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Green Synthesis of Copper Oxide Nanoparticles Using Catharanthus Roseus Leaf Extract and Their Antibacterial Activity

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Abstract- The investigation aims the synthesis of copper oxide nano particles using Catharanthus Roseus leaf extract at room temperature. This method is completely a green method, free from toxic and harmful solvent. The copper oxide nano particles were synthesized by mixing copper sulphate dehydrate (CuSO4.5H2O) and Catharanthus Roseus leaf extract. The bio synthesized copper oxide nano-particles were characterized by XRD, Fourier transform infrared spectroscopy (FT-IR) and UV-vis spectroscopy. Further, the synthesized copper oxide nano particles were tested for antibacterial activity by stand art disc diffusion method. The nano particles were found to have antibacterial activity against pathogenic bacterial strains Escherichia coli by the observation of inhibition zones around each well.

Keywords- *Green synthesis; CuO nano particles; XRD; FTIR; UV;*

I. INTRODUCTION

The field of nanotechnology is one of the most energetic areas of research in modern materials science. Metal nano particles found many applications in different fields because of their unique optical, electronic, mechanical, magnetic, and chemical properties. Various methods have been used for synthesis of nano particles including, chemical, physical, electrochemical, photochemical and biological techniques. Although most of the methods are successful in producing pure and well defined nano particles they are quite expensive or potentially dangerous for the environment. Synthesis of nano particles taking assistance of green methods has attained enormous attention in the recent years [1-5]. Plant systems widely distributed along the co logical boundaries, are easily available and safe to handle. Such studies could prove to have a huge effect in the immediate future, if plant tissue culture and downstream processing procedures are applied in order to synthesize metallic as well as oxide nano particles on industrial scale [2, 17]. As metal nano particles are widely used in the areas of human contact, the necessity to develop eco friendly methods for nano particle synthesis that do not use toxic chemicals has been constantly growing. Green synthesis of copper nano particles is of great interest because of many advantages: copper is highly conductive and also cheaper than silver and gold [1].Copper oxide nano particles are important due to their applications as antimicrobials and in gas sensors, batteries, high temperature super conductors, solar energy conversion tools, and so on [6-9]. Human beings have been using copper (Cu) and copper complexes for various purposes for centuries, such as water purifiers, algaecides, fungicides, and as antibacterial and antifouling agents. Copper-based compounds are efficient biocidal properties, which are generally used in several health related application [10-12].Biosynthesis of metal nano particles by plants is currently under development. The synthesis of metal nano particles using inactivated plant tissue, plant extracts, exudates, and other parts of living plants is a modern alternative for their production [13]. It is a very cost effective method and therefore a prospective commercial alternative for large-scale production. Biosynthesis of copper oxide nano particles using microorganisms such as bacteria, fungi, yeast have been reported in the literature [14-16]. Plants attracted few researchers to use their extracts in green synthesis of copper oxide nano particles such as Garcia papaya leaf extract, Aloe vera leaf extract, and Centella Asiatic leaf extract, Malva Sylvester's leaf extract, and Rosa sahandina broth extract [17-20], The present study reports for the first time, the synthesis and characterization of copper oxide nano particles using Catharanthus Roseus leaf extract.

II. MATERIALS AND METHODS

Materials

All materials were of analytical grade and obtained from Merck Company. Solvents used throughout the reactions

were of high purity and used without further purifications. The Catharanthus roseus leaves were collected from a local source.

Method to synthesis of copper oxide nano particles

In this study, copper sulphate (Merck chemicals) and Catharanthus roseus plant extract were used as the starting materials. The Catharanthus roseus extract solution of prepared using leaves that had been rinsed with de ionized water and finely cut into small pieces. The leaves are grained, filtered and stored. The resulting extract was used as a Catharanthus roseus extract solution. In the preparation of Copper Oxide nanoparticles, samples Copper sulfate (0.1M) was first dissolved in 200 ml of de ionized water and mixed with 50 ml of Catharanthus roseus extract solution under vigorous stirring at room temperature for 3hr. The mixture was maintained at 150° C for 12hr in oven. The obtained powder was calcined at 400° C for 2hr.

Characterization of copper oxide nano particles

Copper oxide nano particles synthesized by this green method were characterized by XRD , UV-Vis spectrophotometer (Shimadzu) and Fourier-transform infrared (FTIR-Shimadzu) spectrum in the range 4000-400 cm^{-1} and also the synthesized copper oxide nano particles were tested for antibacterial activity by standard disc diffusion method.

III. RESULTS AND DISCUSSIONS

A.XRD studies

The XRD measures were carried out at room temperature using panalytical Xpert-Pro with monochromatic beam of Cu Kα radiation (1.5406A). An accelerating voltage of 40 kV and a current of 30 mA with a scan rate of 0.01° s-1 were used. The XRD patterns were recorded in the 2θ range of 10-80°. The values of 2θ, d-spacing, relative intensity and FWHM were obtained from the XRD pattern. Identification of phases was carried out by comparing the diffraction pattern obtained from XRD with standard JCPDS database. The lattice parameters and cell volume were calculated using UNIT CELL software.

Figure .1 shows that XRD pattern of CuO nano particles and Figure .2 shows that Comparison between observed XRD and JCPDS No:48-1548 . A good match of peaks is absorbed when the data is compared with JCPDS no: 48-1548. The peaks at 25.35, 35.46, 38.67 and 43.29 is indexed as (0 1 2), (0 2 2), (0 0 4) (2 2 0) and it is found that Monoclinic crystal system. The lattice parameters are (a) 4.688Å, (b) 4.422Å, (c) 5.131Å , β= 99.506° and α=γ= 90°.

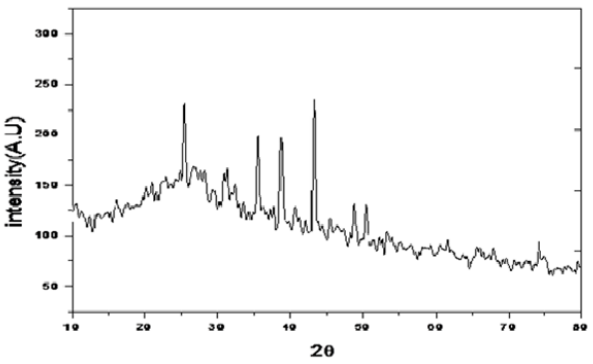

Figure1. XRD pattern of CuO nanoparticles

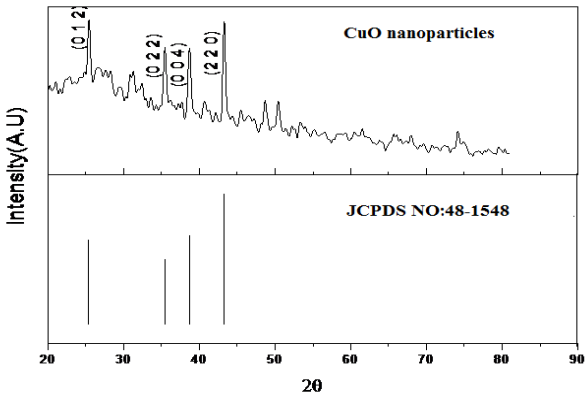

Figure 2. Comparison between observed XRD and JCPDS No:48-1548

1. Crystallite size determination

The crystallite size of Cuo nanoparticles are calculated from the full width at half maximum (FWHM) of the major peak using the following Scherrer formula,

$$D=0.9\lambda/\beta\cos\theta \quad \ldots\ldots..(1)$$

Where,

D – Crystalline size (nm)

λ - Wavelength of the X-ray used (Å)

β - Full width half maximum (radian), it is found to be 35nm

The X-ray diffraction pattern of the synthesized CuO nanoparticles is shown in Fig.1 and .2 the peak details are in Table. 1. [22] reports that absence of spurious diffractions indicates the crystallographic purity. The experimental XRD pattern agrees with the JCPDS card no. 48-1548 (monoclinic) and the XRD pattern of CuO nanoparticles other literature. The 2θ at peak 43.29° confirms the CuO monoclinic structure. Strong diffraction peaks at 38.67° and 43.29° indicating CuO in the monoclinic phase. There is no any spurious diffraction peak found in the sample. The 2θ peaks at 38.67° and 43.29° confirm its monoclinic structure. The intensity of XRD peaks of the sample reflects that the formed nano particles are crystalline and broad diffraction peaks indicate very small size crystallite [23].

Table1. XRD Data of CuO Nanoparticles

FWHM radian	βcos θ	Size (nm)	d-spacing observed	d-spacing calculated
0.00256	0.00256	56.2	3.5135	3.5103
0.01029	0.01015	14.45	2.8784	2.8749
0.00515	0.00226	26.28	2.531	2.5279
0.00343	0.00303	42.16	2.3283	2.3255
0.00343	0.00323	42.2	2.0898	2.0872
0.00257	0.00177	56.27	1.8719	1.8696
0.00686	0.00684	21.08	1.8099	1.8077
0.00515	0.00416	28.1	1.2789	1.2774

Table1. XRD Data of CuO Nanoparticles

2θ	θ	sin θ	cos θ	FWHM θ
25.3501	12.675	0.21942	0.9756	0.1476
31.0700	15.535	0.26782	0.9634	0.5906
35.4669	17.733	0.3045	0.9524	0.2952
38.6725	19.336	0.33111	0.9435	0.1968
43.2960	21.648	0.3689	0.9294	0.1968
48.6409	24.3204	0.41183	0.9112	0.1476
50.4219	25.2109	0.4259	0.9047	0.3936
74.1382	37.0691	0.60277	0.7979	0.2952

2. Instrumental Broadening

When particle size is less than 100 nm, appreciable broadening in X-ray diffraction lines will occur. Diffraction pattern will show broadening because of particle size and strain. The observed line broadening will be used to estimate the average size of the particles. The total broadening of the diffraction peak is due to sample and the instrument. The sample broadening is described by

$$FW(S) \times cos\theta = \frac{K \times \lambda}{Size} + 4 \times Strain \times sin\theta \ldots\ldots\ldots\ldots\ldots\ldots(2)$$

The total broadening β_t equation is described by

$$\beta^2_{t \approx \{0.9\lambda | Dcos\theta\}2 + \{4 \in tan\theta\} + \beta_0^2} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(3)$$

Where D is average particle size, ε is strain and β_0 is instrumental broadening. The size and strain of the experimentally observed broadening of several peaks are computed simultaneously using least squares method and presented in Figure.3. When, particle size becomes smaller, due to size effect, the peaks become broad and widths larger. The broadening of peak may also occur due to micro strains of the crystal structure arising from defects like dislocation and twinning.

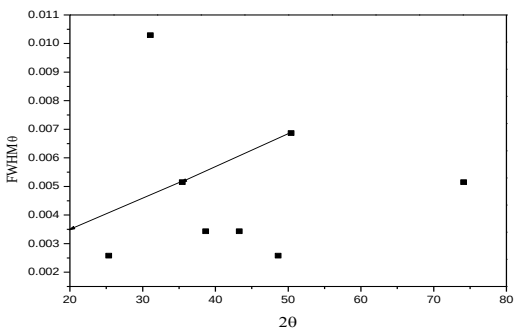

Figure .3. Typical Instrumental Broadening

Williamson and Hall proposed a method for de convoluting size and strain broadening by looking at the peak width as a function of 2θ . W-H plot is shown in Figure.4. It is plotted with sin θ on the x-axis and β cos θon the y-axis (in radians). A linear fit is got for the data and from it; particle size (48 nm) and strain (0.0000175) are extracted from y-intercept and slope respectively.

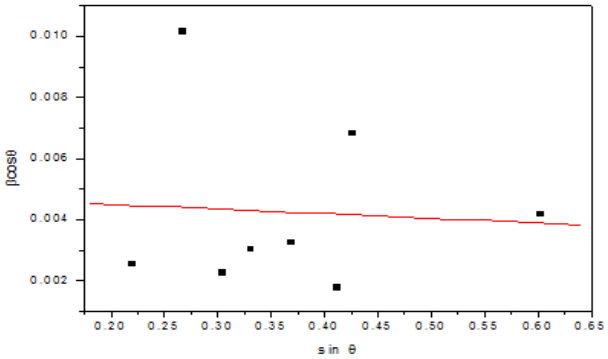

Figure 4. Williamson Hall Plot-CuO nano particles

3. Dislocation Density

The dislocation density is the length of dislocation lines per unit volume of the crystal. A dislocation is a crystallographic defect, or irregularity, within a crystal structure. The presence of dislocation strongly influences many of the properties of materials. Mathematically, it is a type of topological defect. It increases with plastic deformation; a mechanism for the creation of dislocations must be activated in the material. Dislocation formation are formed by three mechanisms i.e. homogeneous nucleation, grain boundary initiation, and interface the lattice and the surface, precipitates, dispersed phases, or reinforcing fibres. The movement of a dislocation is impeded by other dislocations present in the sample. Thus, a larger dislocation density implies a larger hardness. Chen and Hendrickson measured and determined dislocation density and hardness of several silver crystals. They found that crystals with larger dislocation density were harder. Figure . 5. Shows that particle size versus the dislocation density versus the. It has been shown that the dislocation density increases while the grain size decreases with increasing strain and ultimately these parameters reach saturation values.

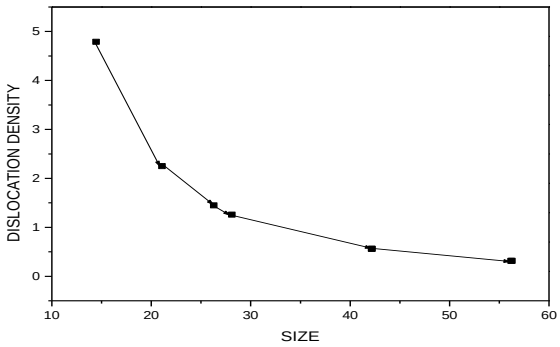

Figure 5.Particle size Vs Dislocation density of CuO nanoparticles

$$\delta = \frac{\delta=15\beta\cos\theta}{4\alpha D} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(4)$$

$$\delta=1/D^2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(5)$$

It is well known that above a certain grain size limit (~20 nm) the strength of materials increases with decreasing grain size. The X-ray line profile analysis has been used to determine the intrinsic stress and dislocation density. The dislocation density (δ) in the sample has been determined using expressions (4 & 5) and results from both the formulas are approximately same. The sample δ is 1.82 x 1014.m^2 and the results are enumerated in Table.2.

Where, δ-dislocation density, β- diffraction broadening - measured at half of its maximum intensity (radian), θ-diffraction angle (degree), α-lattice constant (nm) and D-particle size (nm). It is observed from the tabulated details and from Fig.5; dislocation density of the sample is indirectly proportional to particle size. Dislocation density increases while particle size decreases. It implies that the prepared CuO nanoparticles have more strength and hardness than their bulk (CuO) counterpart.

4. Morphology Index (MI)

It is well known that CuO nano powder is widely used in many diverse industries. Such uses are derived from its unique structural, physical and chemical properties, which are reflected by its hardness, surface properties, particle size and morphology. It is proposed that the specific surface area of CuO nano powder depends on the interrelationships of particle morphology and size. MI is calculated from FWHM of XRD to explore this relationship, based on our earlier report.

MI is obtained using equation,

$$M.I = \frac{FWHMh}{FWHMh+FWHMp} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..(8)$$

Table.2.Morphological Index and Dislocation Density of CuO Nanoparticles

2θ	FWHM(β) radians	Particle size(D_p) nm	Morphology index (unit less)	Dislocation density (m^2)
25.3501	0.002575	56.2	57.142	0.316
31.0700	0.01029	14.45	25	4.789
35.4669	0.00515	26.28	40	1.4475
38.6725	0.00343	42.16	50	0.5625
43.6409	0.00343	42.2	50	0.5614
48.6409	0.002575	56.27	57	0.3158
50.4219	0.006866	21.08	33.3	2.25
74.1382	0.000515	28.108	40	1.26

Where, M.I. is morphology index, FWHMh is highest FWHM value obtained from peaks and FWHMp is particular peak's FWHM for which M.I. is to be calculated. MI range of experimental CuO nano powder is from 25 to 57.14 and the details are presented in Table.2. It is correlated with the particle size (range from 14 to 56 nm). It is observed that MI is directly proportional to particle size and inversely proportional to specific surface area with a small deviation. The result is shown in Figure.6. Linear fit in the figure indicates the deviations and relationships between them. The observed results of the MI confirm the uniformity and fineness of the prepared nano particles.

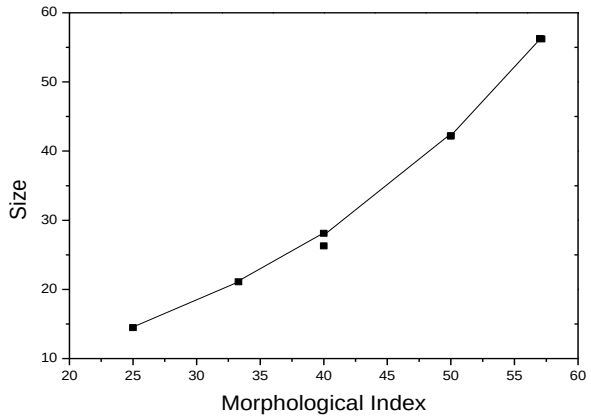

Figure 6.Morphological Index Vs Particle Size of Cuo Nanoparticles

5.Texture Co-efficient (T_c):

In material science, texture refers to the distribution of crystallographic orientations of a polycrystalline sample. A sample in which these orientations are fully random is said to have no distinct texture. If the crystallographic orientations are not random, but have some preferred orientation, then the sample has a weak, moderate or strong texture. The degree is dependent on the percentage of crystals having the preferred orientation. Texture is seen in almost all engineered materials, and can have a great influence on materials properties.

The coefficient of texture is calculated using the relation,

$$TC(hkl) = \frac{I(hkl)/I_0(hkl)}{\frac{1}{N}\sum_N I(hkl)/I_0(hkl)}$$

Where,

Tc is the texture coefficient,

$I_{(hkl)}$ is the measured intensity of the peak

$I_{o(hkl)}$ is the relative intensity of the corresponding peak from a powder reference

N is the number of peaks taken into account

When $T_{c(hkl)} = N$ means that all the atoms are oriented in the hkl plane and lower values of $T_{c(hkl)}$ means random orientation.

N=4 $\Sigma(I_{(hkl)}/I_{o(hkl)}) = 0.65012$

Table.3 Texture coefficient of Cuo nano particles

hkl	I(hkl)/ Io(hkl)	TC(hkl)
012	0.5965	3.67006
022	0.6621	4.0736
004	0.6919	4.2570
220	0.65	3.9923

The texture coefficients of the planes (220), (022) are almost equal to N (=4), proving that the atoms in these planes are well oriented whereas the texture coefficients of the planes (004) and (012) are slightly greater and lesser than N, hence proving that there is slight random orientation of atoms in that plane.

B. FT-IR spectral studies

The FTIR characterization is used to find the molecules and their functional group present in the samples. The Figure .7 shows that FTIR peaks of CuO nanoparticles. The broad peak at 3406 cm^{-1} was assigned to O–H stretching. Another band at 1184 cm^{-1} was attributed to SO_2 absorption of sulfones and 1002cm^{-1} was assigned as absorption peaks of -C-O-C- or -C-O-.Band at 651 cm^{-1} for C–H bend of alkynes, while band at 1384 cm^{-1} indicated the presence of amine groups, as expected due to plant-origin of the samples. 2852cm^{-1} assigned to asymmetric stretching vibration of C-H bond. After the synthesis of copper nano particles by exposing the copper sulphate and garlic leaves extract there was increase in the intensity at 3406 cm^{-1} which may be due to binding of [Cu(SO4)2]+ to –OH groups [24]. The band at 1636 cm^{-1} was assigned to carbonyl and carboxylic (CO) stretching bands of peptide linkages (stretching of amides). The band at 1435 cm^{-1} was assigned to –O– H bend of carboxylates. The red shift was observed from 1636to 2380 cm^{-1} which occurred due to oxidation of –CO groups of the extract components. Similarly, there was increased band intensity at 1475 cm^{-1} due to alkane (germinal) dimethyl [25].

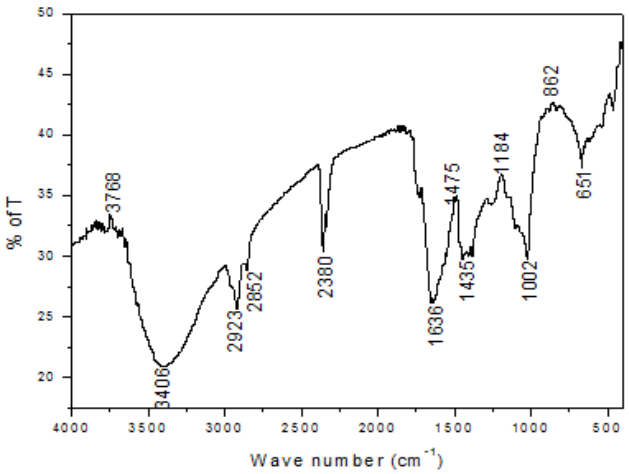

Figure.7 FT-IR spectrum of CuO nanoparticles recorded at room temperature

C. UV spectral analysis

Optical absorption properties of the CuO nanoparticles were investigated at room temperature by UV – vis spectroscopy [26]. Figure.8 shows the absorbance spectrum of the CuO sample with absorption band in 310 nm wavelength range. The optic absorption spectrum was used to study the optical properties of the synthesized CuO nanoparticles, from this the band gap and the type of electronic transitions were determined. When a semiconductor absorbs photons of energy larger than the gap of the semiconductor, an electron is transferred from the valence band to the conduction band there occurs an abrupt increase in the absorbency of the material to the wavelength corresponding to the band gap energy. The relation of the absorption coefficient (α) to the incidental photon energy depends on the type of electronic transitions. When in this transition, the electron momentum is conserved, the transition is direct, but if the momentum does not conserve this transition it must be attended by a photon this is an indirect electronic transition . To analyze the electronic properties of the synthesized CuO nanoparticles, the remission function F(R'∞) of Kubelka-munk was used. F(R'∞) = (1-R'∞)2 /2R'∞ = α/S (3) α is the absorption coefficient (cm-1) and S is the dispersion factor which is independent of the wavelength for particles larger than 5 μm.α is related to the incidental photon energy by means of the Tauc equation, α =A (E- Eg) n , A is a constant that depends on the properties of the material ,E is the photon energy, Eg is the band gap and n is a constant that can take different values depending on the type of electronic transition, for a permitted direct transition n=2 .Figure 9 shows Tauc's plot for direct transitions of CuO nano particles

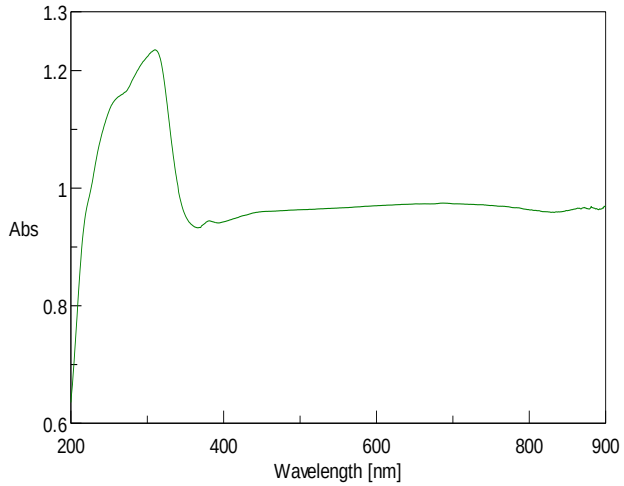

Figure.8:UV-Vis absorption spectrum of CuO nanoparticles recorded at room temperature

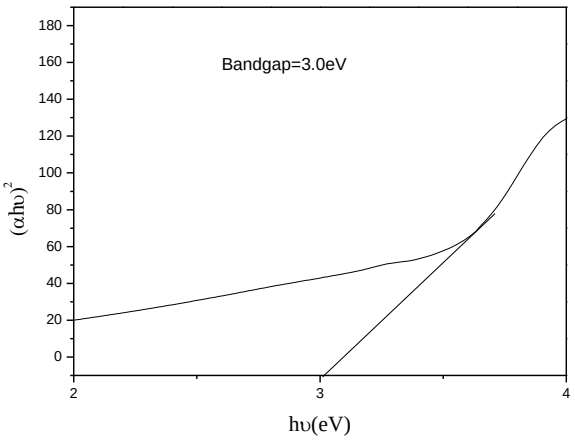

Figure.9: Tauc's plot for CuO UV-Vis spectrum

D. Anti bacterial studies

Streptomycin is an antibiotic used to treat a number of bacterial infections. This includes tuberculosis, Mycobacterium complex, endocarditic, brucellosis, Burk holder a infection, plague, tularemia, and rat bite fever. For active tuberculosis it is often given together with ionized and pyrazinamide. It is given by injection into a vein or muscle.

The antimicrobial activity of bio synthesized CuO nanoparticles was analyzed against Streptomycin, by disc diffusion method for different concentrations. It was observed that microbial growth, decrease with the increase in concentration of biosynthesized CuO nanoparticles. The 10µl, 20µl and 40µl samples of CuO nanoparticles against Escherichia coli bacteria are shown in figure .10. The CuO nanoparticles synthesized from Catharanthus roseus leaf extract are toxic to multidrug resistant microorganisms. From this study it showed that they have great potential in biomedical applications, Also, because of the biological reducing and capping agents these CuO nano particles are also environment friendly.

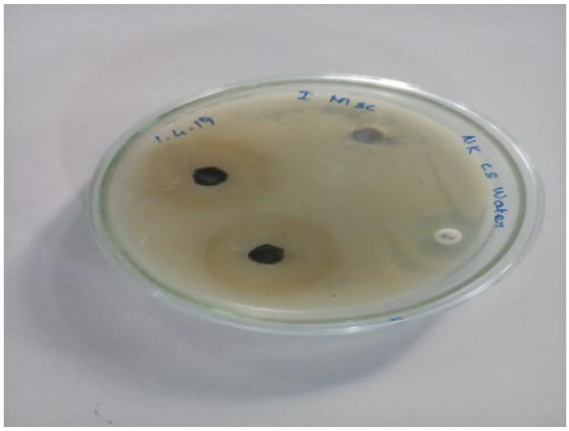

Figure.10:Shows the antibacterial activity of the CuO nanoparticles prepared with different volumes of Catharanthus roseus leaf extract against Escherichia coli

Table 4: Antibacterial Activity data for the CuO nanoparticles.

Sample	E.coli			
CuO nanoparticles	**10µl**	**20µl**	**40µl**	**Streptomycin**
ethanol(mm)	0.8	1.1	1.2	1.5

The zone of inhibition in the gram negative bacteria is tabulated in the table 4. It is observed that 40µl samples showed more inhibition zone.

IV. CONCLUSIONS

In this study, copper oxide nanoparticles were successfully synthesized by using Catharanthus roseus leaves extract which provides cost effective, easy and Proficient way for synthesis of CuO nano particles. The CuO nano particles were characterized by UV-Vis spectroscopy, FTIR, and XRD and their antimicrobial activity was investigated. The reduction of copper sulphate to copper oxide nanoparticles was confirmed by UV-Visible spectro photometer. The bending vibrations and stretching bonds present in the sample was confirmed by the Fourier Transform Infrared spectroscopy (FTIR). XRD study showed that the CuO nanoparticles had monoclinic phase and the average particle size was in the range of 14-56 nm. Morphology index , Texture co-efficient and specific surface area have been calculated from XRD analyses which confirm the nano structure of the samples. The antibacterial activity for the synthesized copper oxide nanoparticles was confirmed by disc diffusion method. These nanoparticles were highly stable with significant antibacterial activity. The antibacterial activity experiment performed *against* Escherichia coli. *coli* demonstrated that the maximum ZOI was higher in gram positive bacteria compared to gram negative bacteria.

Plant extracts are eco-friendly and cost effective can be used in large scale synthesis of copper oxide nanoparticles in nanotechnology processing industries. Copper oxide nanoparticles can be used in nano medicine and cancer treatment because of its varied advantages. Due to the highest conductive properties, these Copper nanoparticles can be implemented in advanced portable gadgets.

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