

## Green Synthesis of Copper Nanoparticles using extracts of *Coldenia Procumbens*

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### Abstract

The aim of this study to obtain copper nanoparticles (Cu NPs) through a method of green synthesis that involves using plant extract of *Coldenia Procumbens*. Cu NPs were successfully synthesized using the green synthesis method in the experiments performed. Ultraviolet-visible (UV-Vis) spectrophotometry of the characteristics of the acquired Cu NPs was performed with scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transform infrared spectrometry (FT-IR). It was found that the results of the SEM and XRD measurements that acquired the Cu NPs were in the size of 50 nm. Absorption peak at 283 nm and 483 nm were observed by UV of Cu NPs. Afterwards, the antibacterial activities of these nanoparticles were measured, and it was understood from the obtained results that Cu NPs have both antibacterial and antimicrobial activities.

**Keywords:** CuNPs; Green synthesis; Nano particles; *Coldenia Procumbens*;

### 1. Introduction

The cell culture is more beneficial for the Nanoparticles which are synthesized from the plants are in rather than from the biological methods. Recent research work has been carried out through Biosynthetic approach to synthesis nanoparticle. Because the nanomaterial research is very useful due to its safe, efficacy and the eco-friendly operations [1-3]. Green synthesis method is used to produce clean, non-toxic, and also environmentally acceptable. In past decades, nanoparticles has customarily extended its application in the making of materials such as integrated circuits, bio-labeling, drinking water, preservations of food, cosmetics, antimicrobials, etc.,

A Huge number of nanoparticles can be synthesized from plants extract. For example, Citrus limon, *Quisqualis indica* pink owers, *Murraya koenigii* Linn. *Canna indica* (red) extract is used to synthesis the polymorphic gold nanoparticles. These were firm and its size is 31-131nm. *Lonicera japonica* leaf extract produce the Ag and Au nanoparticles. In these Ag nanoparticles were spherical in shape and its size is 35-

71nm, and Au nanoparticles were poly-shaped nanoplates and its size is 39-91nm. The reduction of the metal ions into the nanoparticles by the responsible of proteins, carbohydrates, and polyphenols. To reduce the Cu ions to nanomaterial, the reducing agent is from the Mangolia-kobus plant leaf extract was used. Ag, Au, and Pt nanoparticles are extracted from Gum kondagogu (*Cochlospermum gossypium*). From above all Copper nanoparticles had a significant antibacterial activity on all the Grams classes of bacteria.

Copper is widely used as material in the world because of their applications such as electrical, optical as well as bio-medical, catalytic, and anti-microbial like antifungal/antibacterial activity [4]. This metal gives major yield, and the reaction rate is in mild reaction condition when contrast to the other traditional catalysts [5]. Copper nanoparticles signify as anti-microbial agent in many fields. This metal is highly poison to the micro-organism like bacteria (*E.Coli*, *Staphylococcus Aureus*) [6]. This metal is non-toxic to the animal cells, due to efficient bactericidal metal for long period [7]. This is considered as a safe for a human being like food package applications and in the water treatment [8-10].

So, in this investigation, the Cu NPs derived from *Coldenia Procumbens* leaf extract was synthesized. Hence the objectives of this work has summarized by, (i) To synthesis the Cu NPs using plant extract of *Coldenia Procumbens* by biological route. (ii) To characterize the synthesized Copper nanoparticles using various physico-chemical techniques. (iii) To determine the size and shape of Silver nanoparticles and (iv) To study its antibacterial activity against a few common bacteria.

## 2. Materials and methods

### 2.1 Materials required

Copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), Distilled water, Mortar, Beakers, Standard measuring flask, Pipettes and Petri dishes are purchased from Spectrum Reagents and Chemical Pvt. Ltd Edayar, Cochin, India.  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  acts as metal compound. All the glasswares washed with double distilled water for several times before use.

### 2.2 Preparation of extract from plant *Coldenia Procumbens* leaves

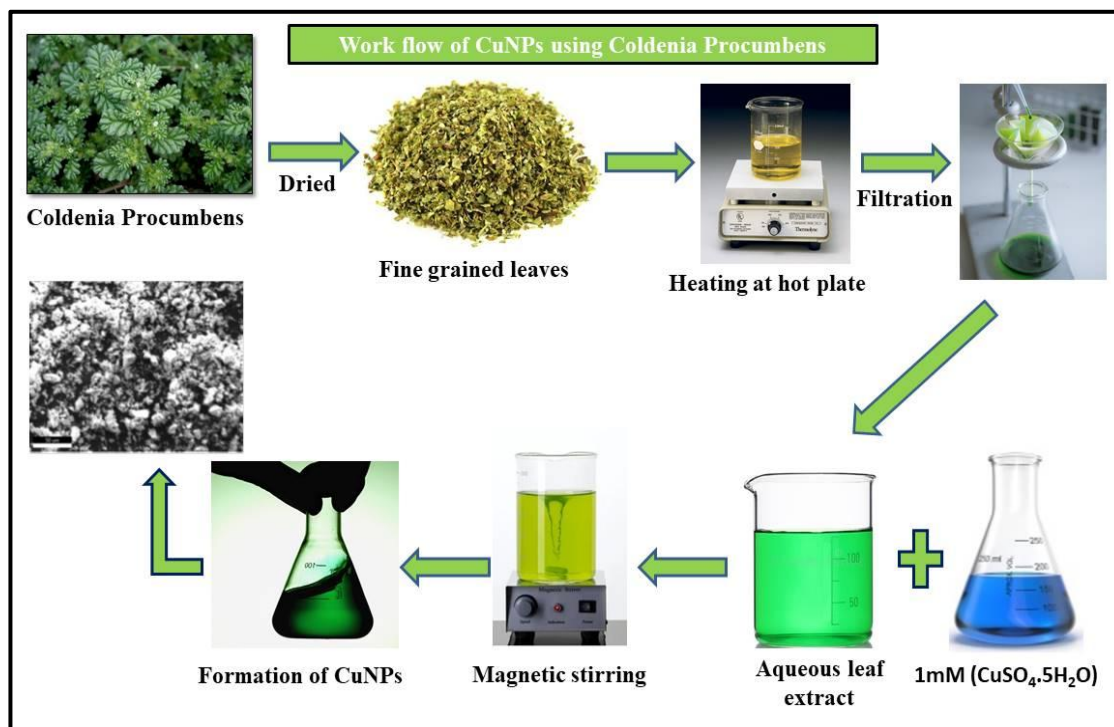
Green and fresh leaves of *Coldenia Procumbens* (Figure 1) were collected from the area of Navlock Horticulure farm, Maniyampattu, Ranipet district, ( $12.2269^\circ \text{ N}$ ,  $79.5168^\circ \text{ E}$ ). Those plants were washed many times with the normal water then with distilled water to remove the dusts and dried slightly through the sun light to reduce the moisture. The fresh leaves of *Coldenia Procumbens* was weighed about 1g of leaves and ground well then about 250 ml of distilled were added. Whatman filter paper is used to filter the final product from the leaf extract and filtered product was used for the reduction of copper ions ( $\text{Cu}^{2+}$ ) to copper nanoparticles ( $\text{Cu}^0$ ).



**FIG 1.** Coldenia Procumbens

### 2.3 Synthesis of Copper Nanoparticles

1m mole of Copper sulphate prepared in a 250 ml of clean standard flask under continuous stirring [11]. By taking different ratios 1:2, 1:4, 1:10 of plant extract and Copper sulphate for the conformation. From the observation the ratio about 50 ml of plant extract, 100 ml of Copper Sulphate the solution becomes changed to dark brown. Then the reduced aqueous solution used to characterize for further antimicrobial activity.



**FIG 2.** Schematic methodology of Coldenia Procumbens CuNPs

#### *2.4 Separation of Cu nanoparticles*

At 5000 rpm the solution is centrifuged to obtain a clear solution for 15 minutes continuously. Process was repeated 2-3 times to confirm the elimination of all the adsorbed constituents existing in copper nanoparticles surface. The obtained final product (copper nanoparticle) was used for the further purposes.

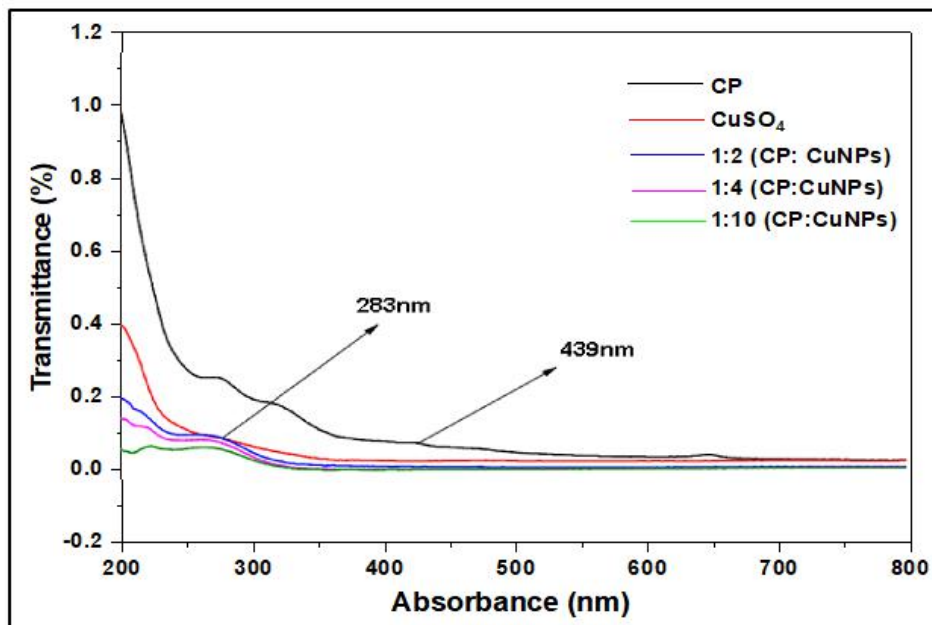
#### *2.5 Characterization of Cu nanoparticles*

From the *Coldenia Procumbens* the obtained Copper nanoparticles were taken for the following characterization Ultraviolet visible spectroscopy (UV-Vis), Fourier transform infra-red spectroscopy (FT-IR), X-Ray Diffraction (Powder-XRD), Scanning electron microscope (SEM) and Anti-microbial activity (anti-bacterial activity). The morphology of the copper Nanoparticles has been studied manually and numerous type of equipment can be employed to characterize these particles. Various parameters are used to determine the quantity of the stabilizing and reducing agent, pH, time of irradiation and power level were also investigated. Degradation of the impurities leads to the enhancement of the catalytic activity of the Nanoparticles [12].

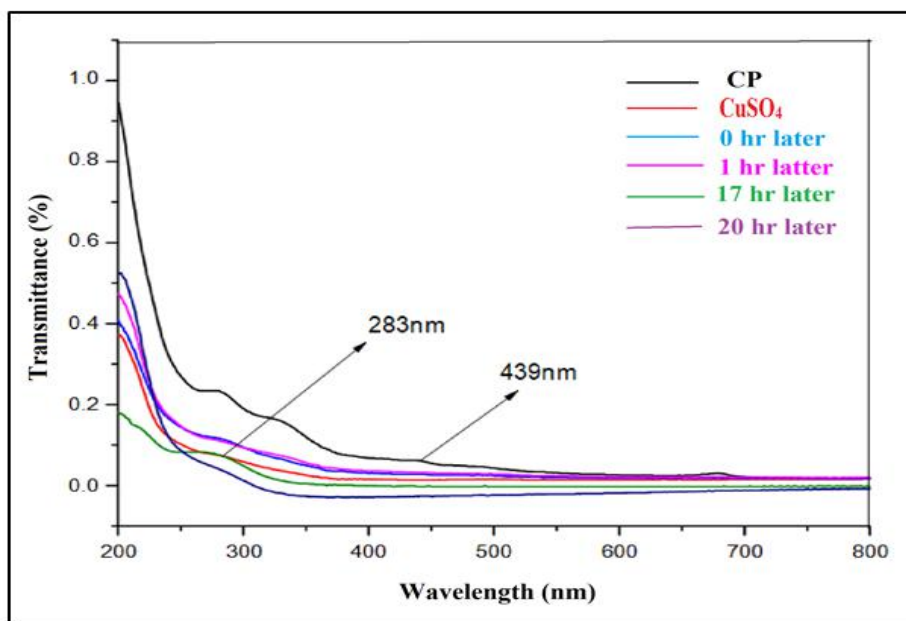
### **3. Results and discussion**

#### *3.1 UV-Vis Spectroscopy*

The different concentration of plant extract diluted to the aqueous solution of  $(\text{CuSO}_4) \cdot 5\text{H}_2\text{O}$ , in the ratio of 1:2 (plant extract and metal solution) combination the maximum displayed a slowly colour changes with time from light green to greenish brown colour. The colour shows the formation of Copper nanoparticles. These colour changes are due to the property of quantum imprisonment i.e., the property of the size of nanoparticles, which affects the optical property of the nanoparticles. The ultraviolet absorption peak of copper nanoparticles is in the range of 200-800 nm. The optical property of synthesized copper nanoparticles was investigated by ultraviolet-visible spectroscopy which is shown in Spectra (Figure 3). The observation of a absorption peak at 283 nm and 483 nm proposed the biosynthesis of copper nanoparticles which further supported by Literature. Figure 4 depict the UV-Vis spectra for different time intervals of Copper nanoparticles.



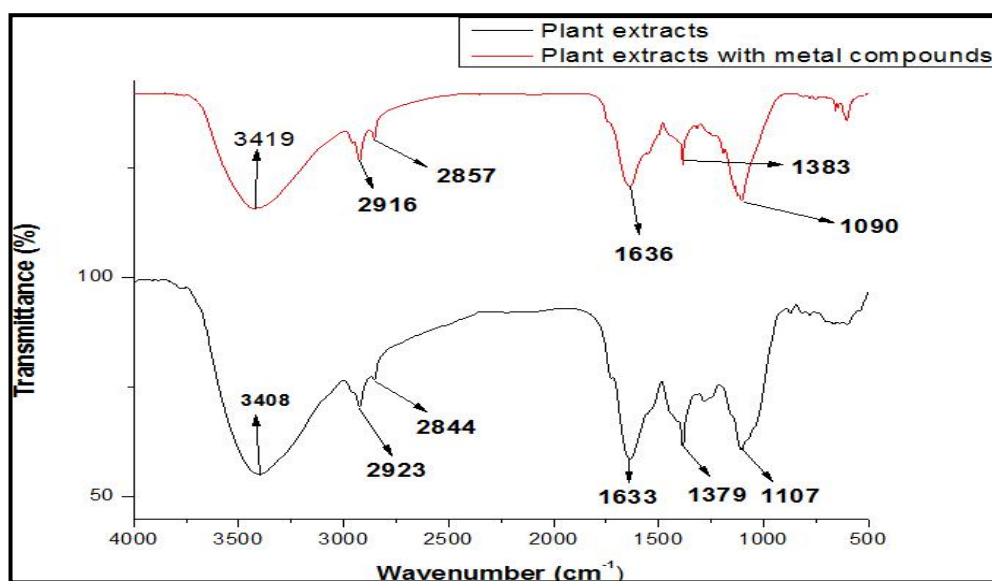
**FIG 3.** UV-Vis spectra of Copper nanoparticles with plant extract and metal solution in the ratio of 1:2, 1:4 and 1:10



**FIG 4.** UV-Vis spectra of Copper nanoparticles at different time intervals

### 3.2 FT-IR Spectroscopy

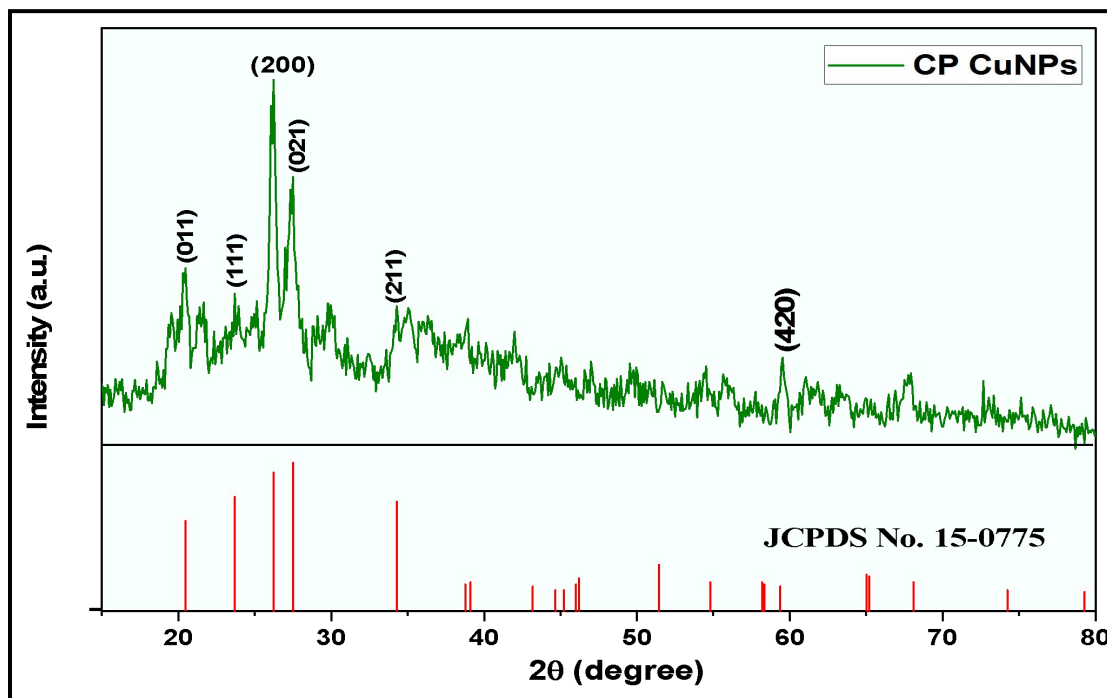
FT-IR gives the information about a functional group detects the biomolecules for covering and effective maintenance of metal nanoparticles synthesized by *Coldenia Procumbens*. The FT-IR spectrum depicts the functional group and binding properties of CuNPs. Figure 5 shows the difference between the plant extract and the Copper nanoparticles of the combination of the plant extract with metal solution. The band shows for the plant extract are at  $3408\text{ cm}^{-1}$ ,  $2923\text{ cm}^{-1}$ ,  $2844\text{ cm}^{-1}$ ,  $1633\text{ cm}^{-1}$ ,  $1379\text{ cm}^{-1}$ ,  $1107\text{ cm}^{-1}$ . Absorption peak at  $3419\text{ cm}^{-1}$  correspond to N-H stretching. The band at  $2916\text{ cm}^{-1}$  and  $2857\text{ cm}^{-1}$  corresponds to the C-H asymmetric stretch and C-H stretching of aldehyde group. C=O stretching occurs at  $1636\text{ cm}^{-1}$ , the band around  $1090\text{ cm}^{-1}$  attributed to the C-X (C-F) stretching. The band at  $1383\text{ cm}^{-1}$  correspond bio reduction of synthesized Copper nano particles.



**FIG 5.** FT-IR spectrum of the synthesized Cu-NPs.

### 3.3 X-Ray Diffraction analysis

X-ray diffraction method shows the characteristics of CuNPs of the plant *Coldenia Procumbens*. In this CuNPs showed the amorphous state or phase were confirmed by the XRD pattern (Figure 6). XRD spectrum at  $2\theta = 20.47^\circ$ ,  $23.69^\circ$ ,  $26.23^\circ$ ,  $27.50^\circ$ ,  $34.28^\circ$ , and  $59.53^\circ$  that can be indexed by (011), (111), (200), (021), (211), and (420) respectively (JCPDS card no. 15-0775). The XRD pattern reveals the orthorhombic cubic structure for copper nanoparticles from *Coldenia Procumbens* plant extract (CP CuNPs).



**FIG 6.** X-ray Diffraction of Copper nanoparticles from Coldenia Procumbens

### 3.3 Surface Characterization of Coldenia Procumbens CuNPs

Scanning electron microscopy (SEM) was used to examine the surface of Chemical and mineralogical compositions of synthesized green CuNPs. Images of CuNPs were magnified 10000 times by Zeiss, Active area 10 mm<sup>2</sup>, (Figure 7). Spherical shape indicates the presence of CuNPs. Figure 7 depict a dispersed CuNPs and identified in the range of 50  $\mu\text{m}$ . Cu nano particles are synthesized by using many plant extracts which are identified in spherical form between 5-200 nm in sizes [13].

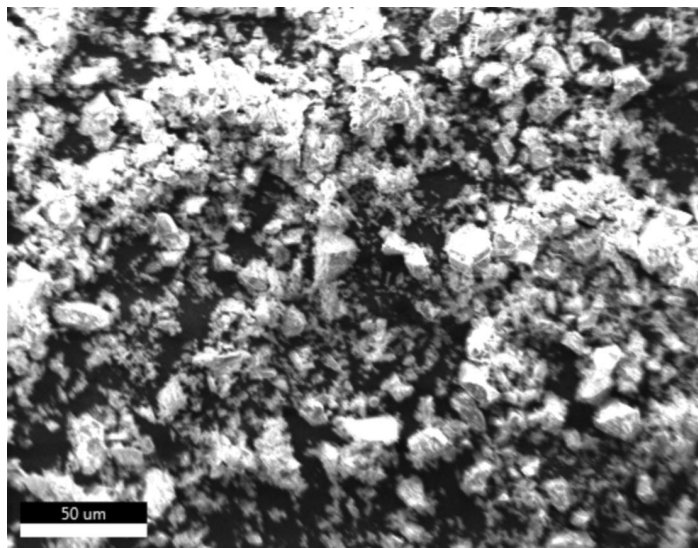
### 3.4 Generalization

When the given interval is  $[1, \infty)$ , the practical running time is the actual or the 'universal' running time of that algorithm M across all inputs.

### 3.5 Caution

Since infinity is a non-reachable entity, we substitute the upper limit in the interval with a suitably high value. This value may vary suitably for various algorithms<sup>[1]</sup>, but the key concept for this calculation is that the current CPU processor speed standards are limited to  $t \cong 10^8$  elementary operations /sec approximately.





**FIG 7.** SEM image of the synthesized Coldenia Procumbens CuNPs

### 3.6 Anti-bacterial activity

The antibacterial activity was confirmed by well diffusion method and then compared with the standard drug like a ciprofloxacin at a concentration of 100  $\mu\text{g/ml}$ . Ethanol and methanol were used as a solvent, the following organisms were used.

Test organisms:

- i. Gram positive bacteria- E. Coli (Escherichia coli)
- ii. Gram negative bacteria- Methanol

Muller hinton agar was dissolved in 200 ml deionized water in a conical flask (250 ml) then it was sealed with a fiber and tin foil sheet and sterilized in an autoclave at 121  $^{\circ}\text{C}$  for half an hour. Then opened the conical flask and poured into petri plate (Figure 8.) for support development [14] and kept for its solidified and then the bacteria called E. Coli was spread around the plate. Then wells were cutted by well cutter then the given nanoparticles solutions were poured in a different concentration like 50 $\mu\text{l}$  and 100 $\mu\text{l}$  along with another two were filled by standard and negative bacteria.

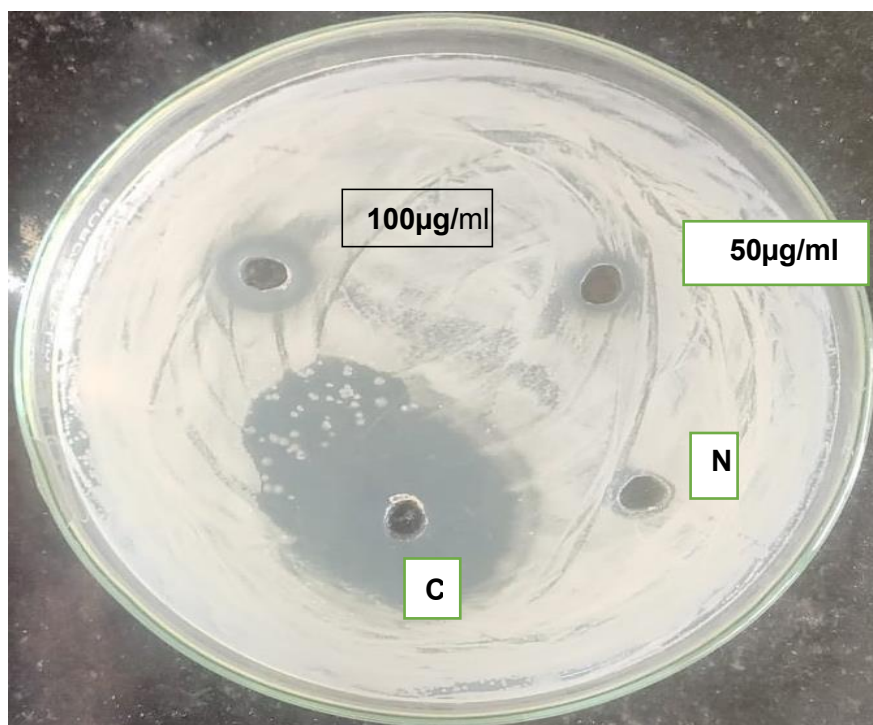
The inhibition assay is utilized to represent the antibacterial activity of the prepared Copper Nanoparticles (Figure 8). At a concentration of Cu-NPs at 50  $\mu\text{g/ml}$ , and 100  $\mu\text{g/ml}$ , the Cu-NPs showed different efficiency against the bacteria called Escherichia coli. At a concentration of Cu NPs at 50  $\mu\text{g/ml}$  and 100



$\mu\text{g/ml}$ , it showed (Figure 9) 3 mm and 6 mm efficiency against *Escherichia coli*. Finally, the CuNPs exhibits the antimicrobial activity which indicates the *Coldenia Procumbens* plant extract.



**FIG 8.** The disk is kept for zone of inhibition for Copper nanoparticles at zero time.



**FIG 9.** This disk shows the zone of inhibition for Copper sulphate with the plant extract with positive result

#### 4. Conclusion

Green synthesis of Copper Nanoparticles with the approaches of green plants is safe, toxicfree, inexpensive, environmentally friendly way of preparing at a big scale. Copper nanoparticle has been successfully sythesised from the extract Coldenia Procumbens. The final product obtained is significantly responsible for the reduction of copper metal into CuNPs. Structure, functional moieties and morphology of the nanoparticle is characterized through analytical techniques. And the synthesized copper nanoparticle from the coldenia procumbens it reveal the major application in anti-bacterial activity. The Coldenia Procumbens plant may be effectively utilized for the production of Cu-NPs will be hopeful application in the area of medicinal field.

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