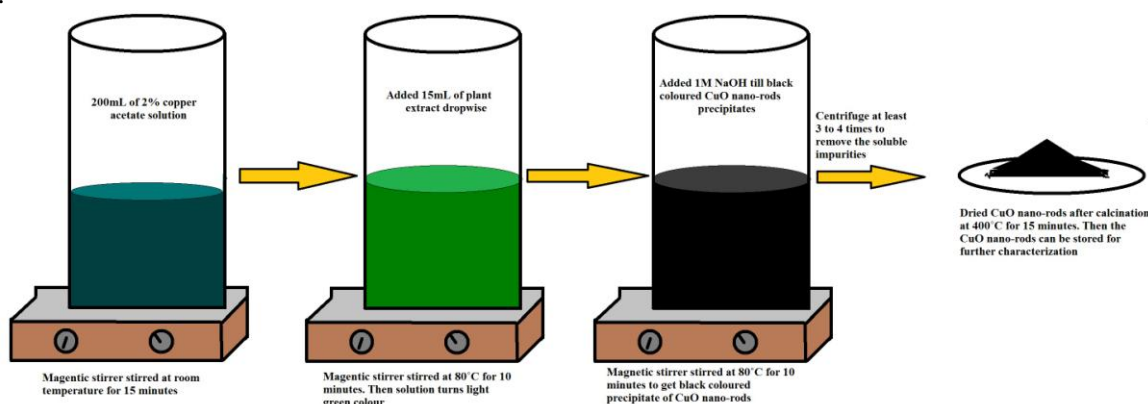


Figure 2: Graphical representation of CuO nano-rods preparation

2.4. Characterization of CuO nano-rods

Phase analysis of prepared CuO nano-rods were carried out by using XRD (RIGAKU SmartLab) in the 2θ range at 20-80° using Cu Kα₁ radiation ($\lambda=1.54056\text{\AA}$). The morphology of the nano-rods was investigated by using scanning electron microscopy (SEM) (TESCAN- MAIA3 XMU). Quantification of the elements present in the prepared CuO nano-rods was studied by energy dispersive spectroscopy (EDS) attached to SEM. Optical properties were studied by using UV-Visible spectroscopy (Shimadzu- UV 3600 Plus); where bandgap was calculated. Thermal property analyses were carried out in Hitachi, STA 7300 model at 6, 8 and 10°/minutes to investigate the activation energy and enthalpy of CuO nano-rods. Antibacterial activities of the prepared CuO nano-rods were studied by using six bacteria (three Gram-positive and negative strains). All the research work starting from the preparation of plant extract, CuO nano-rods, and their characterizations were conducted at Bartın University, Turkey.

2.5. Antibacterial activity

Six bacteria (three Gram-positive and negative strains) were utilized to determine the antibacterial effect of CuO nano-rods prepared from *Tilia Tomentosa* (Ihlamur) leaves extract. All the tested bacteria were collected from the

Department of Molecular Biology and Genetic of Bartın University (Bartın, Turkey). Andrews (2002) explained minimum inhibition concentration (MIC) that inhibits the growth of bacteria under ordinary conditions [27]. Bacterial strains were inoculated to Luria Bertani broth and bacterial growth was standardized to 0.5 McFarland standard turbidity (1.5×10^8 CFU/ml). The same volume of growth medium and solution were added into the first well of 96 well plates and then the mixture was diluted from 50 mM to 1.5625 mM using 96-well microplate that was incubated at 37°C for 24 h after inoculation. The MIC value was determined according to the turbidity of bacterial growth by UV-Visible spectrum at 600 nm. MIC is the lowest concentration in which no trace of bacterial growth is observed. The wells where no bacterial growth were inoculated into petri dishes containing Mueller Hinton Agar and were incubated at 37°C for 24 hours to detect the minimum bactericidal/bacteriostatic concentration (MBC) of it. The MBC assay determines the lowest concentration required to kill microorganisms.

3. Results and discussions

3.1. X-ray diffraction (XRD)

Figure 3 depicts the XRD diffraction pattern of CuO nano-rods prepared by using *Tilia Tomentosa* (Ihlamur) leaves extract. The diffraction peaks at 2θ of 35.58°, 38.71° corresponds to (002) and (111) planes respectively confirming the formation of single-phase CuO with monoclinic structure and all the peaks are well-matched with the JCPDF Card No.: 01-089-5898. All the diffraction peaks are broadened due to the refined size of CuO nano-rods. The Scherrer's formula is used to calculate the average crystallite size of green synthesized CuO nano-rods [5, 8];

$$D = \frac{K\lambda}{\beta \cos \theta} \quad (1)$$

Where, D= Average crystallite size, K= A constant equal to 0.94, λ = the wavelength of X-ray radiation (0.154 nm), β = Full-width half maximum of the peak (in radians) and 2θ = Bragg's angle (degree). We have calculated the crystallite size for the peaks (002) and (111) using Scherrer's equation and the values were found to be ≈ 10 nm and ≈ 9 nm respectively. Lattice parameters and lattice angles were found to be $a=4.69\text{\AA}$, $b=3.42\text{\AA}$, $c=5.13\text{\AA}$ and $\alpha=90^\circ$, $\beta=99.54^\circ$ and $\gamma=90^\circ$ respectively; similarly, space group of prepared CuO nano-rods was found to be 15:C12/c1.

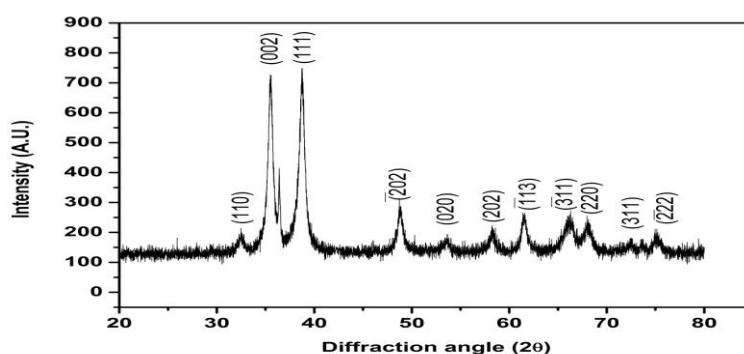


Figure 3: XRD diffraction spectra of CuO nano-rods prepared by using *Tilia Tomentosa* (Ihlamur) leaves extract

The intensities and positions of peaks are in good agreement with the reported values. The peaks are broad due to the nano crystallinity, instrumental errors, strain, etc. [28-30]. Aparna et al. reported that the particles having a crystallite size less than 20nm will exhibit more strain as compared with the particles having more than 20nm crystallite size [31]. The prepared CuO nano-rods depict less than 20nm crystallite size as calculated from Scherrer's formula; therefore our CuO nano-rods are expected to possess more strain.

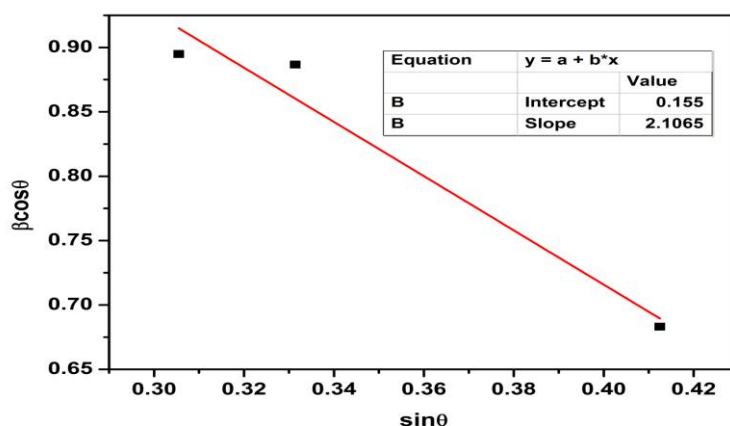


Figure 4: Williamson-Hall Plot for CuO nano-rods prepared by Ihlamur leaves

Lattice strain as well as crystallite size of prepared CuO nano-rods were calculated by using Williamson-Hall equation [6, 32] as follows,

$$\beta \cos \theta = \frac{0.94\lambda}{D} + 4\varepsilon \sin \theta \quad (2)$$

Where 'β' is FWHM, 'ε' is the strain, 'D' is the average crystallite size and 'θ' is the Bragg's diffraction angle. Williamson and Hall proposed a method for de-convoluting size and strain broadening by looking at the peak width as a function of 2θ. Here, Williamson-Hall plot is plotted with sin θ on the x-axis and βcosθ on the y-axis (β in radians). From the linear fit, particle size and strain are extracted from y-intercept and slope respectively [32]. The average crystallite size and lattice strain of prepared CuO nano-rods were found to be ~10nm and 0.052 respectively. Figure 4 represents the Williamson-Hall Plot for CuO nano-rods prepared by Ihlamur leaves.

3.2. Scanning electron microscopy (SEM)

Figure 5 (a) represents the SEM microstructure of green synthesized CuO nano-rods. From the SEM, we can observe the rod-shaped CuO nanoparticles exhibiting almost the same dimension. The average lengths of the CuO nano-rods are 113nm and that of breadth is 32nm respectively. The prepared CuO nanoparticles are homogeneous in nature with little or no agglomeration. The reduced agglomeration is due to the Ihlamur plant extract; as it acts as both reducing agent and capping agent. Therefore, this method is eco-friendly and cost-effective as there is no use of toxic chemicals like expensive capping agents.

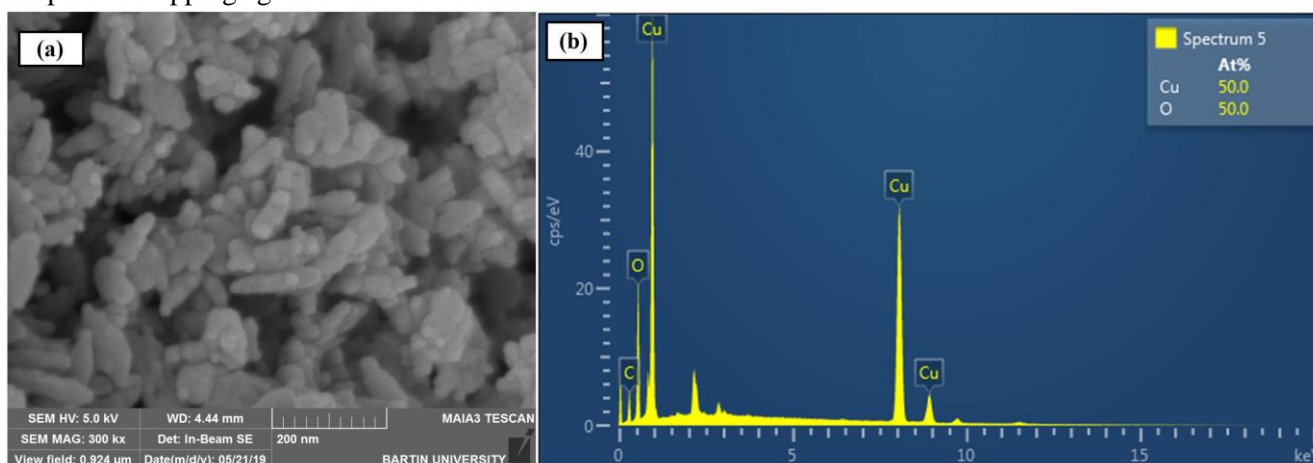


Figure 5: (a) SEM microstructure of green synthesized CuO nano-rods (b) EDS image of CuO nano-rods

Figure 5 (b) shows the EDS image of CuO nano-rods prepared by Ihlamur plant extract. The EDS analysis was performed to study the composition of elements present in the CuO nano-rods. The atomic percentage of copper and oxygen was theoretically calculated and the value was found to be 50% each. Similarly, the experimental atomic percentage of copper and oxygen were found to be 50% each. As we see from the EDS data, both copper and oxygen atoms present in prepared CuO nano-rods are stoichiometric to each other and agree with the theoretical values.

3.3. UV-Visible spectroscopy

Prepared CuO nano-rods are dispersed uniformly in de-ionized water using an ultra sonicator for 2 minutes to get a homogeneous solution. UV-Visible spectroscopy was used to study the optical properties of green synthesized CuO nano-rods and its spectra are depicted in figure 6. The spectrum shows a broad absorption peak at 385 nm and is due to surface plasmon absorption of CuO nano-rods. This surface plasmon absorption phenomenon occurs due to the collective oscillation of the free conduction band electrons when an electromagnetic radiation strikes them. This type of resonance occurs when the wavelength of the incident light far exceeds the particle diameter [33]. The bandgap energy (E) of prepared CuO nanoparticles were calculated by using the following equation [34],

$$E = (h \times C) / \lambda \quad (3)$$

Where, E = Bandgap energy, h = Planks constant = 6.626×10^{-34} Joules sec, C = Speed of light = 3.0×10^8 meter/sec
 λ = Cut off wavelength = 385×10^{-9} meters, *Conversion $1\text{eV} = 1.6 \times 10^{-19}$ Joules

The calculated band gap energy value was found to be 3.22 eV, which is higher than the bandgap of CuO nanoparticles reported [35, 36]. This enhanced band gap energy value may be due to the presence of intra-gap states and quantum confinement effect [37, 38].

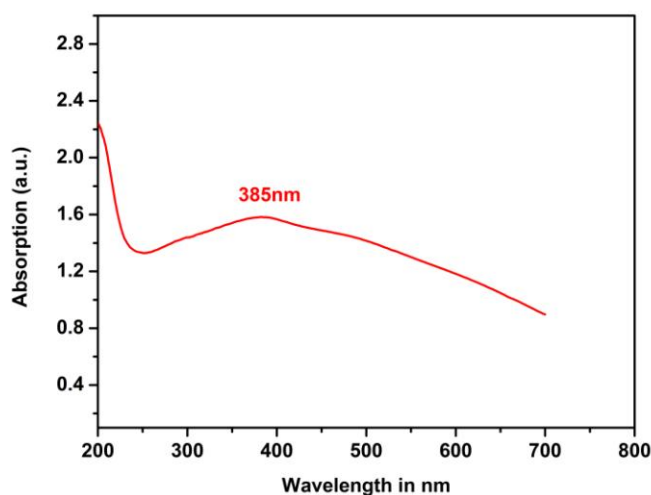


Figure 6: UV-Visible spectra of green synthesized CuO nano-rods and its spectra

3.4. Thermal analysis

Thermal properties of prepared CuO nano-rods were investigated by thermogravimetric analysis (TG) and differential thermal analysis (DTA) at different heating rates over a temperature range of 30–1000°C. Figure 7 (a), 7 (b) and 7 (c) represent the TG and DTA curve of CuO nano-rods at 6, 8 and 10°C/minute heating rates respectively. Prepared CuO nano-rods show good thermal stability over 30–1000°C temperature range and exhibits less weight loss. This is due to the significant resistance of CuO against evaporation and phase change at that temperature range. During a heating rate of 6°C/min, only 2.3% weight loss was observed between 30 to 200°C due to water evaporation and nearly 1.1%

weight loss was observed at 200–800°C due to the decomposition of organic material [39]. The total weight loss during heating rate 6°C/min was found to be 12.9%. In case of 8°C/min, only 2.5% weight loss was observed between 200–800°C due to the evaporation of water and decomposition of organic material [40]. The total weight loss during 8°C/min was found to be 12.41%. Similarly, during 10°C/min, 3% weight loss was observed between 200–800°C and the total weight loss was found to be 11.9%. From the results, we can conclude that as the heating rate increases from 6 to 10°C/min; the percentage weight loss decreases and this confirms the stability of prepared CuO nanoparticles over a wide temperature range (30–1000°C). The prepared CuO nano-rods exhibit superior thermal properties at higher temperatures in terms of lesser weight loss than the CuO nanoparticles reported elsewhere [40, 41], where an average weight loss is 40%.

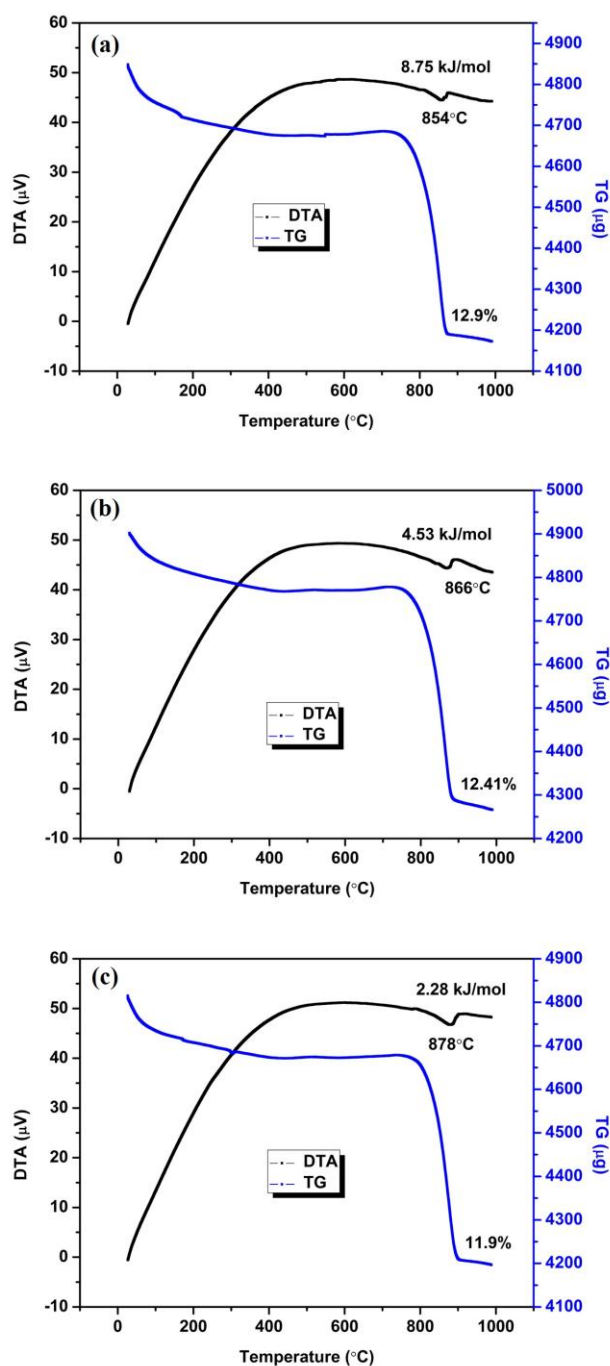


Figure 7: TG and DTA curve of CuO nano-rods at (a) 6°C/minute (a) 8°C/minute and (a) 10°C/minute heating rates respectively

In another set of experiments, we also studied the change in enthalpy, activation energy by using differential thermal analysis. DTA curve at the heating rate 6, 8 and 10°C/min shows endothermic peaks at 854, 866 and 878°C respectively. These endothermic peaks confirm the decomposition of organic matter and CuO nano-rods to Cu₂O and Cu [42-44]. From figure 7 it was observed that, as the heating rate increases from 6-10°C/min, the maxima of the decomposition peak shift towards higher temperatures. This is due to the variation of enthalpy change as well as the temperature of the end of transition at higher heating rates [45]. The enthalpy change of prepared CuO nano-rods at 6, 8 and 10°C/min was found to be 8.75, 4.53 and 2.28kJ/mol respectively. The endothermic peaks represent dehydration, decomposition, and reduction of prepared CuO nano-rods at higher temperatures. The calculated details of weight loss, decomposition peak temperature, enthalpy change of prepared CuO nano-rods are tabulated in table 1.

Table 1: The calculated details of weight loss, decomposition peak temperature, enthalpy change of prepared CuO nano-rods using TG and DTA curves.

Type of nanomaterial	Heating rate (°C/min)	Decomposition temperature (°C)	Percentage weight loss	*Enthalpy change (kJ/mol)
CuO nano-rods	6	854	12.9	8.75
	8	866	12.41	4.53
	10	878	11.9	2.28

*Converted values of mJ/mg to kJ/mol

Activation energy of prepared CuO nano-rods was calculated using the Kissinger method [46, 47] with the help of maxima of the decomposition peaks of DTA curves at 6, 8 and 10°C/min respectively.

$$\ln \frac{\alpha}{T_p^2} = \frac{-E_c}{RT_p} + \text{Constant} \quad (4)$$

Where T_p is decomposition peak temperature, α is the heating rate and R is the gas constant.

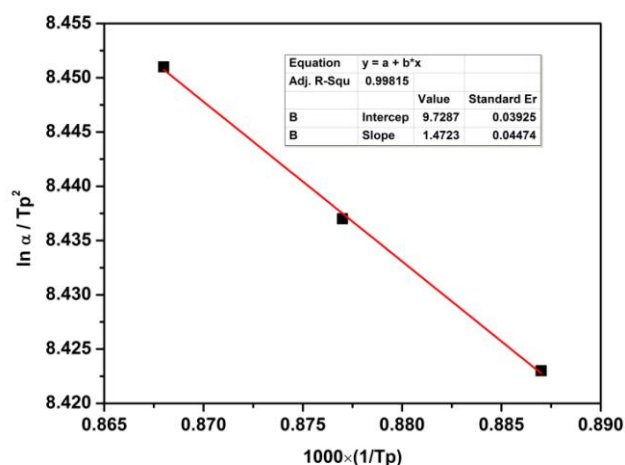


Figure 8: Kissinger plot to calculate activation energy of green synthesized CuO nano-rods

A linear relationship between $\ln (\alpha/ T_p^2)$ and $1000 \times 1/T_p$ for the crystallization temperatures were obtained using equation (4) and then the activation energy required for decomposition reaction was calculated. Figure 8 depicts the Kissinger plot of the activation energy of green synthesized CuO nano-rods and the value was found to be 12.23 kJ/moles. The calculated values of activation energy using the Kissinger method is tabulated in table 2.

Table 2: The calculated values of the activation energy of CuO nano-rods using the Kissinger method.

Type of nanomaterial	Heating rate α (K/min)	Peak temperature T_p (K)	$\frac{\alpha}{T_p^2}$ ($\times 10^{-4}$)	$\ln \frac{\alpha}{T_p^2}$	$1000 \times \frac{1}{T_p}$	Activation energy E_a (KJ/mol)
CuO nano-rods	279	1127	2.19	-8.423	0.887	12.23
	281	1139	2.16	-8.437	0.877	
	283	1151	2.13	-8.451	0.868	

3.5. Antibacterial activity

The antibacterial activity of CuO nano-rods was analyzed against six bacteria (three Gram-positive and negative strains). CuO nano-rods showed good antibacterial activity against tested bacteria except *S. aureus* strain as depicted in Table 3.

Table 3: The calculated values of the activation energy of CuO nano-rods using the Kissinger method.

Bacteria	50 mM	25 mM	12.5 mM	6.75 mM	3.375 mM	1.6875 mM
- <i>P. fluorescens</i>	M					
+ <i>E. faecalis</i>	M					
- <i>S. enteridis</i>	*	M				
+ <i>S. aureus</i>						
+ <i>B. subtilis</i>	M					
- <i>E. coli</i> CFAI		M				

The CuO nano-rods exhibit significant antibacterial effect at 50 mM concentration against *P. fluorescens*, *E. faecalis* and *B. subtilis* respectively, whereas, the antibacterial effect against *S. enteridis* and *E. coli* was exhibited at 25mM concentration. The MBC result obtained only against *S. enteridis* at 50mM. Figure 9 depicts the MBC result of *S. enteridis* at 50 mM and MIC of remaining bacteria at 25mM and 50mM. Jadhav et al. [48] and Černík and Thekkae Padil [49] have studied the antibacterial activity of copper oxide against gram (-) *E. coli* and gram (+) *S. aureus* using MIC assay. They clarified that copper oxides at low concentrations got antibacterial effect. Ananth et al. [50] analyzed that the antimicrobial activity of copper oxides against gram-positive (*Streptococcus iniae* and *Streptococcus parauberis*) and gram-negative (*E. coli* and *Vibrio anguillarum*) using MIC and disc diffusion method. According to their results, the growth of all bacteria was inhibited at low concentrations. As the literature suggests that CuO nanoparticles were proved to be an excellent antibacterial agent. This is because; CuO nanoparticles can give rise to reactive oxygen species that may inhibit bacterial growth [51]. The shape of CuO nanoparticles plays an important

role in the inhibition of bacterial growth and its killing. The shape of CuO nanoparticles used in this experiment was nano-rods and they might interact very well with the cell wall, organelles, and enzymes. Due to these interactions, CuO nano-rods can cause inhibition of DNA replication, deactivation of enzymes, tearing the cell wall of bacteria and ultimately results in the death of the bacteria. Therefore, CuO nano-rods act as a good antibacterial agent and can be used in food industries, cosmetics, agri-industries, etc. Therefore, we can conclude that prepared CuO nano-rods proved to be the potential candidate for killing bacteria.

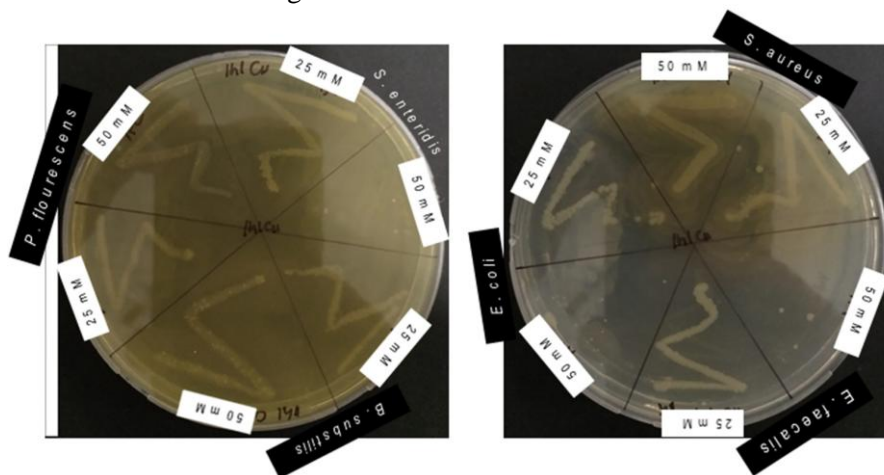


Figure 9: MBC result of *S. enteridis* at 50 mM and MIC of remaining bacteria at 25mM and 50mM

4. Conclusion

The CuO nano-rods were successfully prepared by simple and eco-friendly plant-mediated biological methods using leaves of *Tilia Tomentosa* (Ihlamur). The prepared CuO nano-rods exhibit monoclinic structure with an average crystallite size of 10nm and a lattice strain of 0.052 as calculated from the Williamson-Hall equation using XRD spectra. SEM images confirm the rod-like shape of prepared CuO nanoparticles with very less agglomeration and EDS data confirms the stoichiometric ratio of copper and oxygen matches with theoretical values. UV-Visible spectroscopy of CuO nano-rods depicts a broad absorption peak at 385 nm and it is due to surface plasmon absorption. We successfully calculated the bandgap of CuO nano-rods and it was found to be 3.22 eV. The thermogravimetric analysis of CuO nano-rods shows a decrease in weight loss with an increase in the heating rates from 6 to 10°C/min over a wide temperature range of 30–1000°C. Therefore, we can conclude that the prepared CuO nano-rods depict better thermal stability than CuO nanoparticles reported by the researchers elsewhere. DTA analysis shows the endothermic peaks at 854, 866 and 878°C respectively over heating rates of 6, 8 and 10°C/min. DTA analysis confirms the shift of decomposition peak towards higher temperatures due to the variation of enthalpy change. The enthalpy change of prepared CuO nano-rods at 6, 8 and 10°C/min was found to be 8.75, 4.53 and 2.28kJ/mol respectively. We calculated the activation energy of prepared CuO nano-rods using the Kissinger method and the value was found to be 12.23 kJ/moles. We successfully investigated the antibacterial activity of CuO nano-rods against six bacteria (three Gram-positive and negative strains). The CuO nano-rods demonstrated a significant antibacterial effect against all the bacteria. But the MBC result obtained only against *S. enteridis* at 50mM and the MIC result obtained to the remaining bacteria at 25 and 50mM respectively. Therefore we conclude that the CuO nano-rods prepared from *Tilia Tomentosa* (Ihlamur) leaves can be a potential candidate for antibacterial material.

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