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**Neha Chauhan**
Department of Life Sciences, ITM University, Gwalior, Madhya Pradesh, India

**Rita Sharma**
Department of Life Sciences, ITM University, Gwalior, Madhya Pradesh, India

**Yash Dutt**
Department of Life Sciences, ITM University, Gwalior, Madhya Pradesh, India

**Nikita Jaiswal**
Department of Life Sciences, ITM University, Gwalior, Madhya Pradesh, India

**Tejasv Gupta**
Department of Life Sciences, ITM University, Gwalior, Madhya Pradesh, India

**Corresponding Author:**
**Rita Sharma**
Department of Life Sciences, ITM University, Gwalior, Madhya Pradesh, India

# Characterization of copper oxide nano particles using *Prosopis juliflora* extract and their antioxidant and photocatalytic activities

**Neha Chauhan, Rita Sharma, Yash Dutt, Nikita Jaiswal and Tejasv Gupta**

**Abstract**
Present investigation involves the green synthesis of nanoparticles from the chemicals and utilizing the bio components of leaves extract of *Prosopis juliflora***.** Metal nanoparticles are obtained using plant extracts as the components of green approach is considered to be ecofriendly and economically important. Present study reveals the green synthesis of metallic nanoparticles, the polyol components present in the plant extracts are responsible for the bioreduction of metal ions whereas water soluble heterocyclic components stabilize the existing nanoparticles. The study also deals with the synthesis of pure copper oxide nanoparticles (CuO) with appropriate reactants at room temperature. Characterization of the synthesized product was performed by UV vis spectroscopy, followed by Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction analysis (XRD). XRD pattern confirmed the crystalline nature and monoclinic structure of synthesized composition. The functional groups present in the sample were identified by FTIR spectroscopy. These analytical techniques clearly confirm the formation of copper oxide with monoclinic structure.

**Keywords:** CuO, NPs, green synthesis, *Prosopis juliflora*, XRD, FTIR

## 1. Introduction
The development of rapid and reliable processes for the preparation of nano sized metal particles has attracted significant attention due to their unusual size dependent optical and electronic properties. Till now several physical, chemical and biological methods were available to synthesize different types of nanoparticles. However, it is a fact that reproducibility and stability of the nanoparticles with appropriate size are very difficult to achieve through popular chemical reduction method [1, 2]. Copper oxide nanoparticles have gained a lot of attention because they are the simplest members of the family of copper salts, and they possess several useful physical properties such as electron correlation effects, spin dynamics and high temperature superconductivity [4]. Copper oxide nanoparticles are used to improve viscosity of energy transferring fluids, thereby boosting thermal conductivity. Copper oxide nanoparticles are potentially high valuable antimicrobial agents because during synthesis, they possess extremely unusual crystal morphology and high surface areas [7]. The use of plant extract for the synthesis of nanoparticles is a gradually evolving research area known as green synthesis of nanoparticles [8]. Nanoparticles have various properties like catalyst in organic chemistry, thermal and optical properties and biological application etc. [9]. In recent years, Cu nano particles have attracted much attention of researchers due to its application in industrial and medical application. The biological property shown by Cu nanoparticles are wound dressings and biocidal properties, antibacterial, potential industrial uses such as gas sensors, catalytic process, high temperature superconductors and solar cells [10, 11]. Nanoparticles provide solutions to technological and environmental challenges in the areas of solar energy conversion, catalysis, medicine, and water-treatment [14]. Copper nanoparticles have diverse applications as heat transfer systems, as super strong materials, as sensors and as catalysts. Their other properties like antimicrobial activity, disinfecting property and stability as matrix bound particles can be further exploited for use in wall paints and plasters to coat hospital equipment [15, 16]. Considering the vast potentiality of plants as sources for the green synthesis of different nanoparticles, and especially copper nanoparticles researchers worked with plant extracts, some specific plant parts or whole plant for the green

synthesis [17]. The present study also concentrated on green synthesis of copper nanoparticles using concentrated and wet leaf extracts of *Ricinus communis*, *Punica granatum*, *Psidium guajava*, *Eucalyptus globules*, *Ocimum tenuiflorum*, Tagetes sp., and *Phyllanthus emblica* as both reducing and stabilizing agent [18]. Medicinal plants have provided the modern medicine with numerous plant-derived therapeutic agents [19]. The plant has been reported to possess antipyretic, hepatoprotective, antidiabetic, antiperoxidative and antihyperglycemic, microbicidal and antihyperlipidaemic activities. The flowers are used to treat urinary discharges, nocturnal emissions, diabetes and throat irritation [20, 21]. It is generally analysed that UV-Visible Spectroscopy could be used to examine formation of copper nanoparticles. On comparing the FT-IR spectra of *Cassia auriculata* leaves extract and the copper nanoparticles revealed the presence of bonded – OH group whereas stretching indicate the presence of >C=O group. Copper nanostructure was confirmed by the characteristic peaks observed in the XRD pattern. The formation of copper nanoparticles as well as their morphological dimensions in the FE-SEM observation showed that the average size was from 38.1-43.5 nm with interparticle distance, whereas the shapes were uniformed spherical. However, further observation with high magnification reveals that these Cu nanoclusters are assembled by smaller nanoparticles, which exhibit good uniformity and the average diameter is about 38.1 nm [29].

*Prosopis juliflora* belongs to the family Fabaceae, is a shrub or small tree and is native to South America, Mexico and Caribbean. It has been established as an invasive weed in Australia, Africa and Asia [30]. This mesquite tree grows up to a height of 12 meters (39 ft) and a trunk along with a diameter of up to 1.2 metres (3.9 ft) [32]. Its leaves are deciduous, bi-pinnate, compounded and light green with 12 to 20 leaflets. Pods are 20 to 30 cm long and contain between 10 and 30 seeds per pod. A mature plant can produce hundreds of thousands of seeds. Seeds remain viable for up to 10 years. The tree reproduces through seeds and not Vegetatively [33]. The heartwood of this plant possesses and unusual amount of Mesquitol and Flavonol [34].

## 2. Material and Methods

### 2.1 Plant material and its preparation

Leaves of *Prosopis juliflora* were procured from the Botanical garden. The plant leaves were dried in shaded area, then finely powdered using mortar and pestle and stored in glass vials until the extraction. Extract of leaves were prepared by using 5 gm powdered leaves of *Prosopis juliflora* which were squashed with 100 ml of distilled water in 250ml conical flask at room temperature. The mixture boiled for 60 minutes on heating mantle. The extract was cooled, filtered and stored in refrigerator for its further use.

### 2.2 Synthesis of Nanoparticles

For the preparation of nanoparticles, 50ml leaf extract was boiled at 60-70°C on heating mantle followed by addition of 5gms $Cu(NO3)_2.5H_2O$ to the boiling leaf extract and was left for boiling until it gets converted to a fine paste. The paste was then collected and dried in hot air oven and finally the obtained powder was packed and stored in sealed capped bottle for further characterization and testing.

### 2.3 Evaluation of secondary metabolites

**2.3.1 Qualitative analysis for tannins:** Crude extract was mixed with 2 ml of 2% solution of Ferric Chloride. A blue – green black coloration indicated the presence of phenols & tannins.

**2.3.2 Qualitative Analysis for Saponins:** Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 seconds. For motion of 1cm layer indicate the presence of saponins. Froth becomes stable for 1 min.

**2.3.3 Qualitative Analysis for Flavnoids:** Extract was treated with few drops of sodium hydroxide solution. Formation of yellow colour which become colorless on addition of dilute acid, indicate the presence of flavnoids.

**2.3.4 Qualitative Analysis for Alkaloids:** Test with Wagner's reagent: -2ml of extract was treated with 2ml of Wagner's reagent. Formation of brownish red precipitate indicates the presence of alkaloids.

**2.3.5 Qualitative Analysis for Phenols:** Ferric chloride test: - A fraction of the extract was treated with 5% of $FeCl_3$ reagent and observed for the formation of deep blue- black colour.

**2.3.6 Qualitative Analysis for Terpenoids:** 2ml of extract was mixed with 2ml of chloroform and concentrated sulphuric acid was carefully added so form a layer. A reddish brown indicates the presence of terpenoids.

### 2.4 UV Visible Spectroscopic Characterization

UV-vis spectroscopy is a very useful and reliable technique for the primary characterization of synthesized nanoparticles. The UV–vis spectra of the formed CuO nanoparticles dispersed in water exhibiting the maximum absorption peaks at about 380 nm. In addition, UV-vis spectroscopy is fast, easy, simple, sensitive, selective for different types of NPs, needs only a short period time for measurement, and finally a calibration is not required for particle characterization of colloidal suspensions [35-37].

### 2.5 Catalytic Properties of CuO Nanoparticles

The catalytic activity of CuO nanoparticles that were synthesized from the extract of *Prosopis juliflora* was evaluated using UV–Vis spectrophotometer, Cary E 500, is used to monitor the absorbance peaks. The absorbance is usually measured in the range of 350–800 nm at room temperature. In the first reaction mixture, the aqueous solution of methyl orange (MO) was monitored by the measurement of the intensity peaks. In the second reaction mixture, 4 ml of methyl orange (MO), 0.5 ml of *Prosopis juliflora* water extract and 3 ml of distilled water were analyzed. The study of decomposition of methyl orange (MO) used the same amount of methyl orange and *Prosopis juliflora* water extract, i.e. 4 ml of MO and 0.5 ml of *Prosopis juliflora* water extract. For the variant MO I, 0.5 ml of the prepared solution of CuO nanoparticles and 2.5 ml of distilled water were used. For other variants, i.e. from variant MO II to variant MO V, the amounts of the prepared solution of CuO nanoparticles were successively increased by 0.5 ml, and the amounts of distilled water were successively decreased by 0.5 ml. All variants were exposed to sunlight for 120 min [38].

### 2.6 Reducing Power Estimation

The reducing power of nanoparticles of methanolic extracts increases with the increased in concentration of the extract in the solution. The reducing power was best observed at

1mg/ml concentration. The presence of the reducers (i.e. antioxidants) in the solution causes the reduction of $Fe^{3+}$ or ferricyanide complex into the ferrous form. Therefore, measurement of the formation of Prussian blue at 700 nm gave the indication of $Fe^{2+}$ concentration in the solution which again shows the reducing capability of the extract [39].

### 2.7 Metal Chelating Activity
Metal chelating activity was measured, by the addition of 0.1 mM FeS04 (0.2 mL) and 0.25 mM ferrozine (0.4 mL) subsequently into 0.2 mL of plant extract. After that the solution was kept for incubation at room temperature for 10 min, than the absorbance of the obtained mixture was recorded at 562 nm [40].

### 2.8 Nitric Oxide Scavenging Activity
Extract of different dilutions was prepared and dissolved in the PBS solution. 200µL of PBS solution was diluted up to 800µL. Then the dilutions were kept in incubator at 37ºC for 2 hours under normal light followed by incubation for 20 minutes in dark. Griess reagent was added and followed by the incubation for 40 minutes at the room temperature. Afterwards absorbance was noted at 540nm with a suitable blank. Control solution was prepared and the percent of inhibition was calculated using the equation: percent inhibition = (OD of control – OD of extract/ OD of control)*100 [41].

### 2.9 Total Antioxidant Assay (TAA)
The antioxidant activity of the CuO nanoparticles was determined by the total antioxidant activity. CuO nanoparticles and 4ml of reagent solution (23mM sodium phosphate, 4mM ammonium molybdate and 0.6M sulphiric acid) were put in the sealed pack test tube and then incubated in water bath at 95º C for 90 minutes. The sample was kept for cooling at room temperature and absorbance was taken at 695 nm with a standard blank. The antioxidant activity was expressed along with BHT which was used as the standard [42].

### 2.10 FTIR
The functional and other groups attached to the surface are detected by the use of FTIR. The characterization involved is Fourier transform infrared spectroscopy; analysis of the CuO nanoparticles and it is done by Perkin Elmer Spectrum 1000 spectrum is attenuated at total reflection mode, using the range of 4000-400 $cm^-$ with the resolution of 4 $cm^-$ [43].

### 2.11 XRD
The XRD technique is used to determine the size and structure of the nanoparticles. X- ray diffraction peaks was observed by using X-ray diffractometer (MiniFlex 600) with Cu having detector (D/teX Ultra). The instrument was operated at voltage 40kW and current 15mA. Diffraction patterns were run at 5-10 °C /min in terms of 2θ; crystal and physical state of nanoparticle characterized [44, 45].

## 3. Result and Discussion
### 3.1 Qualitative estimation of Secondary metabolites
Flavonoids and phenolics show effective scavenger activity of free radicals due to the presence of hydroxyl group. Their presence indicates high analgesic, anti-inflammatory effects and there is high concentration of these metabolites in aqueous extract of *Prosopis juliflora* leaves. Tannins are present in higher concentration in aqueous extract of *Prosopis juliflora* leaves and it possesses antiviral, antibacterial and antiparasitic effect. Saponins are present in very low quantity in the aqueous extract of *Prosopis juliflora*. Terpenoids are present in the moderate amount in the aqueous solution of *Prosopis juliflora* show the presence of aromatic compounds [66]. The proportion of various secondary metabolites present in the leaves of *Prosopis juliflora* is depicted in table 1.

**Table 1:** Secondary metabolites Analysis

| Tests | Ss Amount | Moderate Amount | High Amount |
|---|---|---|---|
| Tannins | | | ++++ |
| Flavonoids | | | ++++ |
| Saponins | + | | |
| Phenolics | | | ++++ |
| Alkaloids | | | ++++ |
| Terpenoids | | ++ | |

### 3.2 UV Vis Spectrophotometry
The absorption spectrum of the copper nanoparticles solution shows the higher absorbance at
220nm as shown in the figure 1, along with the excepted value of copper nanoparticles. Hence, the λmax for the copper nanoparticles synthesized from *Prosopis juliflora* is 220nm [47].

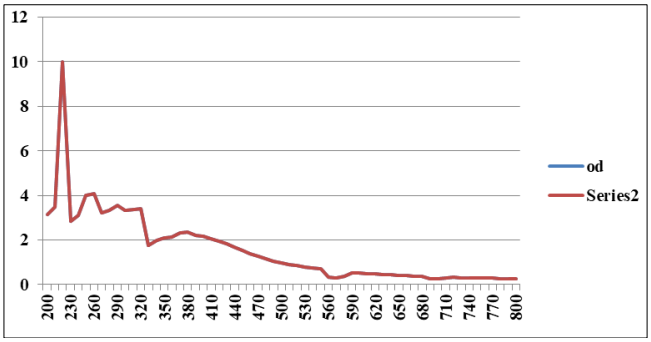


**Fig 1:** UV Vis absorption spectrum of Cu NPs; X axis – wavelength (nm), Y axis – absorbance

### 3.3 Catalytic activity
The CuO nanoparticle was used as a catalyst for the disintegration of Methyl Orange. All dilution was exposed to sunlight for around 120 min for the reaction of nanoparticles on Methyl Orange and its degradation. Dilution was used from MO1 to MO5 as shown in the figure. The maximum absorbance wavelength of MO was recorded between 400-500 nm. The figure 2 shows that the disintegration of MO that occurs due to the increase in the concentration of the amount of CuO nanoparticles prepared of different dilutions. This confirms the presence of catalytic properties of the synthesized CuO nanoparticles [48].

### 3.4 Reducing Power Estimation
The reducing power of the nanoparticles increases with the increase in the concentration of the solution. The best result for the reducing power was estimated at 1mg/ml concentration. Due to the presence of the reducers in the solution the causes the reduction of $Fe^{2+}$ to $Fe^{3+}$. Thus, the measuring of the reducing power at 700nm shows the reduction of $Fe^{2+}$ and the presence of reducing capacity in the solution [49].

### 3.5 Metal Chelating activity
As excess free irons have been implicated in the induction and formation of free radicals in biological systems, it can also

stimulate lipid peroxidation and is the most powerful pro-oxidant among various species of metal ions. In the concentration range of 0.2 to 0.8 mg/mL, estimated to show the best result and maximum metal chelating activity by the green synthesized CuO nanoparticles of *Prosopis juliflora* leaves [50].

### 3.6 NO scavenging activity

NO is produced by almost all type of cells in the human body, and it is one of the important molecules found in blood vessels, epithelial cells and it is also a heteronuclear diatomic molecule. The presence of these compounds in the excess amount in body can cause different harmful disease. NO scavenging activity can be detected in almost all of the plants extract which has been detected in different concentration. There is a decrease in the absorbance at 540nm shows the presence of good amount of NO scavenging activity that is at 0.8mg concentration [51].

### 3.7 Total Antioxidant Activity

The absorbance obtained in the total antioxidant activity was 0.111, 0.14, 0.314, 0.311, 0.020 for 500, 250, 125, 75 and 30 µg of CuO nanoparticles respectively. The absorbance of the standard BHT was 0.68±0.04 to 0.1±0.01. Thus, this shows the conformation of the absorbance of extracts with the standard solution. The maximum absorbance was observed at 125 µg [52].

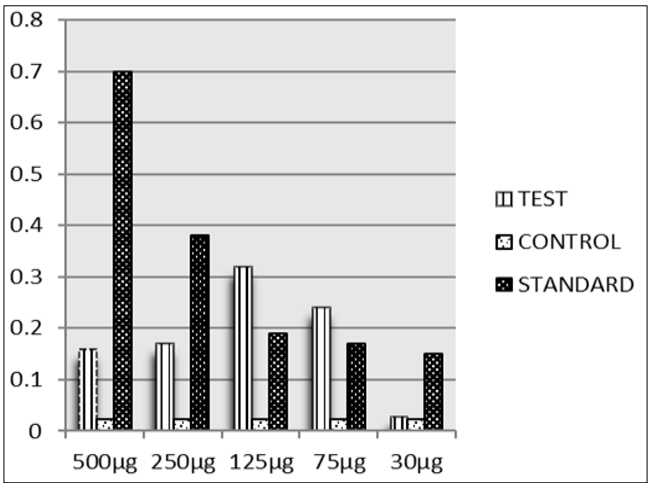


**Fig 2:** TAA assay of CuO nanoparticles; X axis – concentration, Y axis- Absorbance

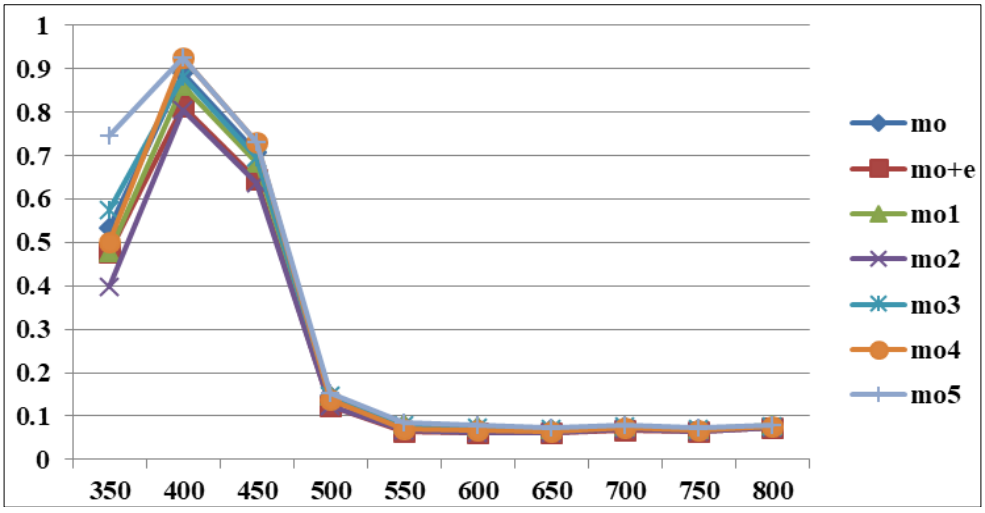


**Fig 3:** Absorption Spectra of MO by CONPs synthesized using *Prosopis juliflora*; X axis-wavelength (nm), Y axis- absorbance

### 3.8 FTIR

A characteristic absorption band were exhibited at 3994 cm-1 for OH stretch, 2502 cm-1 for CH stretch, 1938 cm-1 for CO stretch, 1330 cm-1 for C=C stretch,929 cm-1 for =CH bending and 1496 cm-1 for CO stretch. (Fig 4)

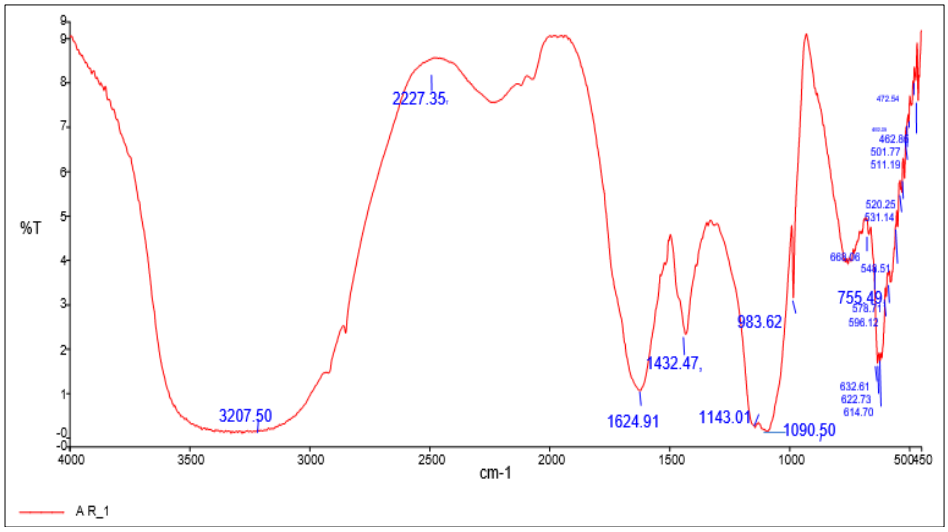


**Fig 4:** FTIR Analysis

### 3.9 XRD

The major peaks obtained at 2θ values of 32.15(3), 35.46(8), 46.40(2), 51.089(19) and 66.582(5) in the high angle XRD of CuO nanoparticles indicate the existence of crystalline nature of nanoparticles (fig 5). The major peak positions closely matches with the Joint committee for powdered X-ray diffraction standard (JCPDS NO 02-1225) and also matches with values of monoclinic phase copper oxide nanoparticles [52]. The size of particle may be 76.8nm.

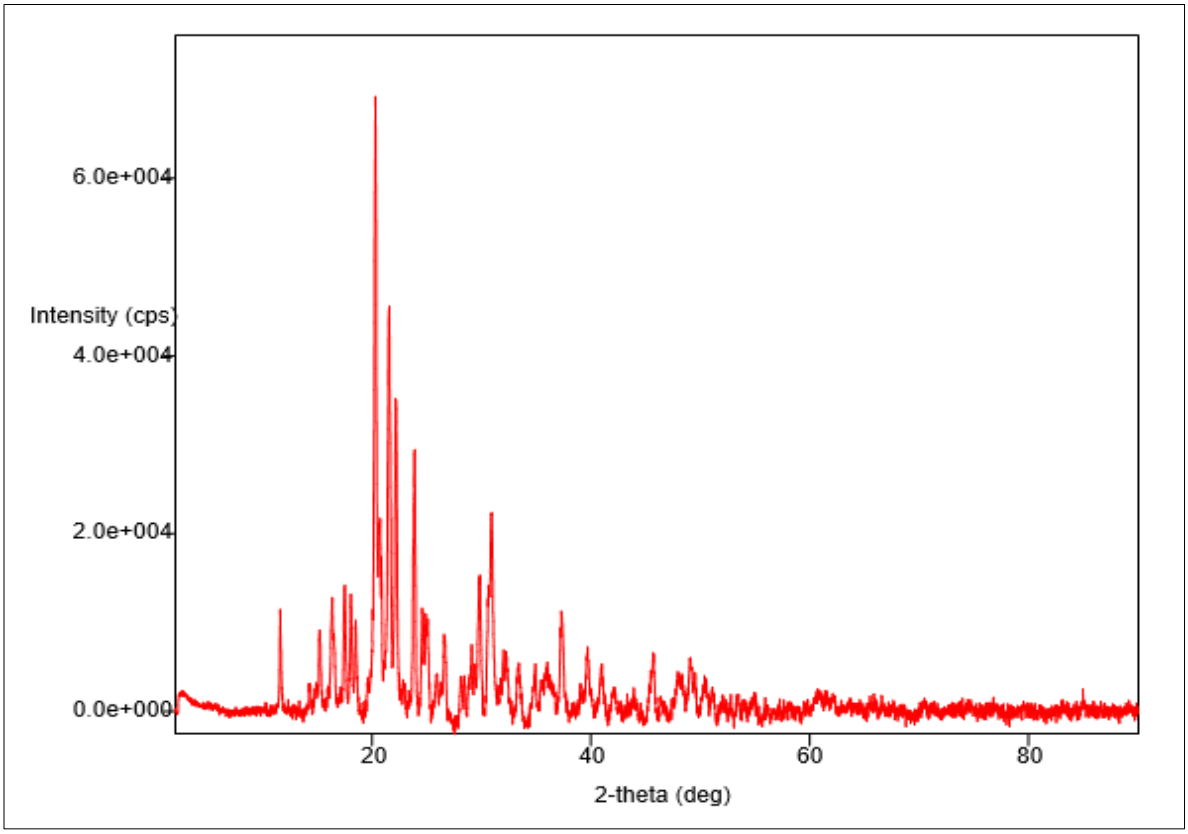


**Fig 5:** XRD Analysis

### 4. Conclusion

It is hereby concluded from the present study that the flavonoids and phenolics of leaf extract of *Prosopis juliflora* had surface active stabilizing molecule for the synthesis of Copper Oxide nanoparticles.

This study also examined the role of aqueous extract of *Prosopis juliflora* in the formation and stabilization of Copper Oxide nanoparticles. The synthesized nanostructures have been characterized by UV Vis, FTIR & XRD profile. The potential particles had good catalytic & antioxidant activity. To conclude, this study presents the ecological method for preparing nanoparticles without any harmful effects.

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