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# Cytotoxic activity and DNA fragmentation study of nano structured assemblies of copper sulfide nanoparticles using single route molecular precursor source of copper

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In this paper, we have reported an easy, template free and squashy solution chemistry path was applied to synthesize variegated nanostructured congregation assemblies of copper nanoparticles at room temperature using hydrazinecarbothioamide coordinated Cu(II) complex through a single route molecular precursor source. The parent compounds were heated under microwave irradiation to obtain diversified CuS nanostructures in the shapes of spheres and nanotubes were found to be assemblies of either nanoplates or nanoparticles. The formation of nanostructured CuS nano particles was detailed studied by differing the synthetic conditions such as reaction time, temperature, parent compounds ratio, and the presence of counter ions. The presence of $NO_3^-$ and $SO_4^{2-}$ ions as counter ions were found to be suitable for the formation of nanotubes whereas the presence of $Cl^-$ ions initiate the formation of spherical nano assemblies of CuS nano material is obtained. The synthesized samples were characterized using various structural, morphological and optical characterization techniques like elemental analysis, FT-IR, UV-Vis, and thermogravimetric analysis/differential thermogravimetric (TGA/DG). Important changes were observed in the FT-IR spectra of the copper complex compared to the FT-IR spectrum of ligand. X-ray diffraction studies confirms the formation of hexagonal crystalline phase of CuS nano particles: Transmission electron microscopy exhibit nanotube like structures with an average particle size of 78nm. Strong UV absorption band at 425nm confirms the formation of good quality CuS nano particles. The microwave irradiation of parent compounds in the presence of nonpolar solvents like DMF, DMSO environment played a significant role in decreasing the reaction time, decreases the possibility of side reactions and proceeds the reaction in the formation of good quality nanoparticles. The SEM analysis of CuS nano particles confirms the agglomerated grain like surface morphology of nanoparticles. All compounds showed the various pharmacological activities like antioxidant, antibacterial activities due to the presence of strong electron withdrawing and electron releasing functional groups are present in molecular precursors. The microwave synthesis of CuS nanostructured assemblies in nonpolar solvent in a proper stoichiometric ratio is an excellent method for preparing highly efficient bio active agents like antibacterial & antioxidant agents which can be considered as a good drug candidate for medicinal chemists in future.

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*Keywords:* CuS , Hydrazinecarbothioamide, Single molecular precursor, Antibacterial, Antioxidant activities

## 1. Introduction

Nanobiotechnology, a vibrant research field over the last few decades, has had an extraordinary impact on many fields of the modern society. A vast variety of nanomaterial have showed tremendous attention from researches and academician due to their special properties which can be different from those materials which are exhibited in a bulk state. Advance nanotechnology includes electronics, energy, textile, biomedical sciences etc[1]. Dr. Richard Feymann used the term nanosurgeons for nanomaterials in biomedical science because nanomaterials could enter the body and interact with the surrounding environment at the cellular

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level and biomedical sciences have eye witnessed the applications of novel and better nanomaterials which could assist with disease / diagnosis therapy and improve patient management. The main aim of nanobiotechnology in biomedical applications are to introduce novel technologies, and improve the existing technologies for more sensitive, accurate, efficient and timely medical procedures [2] and developed promising molecular discoveries will be efficiently translate into the clinic to benefit for cancer patients in both for public and private sectors. Tunable physicochemical characteristics properties, as well as the ability to be immediately applied/investigated in biological systems upon appropriate functionalization, make nanomaterials very important for a range of applications including bio sensing [3-4], imaging [5-9], diagnosis [10], drug delivery [11] and therapy [12-13]. Semiconducting nanoparticles have very unique properties like charge transport, light emission, mechanics, thermal diffusion and characteristic properties of the size scaling effects at nano size dimensions. These semiconducting nanoparticles played a very important role in biomedical sciences, which represent a dynamic area of research in molecular and translational medicine. The increasing importance of semiconducting nanoparticles in biomedical sciences is due to the detection and treatment of cancer and other diseases, drug delivery, and in-vitro bio sensing applications can be partly attributed to their favorable and easily tunable physical, chemical, magnetic and optical properties [14-15]. Copper Sufide (CuS) is a p-type semiconductor which has excellent optical and electrical properties has been elaborately studied for various industrial applications [16-21]. Now-a-day the applications of CuS nanoparticles are gradually increasing as a promising platform for sensing [18, 22-29], molecular imaging [30], photo thermal therapy [31-34] drug delivery [35] as well as multifunctional agents that can integrate both imaging and therapy [36]. Very fine quality, mono dispersed, highly crystalline nanoparticles of copper sulfide were synthesized by various physical and chemical pathway [27-30] like sol gel, electro deposition, dipping, CVD, spray pyrolysis, inert gas condensation, and solvo thermal method. But for maintaining the size and sustaining the proper molecular stoichiometry for substantial production via low-cost methods are still a great challenge for environmentalists because top-down conventional technologies create a huge environmental pollution [31]. To solve this problem, researchers used various metallic organic compounds and isolated molecular precursors for synthesis of metallic nanoparticles. This method involves the pyrolysis of unique molecular precursor which contain both metal ions as well as heteroatoms like O, N, S etc. The use of isolated molecular adducts/ metal complexes as a single source precursor in the synthesis of metallic nanoparticles has various advantages over classical methods because classical methods need costly volatile solvents, releases toxic and harmful gases in environment and time-consuming reactions. In a single source precursor method, we can easily maintain the molecular stoichiometries of metal and ligand composition in the proper formulation of metallic nanoparticles as well as the purification of precursors are very easy than classical methods. Hence, in this process, there are very less probability of impurities being introduced into the nanoparticles. In this procedure, schiff base ligand was used for synthesis of single molecule precursors. Then single molecule precursors were used for synthesis of metallic complexes. The synthesis of metallic complexes from single source precursor is very interesting area of research due to variable coordination behavior of metal ions. Recently microwave assisted synthesis has been reported as a newer way to synthesize large scale production of high-quality nanoparticles. It has been shown that the microwave irradiation methods are very useful for the formulation of uniform nucleation and rapid crystal growth of nanoparticles than conventional methods. In our present research work, we have first time reported the controlled synthesis, and pharmacological activities of copper sulfide nanostructured assemblies using hydrazinecarbothioamide ligand as a single molecular precursor source and their structural, morphological and optical characterizations via various spectroscopic techniques.

## 2. Experimental

### 2.1. Materials Used

Substituted aniline, 4-chloroacetophenone, $CuCl_2.2H_2O$, hydrazine hydrate, thiosemicarbazide hydrochloride, methanol, ethanol, 4-nitroacetophenone, dimethylsulphoxide (DMSO) are obtained from Ranbaxy and Merck Pvt. Ltd. and used as received without further purification. Distilled water was used for all the experiments.

### 2.2. Instrumentation

Mechanical Stirrer, water bath, ultrasonicator , Microwave

### 2.3. Spectroscopic characterization of compounds

Nanoparticles of CuS were collected and characterized by structural, morphological, and optical characterization. UV-spectra of all synthesized compounds were recorded on a Perkin Elmer UV- visible Lambda 25 spectrophotometer in the range of 200-900nm. FT-IR spectra of compounds were recorded in KBr pellets on Perkin Elmer FT-IR spectrophotometer in the range of 4000-400$cm^{-1}$. The conductance of measurement of coordinated copper (II) complex was carried out at room temperature in $10^{-3}$m DMSO solution using Digisun electronic digital conductivity meter. In the conductance measurement 0.01 mole KCl solution is used for the calibration of conductivity meter. Powder X-ray diffraction patterns were obtained from using X pert Pro PAN analytical X-ray diffractometer in the $2\theta$ ranging from 20 to 80º with CuK α radiation of wavelength 1.54Å at 40KV/50mA. XRD measurements were taken using a glancing angle of incidence detector at an angle of 2 for 2θ values over 10 to 80 in steps of 0.05 with a scan speed of 0.012. TEM micrographs were recorded using a JEOL-IEM-1230 microscope. The synthesized compounds were suspending in ethanol, followed by ultrasonication for 30 minutes. Then, a drop of the suspension was added to a carbon coated copper grid allowing the solvent to be evaporated before its solvent to be evaporated before its introduction into the TEM. SEM micrographs were recorded on Hitachi-PU 5.0KV 7.2mm×600 K SE(UL). The thermal gravimetric analysis (TGA) was performed on a PCT- thermo balance analyzer in the presence of constant air supply at a heating rate of 100ºC/minute from room temperature to 1000ºC.

### 2.4. Biomedical Applications

Antibacterial activities of compounds were screened against standard antibiotic vancomycin for both gram positive and gram negative strains of bacteria. Zone of inhibition was measured by disc diffusion method. Antioxidant activities of compounds were screened against DPPH compound using in-vitro antioxidant protocol. The in-vitro cytotoxic activity was performed for compounds on human skin cancer (A-431) cell line to find a toxic concentration of compounds by MTT assay protocol. DNA fragmentation assay of coordinated Cu (II) complex was performed against human skin cancer cell lines A431 cells and one by using MTT assay in which the cell viability was measured based on the mitochondrial dehydrogenase enzyme activity.

### 2.5. Preparation of CuS nanoparticles

The whole process of synthesis of CuS nanoparticles was splitted into three steps:

#### *2.5.1. Synthesis of hydrazinecarbothioamide ligand*

The hydrazinecarbothioamide ligand was prepared by refluxing method as shown in figure 1. Acid hydrazone (1mmol) in hot ethanolic solution(20mL) was mixed with 30mL hot ethanolic solution of thiosemicarbazide hydrochloride (2mmol). The reaction mixture was stirred for 2 hours on a water bath at 80ºC. On cooling the contents to room temperature, the precipitate was separated out, filtered, washed with acetone and dried in a desiccator. The ligand was obtained as white crystals. Percentage yield: 80%, m.pt, 50ºC, Anal. Calc. For $[C_{36}H_{34}N_{14}S_2O_4]$: C, 57.28; H, 6.80; N, 26.25; S, 12.56. Found: C, 56.18; H, 6.20; N, 25.24; S, 11.92%. Significant IR bands ($cm^{-1}$): υ ($NH_2$): 3363, υ(N-H): 3265, υ(C=N): 1586, υ(C-H): 1388, υ(N-N): 1089, υ(C=S); 959

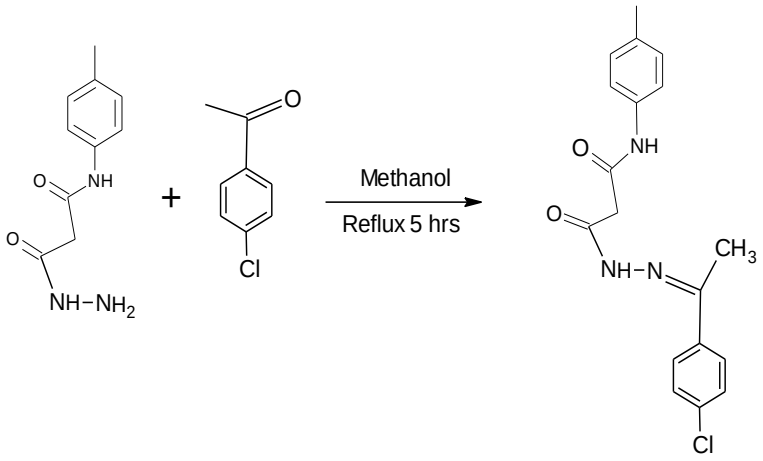
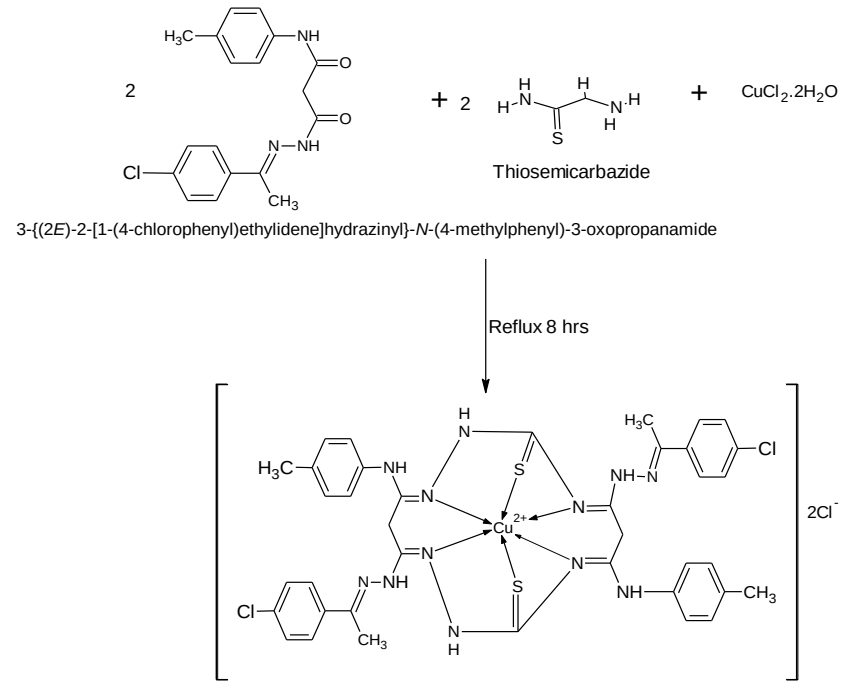



*Fig. 1. Synthesis of hydrazine carbothioamide ligand*

### *2.5.2. Synthesis of Coordinated Cu(II) complex*

The synthesis of Cu(II) complex from ligand was performed by general procedure reported previously[32,33]. A hot solution of hydrazinecarbothioamide ligand (0.266g, 5mmol) in ethanol 50ml was added to a hot solution of $CuCl_2.2H_2O$ (0.276g, 5mmol) in 20ml ethanol. The reaction mixture was stirred and refluxed for 1hr at a temperature of 80ºC. The dark brown colored solution was filtered hot to remove traces of unreacted materials and concentrate at room temperature. The colored crystalline product was obtained after 24 hours of ageing process. The product was filtered, washed 2 times with ethanol and dried under vacuum.




3-{(2*E*)-2-[1-(4-chlorophenyl)ethylidene]hydrazinyl}-*N*-(4-methylphenyl)-3-oxopropanamide

*Fig. 2. Synthesis of Coordinated Cu(II) complex from hydrazinecarbothioamide ligand.*

### *2.5.3. Synthesis of CuS nanoparticles from Synthesised Coordinated Copper (II) Complex*

For preparing CuS nanoparticles from synthesized coordinated copper (II) complex of schiff base ligand. Take 1gm of synthesized copper complex and dissolved in 50ml

dimethylsulphoxide solvent in a round bottom flask. An ultrasonic treatment (using piezoelectric sandwich transducer at 40 KHz resonant frequency) was given to reaction mixture for 30 minutes at 75ºC for proper mixing and them reaction mixture was kept for ageing for further 30 minutes.

***2.5.4. Microwave assistance of Reaction mixture***

Finally reaction mixture was heated in microwave oven for 15 min with 800W power microwave resulted immediate formation of CuS nanoparticles. The solution containing CuS nanoparticles was then cooled, centrifuged, and washed several times with methanol and chloroform.

## 3. Results and Discussion

The coordinated Copper (II) complex was synthesized using an easy and shorter method .For this synthesized schiff base ligand reacted with $CuCl_2.2H_2O$ to form Cu (II) complex This Cu (II) complex was used as a precursor for the synthesis of CuS nanoparticles. The ligand and its Cu (II) complex were characterized by elemental analysis which confirmed the elemental and structural composition of ligand and complex. Brown color CuS nanoparticles were synthesized within a short period of time using microwave radiation in the presence of nonpolar di methylsulphoxide solvent. Microwave radiations in synthesis of CuS provide fast , uniform heating, selective in nature, high reaction speed, and low energy consumption in the process of synthesis of nanoparticles and such type of microwave heating help in delivering energy into the reaction vessel and increases the reaction rate and efficiency of conversion of Copper (II) complex into copper sulfide nano particles because microwave heating of compounds reduced the crystallization time of nanostructures and improved the crystalline behavior of the final product of reaction in a unique way like when Cu(II) complex of ligand was irradiated with microwave radiations, it helps to obtain good quality nanotubes of CuS. In this reaction mechanism, dimethylsulphoxide solvent play a very important role in the synthesis of nanotubes of copper sulfide because DMSO considered as an excellent absorber for the microwave for the microwave radiations, which can take up the energy from the microwave region and develop the polar reaction conditions heated up to high temperature immediately [34, 35] . The presence of nonpolar solvent in reaction medium can effectively absorbs and stabilize the surface morphology of nanoparticles and helps in producing nanotubes of CuS nanoparticles [36] . The application of microwave radiations reduces the reaction time by increasing product purities by decreasing the amount of unwanted side reactions as compared to old classical heating methods and microwave irradiation of samples allow convenient access to high temperature and pressures.

### 3.1. Structural Studies of Synthesized Compounds

The characteristic stretching vibration modes of respective ligand and its Cu(II) complex were investigated confirming the distorted octahedral geometry .It also revealed that the bonding of that the bonding of the copper metal to the ligand is coordinated through N and S elements of the ligand. The strong and high intensity IR bands for the ligand and Cu(II) complex spectra were observed in the 3379,3368,3372,and 3362 $cm^{-1}$which attributed to $NH_2$ vibrations. The strong absorption peaks at 1580,1628, 1620 and 1618 $cm^{-1}$ in a free ligand and its coordinated copper (II) complex can be attributed to C=N stretching vibrations of the bonding bridge between hydrazone and thiosemicarbazide hydrochloride shown Figure -3 .The presence of strong bands in the complex is due to the stretching of C-N groups are found at 1404,1408,and 1410 $cm^{-1}$with higher frequencies(i.e. blue shift) compared to free Schiff base ligand which absorbed at 1387 $cm^{-1}$which is an indication of the hexadentated bonding behavior of the ligand ..The absorption peaks at 1080,1026 , and 1020 $cm^{-1}$ were assigned with N-N stretching vibrations from the aromatic ring. The high intensity absorption peaks found from 952 to 956 $cm^{-1}$ region are corresponding to the C=S vibrations which show the typical coordination behavior between the ligand and the $Cu^{2+}$ cations through S &N atoms [37-39],shown in figure 4.The FT-IR spectra results showed that the new absorption bands at 542,546,and 538 $cm^{-1}$ which do not exist in the schiff base ligand spectrum that are attributed to Cu –S bond which are the explanation for the consideration of Cu

ions to S atom. This type of performance may be explained to the electron releasing tendency of the aromatic amines which forces high electron density towards the S atom [40]. The absorption studies of the CuS nanoparticles were studied at room temperature in the visible range 200-800 nm. According to quantum confinement theory, the free electrons in the conduction band as well as holes in the valence band are confined by the potential barrier of the surface of nanoparticles. Due to this confinement of electrons and holes, the lowest, the lowest energy optical transition from the valence to conduction band will increase in energy as a result of this energy band gap increases simultaneously. [41].Either the shoulder or the peak of the spectra that is corresponding to the fundamental absorption edges in synthesized samples can be used to estimate the energy band gap of the nanomaterial [42]. CuS nanoparticles gave absorption band edges with the largest blue shifts as compared to their bulk coordinated Cu (II) complex from schiff base ligand which is at 573nm. The absorption spectra gave absorption peaks at 280 (4.32ev) nm for CuS nanoparticles.

### 3.2. Morphological Studies

The synthesis of uniform and mono disperse nanoparticles is of utmost importance due to their biomedical applications. Therefore, techniques for both physical and chemical characterization of the synthesized nanomaterial are indispensable. Characterization of CuS nanoparticles can be performed using a variety of techniques, including but not limited to X-ray diffraction (XRD), Scanning electron microscopy (SEM), Energy dispersive X-ray spectroscopy (EDS) and Transition electron microscopy (TEM), Fourier transform infrared spectroscopy (FT-IR), Dynamic light scattering (DLS), UV- Visible and Photoluminescence (PL) spectroscopy etc. These techniques can provide important information on the elemental, structural (e.g. size & shape) and optical properties of CuS nanoparticles. X-ray diffractogram for CuS nanoparticles obtained from Cu(II) complex of hydrazinecarbothioamide ligand as a single route molecular precursor source. The crystallinity of synthesized CuS nanotubes was examined by XRD. The XRD pattern shown in figure 5 indicates that peaks could be perfectly indexed to a pure single phase of hexagonal CuS nanoparticles according to JCPDS card 06-0464. No peaks of any other phase impurities were detected from XRD patterns, indicating the high purity of the product. It has been demonstrated that Raman spectroscopy is a fast and nondestructive tool for appreciating crystalline material qualities. So, structured characterization of the synthesized CuS nanoparticles was carried out using Raman spectroscopy. Raman spectrum collected using the 516.5nm excitation line at room temperature for the Cu S nanotubes. The band centered at 484 $cm^{-1}$ may attributed to the lattice vibration, which is identical to the ones recorded for the corresponding thin films [43, 44] and CuS nanospheres [45]. The corresponding EDX analyses give clear evidence for the FE-SEM observation of the samples obtained at different reaction times. We can observe the successful incorporation of Cu and S elements into the CuS nanotubes in the compositional information and the Cu/S stoichiometric ratio is 0.54. The signal of S originates from the Cu (II) complex of hydrazinecarbothioamide ligand as a single route molecular precursor source. In order to follow the morphology evaluation of the CuS nanotubes in depth, the products obtained at different reaction stages were collected and characterized by TEM fig-7. The product obtained at 10 minutes exhibits slightly rough surfaces, indicating the initial deposition of CuS nanocrystallites on the surface of the original synthesized Cu(II) complex via Schiff base ligand. When the reaction time is proceed to 30 minutes, the layer of deposited CuS crystallites become slightly dense and thicker, suggesting the formation of CuS nanotubes . Prolonged reaction times will lead to the formation of numerous CuS nanocrystallites on the outer surface (Fig.7) when the reaction time is prolonged to ½ hour, we are able to realize uniform and pure CuS nanotubes composed of nanoparticles with about 85 nm in diameter. Figure-7 displays the HRTEM image for the wall of individual CuS nanotube. It is clear that the CuS nanotubes are polycrystalline and clearly observed crystal fringes formation demonstrate that the CuS nanotubes are highly crystalline in nature, and free from dislocation and stacking faults. Moreover, the corresponding SAED pattern with characteristic ring diffraction also explains the polycrystalline nature of CuS nanotubes. A series of SEM images in figure-8 show the morphology at different reaction stages corresponding to the reaction time of synthesis of CuS nanotubes. When the ligand and Cu (II) complex react at 80ºC for 10 minutes, some CuS nanoparticles on the surface of coordinated Cu(II) complex are

observed because ions exchange happens as $Cu^{2+}$ reacts with $S^{2-}$ ions slowly dissolved from the surface of coordinated Cu(II) complex to form initial CuS shells, as depicted in Fig.8 the nanotube surface becomes more rough, revealing that more Cu S nanoparticles piled up on the initial surface of CuS shells. When the reaction time proceeds, large number of CuS nanoparticles come into being and arrange along the 1 dimensional direction. Finally, some were defined CuS nanotubes composed of nanoparticles with diameter of about 85nm and lengths of about 200-400nm appear. When further proceeding the reaction time to 1 hour under hydrothermal conditions.

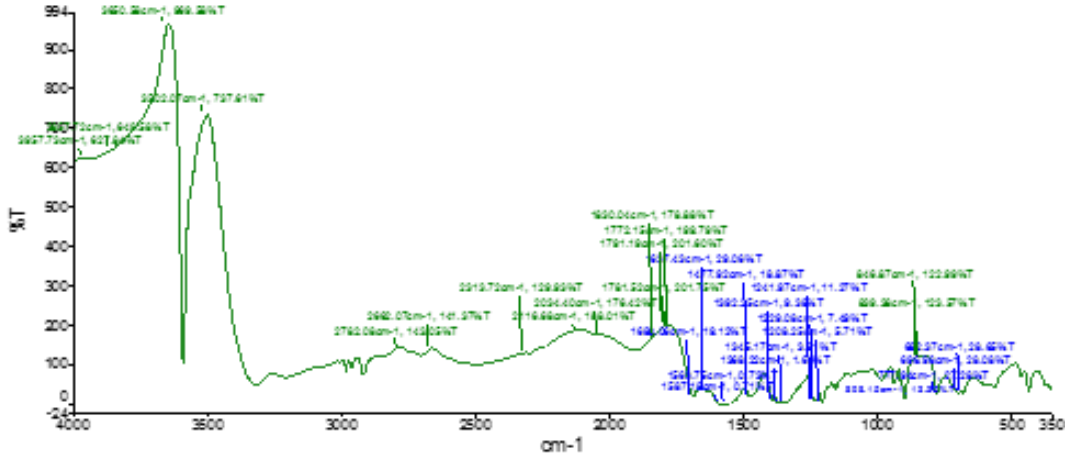



*Fig. 3. FT –IR spectra of Ligand and its Coordinated Cu(II) Complex.*

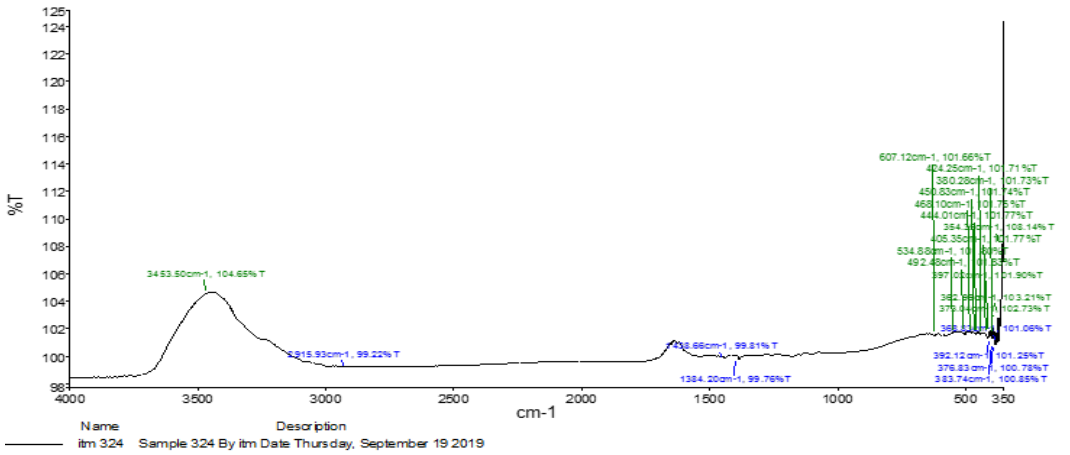



*Fig. 4. UV- Visibe Spectra of Coordinated Cu (II) complex and CuS nanoparticles.*

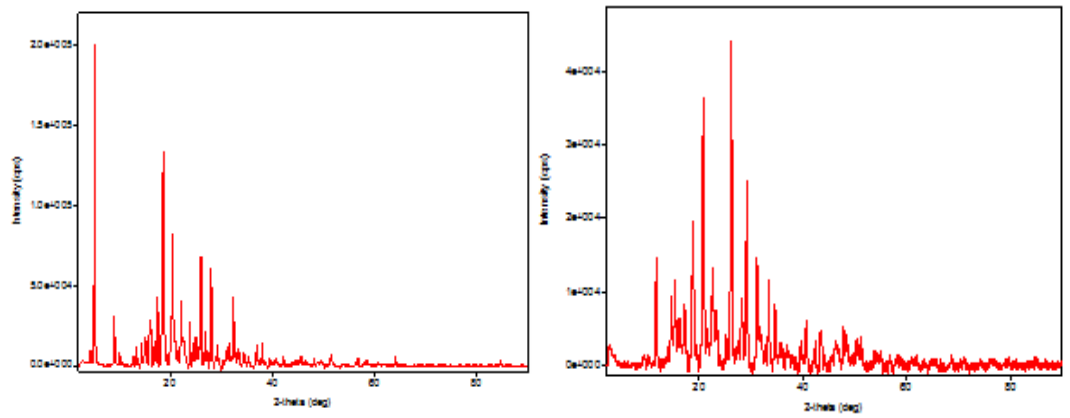


*Fig. 5. XRD Spectra of synthesized copper complex (a) & copper sulphide nano particles.*

### 3.3. Particle size of Copper Sulfide nanoparticles

The particle size of copper sulfide nanoparticles are range in between 100 nm. These are in the range of nanoparticles.fig.6shows the particle size of microwave assisted synthesized copper sulfide nanoparticle figure -6

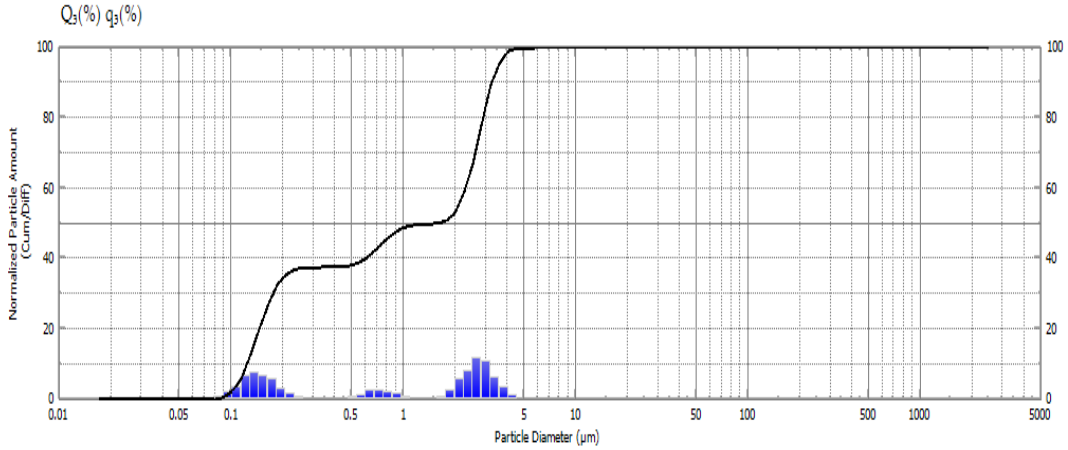



*Fig. 6. Particle size analysis of copper sulfide nanoparticles.*

### 3.4. TEM Studies of CuS Nanoparticles

The TEM images of microwave assisted CuS nano particles are shown in figure 7. According to TEM images of nanoparticles ,it is indicated that the coordinated Cu(II) complex of single molecular precoursor and microwave assisted CuS nanoparticles are arranged in a crystalline form, approximately 100nm in size, which are expected to accumulated around the nucleus through nucleation process of crystal formation and prevent their further growth. Figure 7 shows that the CuS nanoparticles are arranged in an oval shaped geometry and all particles are arranged in a definite geometry. TEM images of CuS nanoparticles are not combined but they are separated by equal interspace between the nanoparticles, which was confirmed by the microscopic visualization under the high resolution in transmission electron microscope. The TEM images explained that the CuS nanoparticles are bounded by the weak vanderwaal's forces.

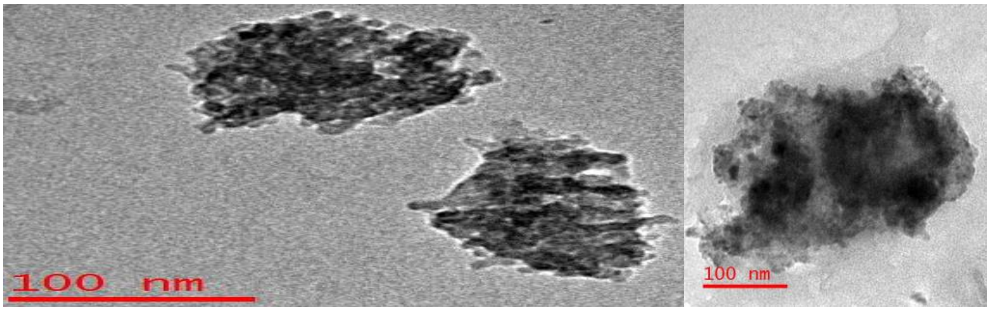



*Fig. 7. TEM images of CuS nano particles.*

### 3.5. SEM studies of CuS nanoparticles

SEM monographs explains the well dispersed, versatile and elongated shape of CuS nanoparticles when synthesized coordinated Cu(II) complex from Schiff base ligand irradiated with microwave radiations but the size of Cu S nanoparticles increases when we increases the concentration of Cu(II) complex which acts single route molecular precursor for the formulation of nanoparticles. According to monographs of nanoparticles it is indicated that the synthesized CuS nanoparticles were arranged into a ver open and quasi- linear structure than a closely packed dense assembly shown in figure -8.

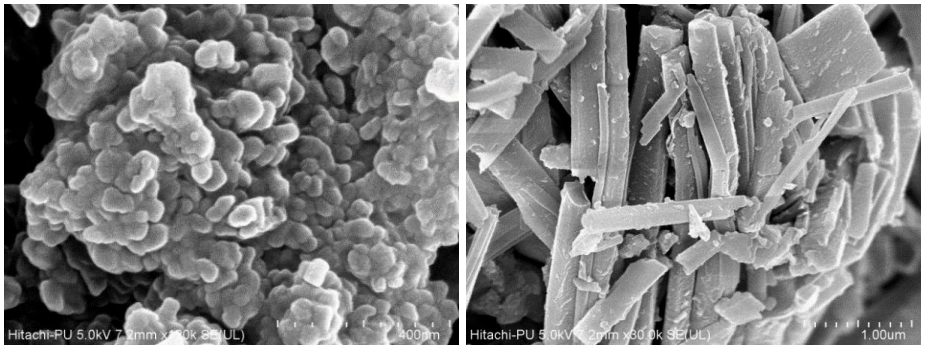


*Fig. 8. SEM images of synthesized CuS nanoparticles.*

### 3.6. EDX Study

Figure 9 shows the EDX spectrum of microwave assisted CuS nanoparticles. Copper ions and Sulfur element signals comes from CuS nanoparticles and the atomic percentage of copper element is 19.48%. Except for Cu element there were some other peaks are also appeared in the EDX spectra of nanoparticles. The atomic composition percentage of various elements are 24.18% (Cu),42.73% (C),21.08% (O) and 12.04% (S) and the atomic composition is then calculated as 6.75%,63.19%,23.39% and 6.68% respectively. The signal from the EDX spectra confirms the presence of S and Cu elements in the CuS nanoparticles. The results of EDX studies indicates that the reduction of coordinated Cu(II)complex takes place due to application of microwaves .The other impurities like C ,O element are also identified due to interaction of copper (II) complex with high energy ecofriendly microwave radiations during chemical process of formation of CuS nanoparticles.

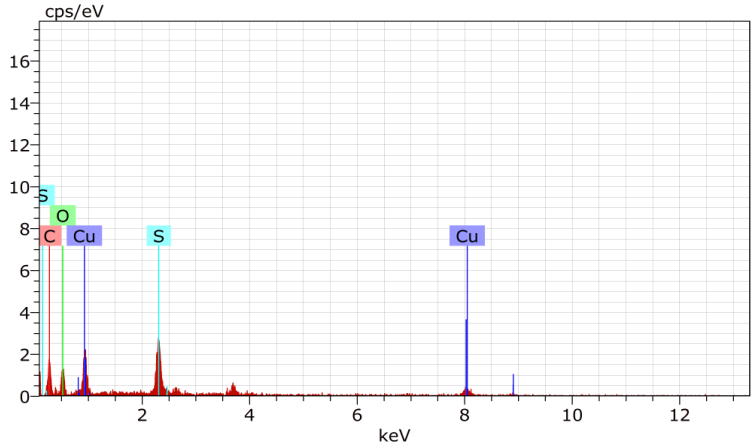



*Fig. 9. EDX spectra of copper sulphide nano particles which shows the presence of Cu , S, C ,O Elements.*

*Table 1. EDX Spectrum of Copper sulfide nanparticles.*

| Element [wt.%] | Series [wt.%] | unn. [at.%] | C norm [wt.%] | C Atom. | C Error (3 Sigma) |
|---|---|---|---|---|---|
| Carbon | K-series | 34.10 | 42.74 | 63.20 | 29.33 |
| Oxygen | K-series | 16.81 | 21.06 | 23.38 | 15.58 |
| Copper | K-series | 19.28 | 24.17 | 6.75 | 5.09 |
| Sulfur | K-series | 9.59 | 12.02 | 6.66 | 1.53 |
| Total | | 79.78 | 100.00 | 100.00 | |

### 3.7. Thermal behavior of compounds

The thermal properties of the compounds were studied by thermo gravimeter analysis at room temperature range from 20 to 800°C under nitrogen atmosphere. Figure 10 shows the resulting TGA behavior of Cu (II) complex of Schiff base ligand. The TGA figures indicate that the Cu (II) complex begin to decompose at 262°C and the weight loss happens in the temperature range of 180-400°C. The weight loss may be attributed to the decomposition of the Cu (II) complex with the weight loss of about 80°C. TGA figure also shows the first decomposition at 180-220°C temperature range which may be attributed to the loss of C-N and C-C bonds from schiff base ligand. The second decomposition comes shortly after the first decomposition at a temperature of 240-320°C which corresponds to about 10% weight loss. The third decomposition occurs at 550-720°C temperature range, leaving a very low percentage of residue. The residual CuS nanoparticles were dispensed in toluene and the ultrasonic bath was used to speed up the dissociation of the nanoparticles in order to obtain better quality nanoparticle dispersions. The absorption studies of the CuS nanoparticles were studied at room temperature in the visible range 200-800 nm.

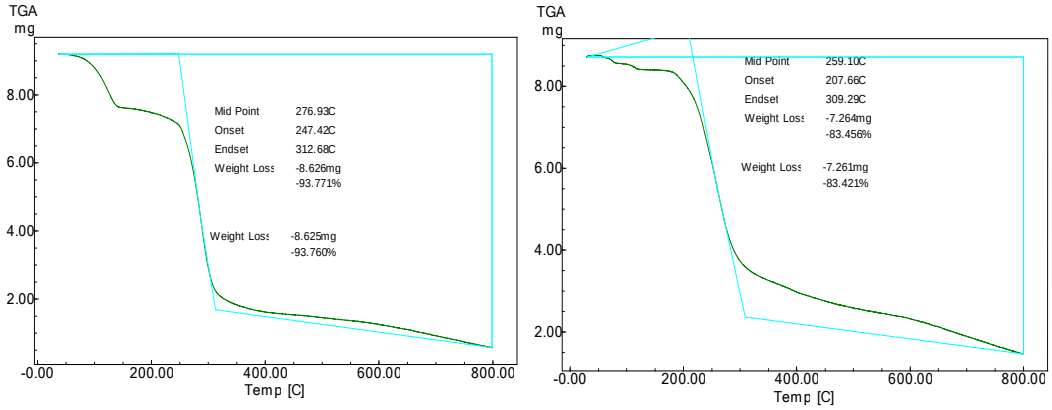



*Fig. 10 .TGA Spectra of Copper complex & copper sulphide nanoparticles.*

### 3.8. Antioxidant Activities

All synthesized compounds were screened for antioxidant activity using DPPH assay. DPPH is a stable free radical compound and has been widely used to test the free radical scavenging activity of numerous chemicals, includes herbal products, synthesized metal complexes and metallic nanoparticles. It is very good to note that DPPH free radical scavenging activity of all synthesized compounds were greater than standard ascorbic acid: The results showed that the compounds showed greater antioxidant activity than quercetin (Positive control) and the CuS nanoparticles showed greater antioxidant than ligand and its coordinated Cu(II) complex. All results of antioxidant activities of synthesized compounds are in accordance with the theoretical aspects, because the position and the number of different functional groups as well as the degree of conjugation of the whole coordinated Cu (II) complex molecule are important. The antioxidant efficacy of all compounds is directly proportional to the presence of total number of electron withdrawing and electron releasing functional groups in the benzene ring [46]

### 3.9. Mechanism of antioxidant activity of Synthesized Compounds

An unshared electron present on the N atom of DPPH molecule is responsible for the absorbance of light at 517nm and also for formation of visible deep purple color. When DPPH radical accepts an unpaired $e^-$ donated by an antioxidant compound, the color of DPPH solution fades, which can be quantitatively measured from the changes in the absorbance of light. The reverse reaction is evaluated by adding DPPH-H at the end of the reaction. If there is an increase in the percentage of remaining DPPH free radical at the plateau, the reaction is reversible, otherwise it is a complete reaction [47]. The DPPH assay evaluates the ability of the sample to donate H to the DPPH radical, resulting in de-colorization of DPPH solution. The greater the de-colorization

action of solution, the higher the antioxidant activities of compound and this was reflected in a lower $IC_{50}$ value of samples.

*Table 2. Trolox[a] equivalent antioxidant activity.*

| S. No. | Compound | DPPH activity ($IC_{50}$ $\mu$g/ml) |
|---|---|---|
| 1. | Schiff base ligand $[C_{36}H_{34}N_{14}S_2O_4]$ | 3.58 |
| 2. | Coordination Cu (II) complex $[Cu\ (C_{36}H_{34}N_{14}S_2O_4)]\ Cl_2$ | 2.59 |
| 3. | CuS Nanoparticles | 1.84 |
| 4. | Ascorbic Acid | 3.76 |

*TEAC- Troloxequivalent antioxidant capacity has been calculated from molar absorptivity by dividing $1.64\times10^4$.*

### 3.9. Antibacterial Activities of Synthesized Compounds

In-vitro antibacterial efficacy of synthesized compounds were studied by using different concentration of compounds on agar plate that was incubated with 6µgm/ml with different bacterial strains like E. Coli (MTCC-1687), E. faecalis (MTCC-439), S. aureus (MTCC-737) and indigenous methicillin resistant S. aureus isolates and the same concentrations of control antibiotic vancomycin was used to compare the antibacterial activities of synthesized compounds. The results of antibacterial activities of compounds showed that the growth of bacteria drastically increased in the case of CuS nanoparticles. The results also revealed that all compounds are nontoxic in nature for broad spectrum bacterial species.

*Table 3. Zone of Inhibition of compounds against standard drug vancomycin.*

| S.No. | Test Microbes | Diameter of zone of inhibition (in mm) at different concentration | | | | |
|---|---|---|---|---|---|---|
| | | 50µgm/ml | 25µgm/ml | 12.5µgm/ml | 6.25µgm/ml | 3.125µgm/ml |
| 1. | E. coli (MTCC-1687) | 10mm | 9mm | 6mm | 5mm | 4mm |
| 2. | E. faecalis (MTCC-439) | 18mm | 20mm | 19mm | 21mm | 22mm |
| 3. | S. aureus (MTCC-737) | 25mm | 21mm | 23mm | 20mm | 28mm |
| 4. | MR. S. aureus (Indigenous) | 20mm | 22mm | 18mm | 17mm | 19mm |

*Table 4. Antibacterial activity of Schiff base ligand, coordinated Cu (II) complex of ligand and CuS nanoparticles.*

| S.No. | Concentration of compounds in µgm/ml | E. Coli (MTCC-1687) | | | | | S. aureus (MTCC-737) | | | E. faecalis (MTCC-439) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Schiff base ligand | Cu (II) Complex | | | | CuS Nanoparticles | Schiff base ligand | Cu (II) complex | CuS Nanoparticles | Schiff base ligand | Cu (II) complex | CuS Nanoparticles |
| 1. | 100µgm/ml | 12 | 14 | | | | 18 | 13 | 16 | 20 | 10 | 12 | 16 |
| 2. | 50µgm/ml | 14 | 16 | | | | 20 | 11 | 14 | 19 | 13 | 16 | 18 |
| 3. | 25µgm/ml | 11 | 14 | | | | 18 | 14 | 20 | 24 | 15 | 17 | 19 |
| 4. | 12.5µgm/ml | 8 | 10 | | | | 15 | 10 | 14 | 18 | 9 | 11 | 13 |
| 5. | 6.25µgm/ml | 15 | 18 | 22 | 12 | 16 | 20 | 10 | 12 | 14 | | | |

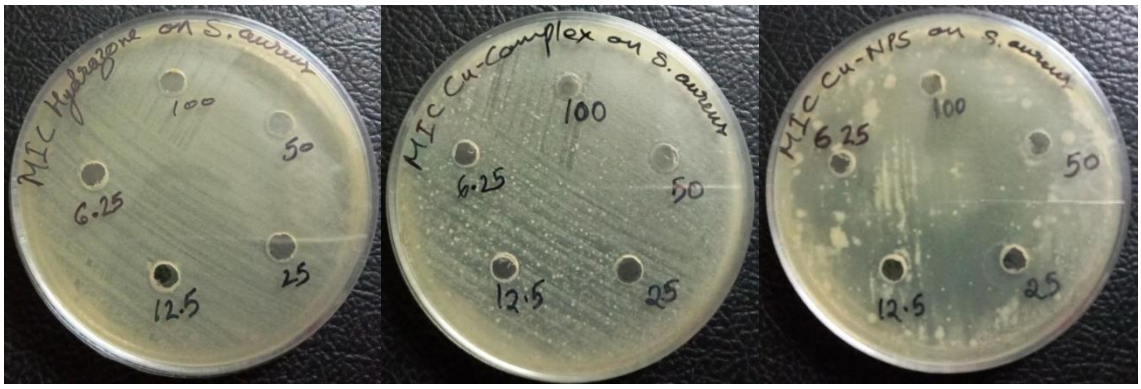



*Fig. 11. Antibacterial activity of Schiff base ligand, Copper (II) complex & Copper Sulphide nanoparticles against S. aureus (MTCC-737 using MIC method).*

### 3.10. Cytotoxic activity of Synthesized compounds

In the cytotoxic activity of synthesized compounds, compounds were tested on human skin cancer cell line (A-431) by MTT assay. In this assay, cells were exposed to different concentration of compounds ranging from 1000 to 7.8µg/ml to determine the percentage growth inhibition on A-431 cells. The coordinated copper (II) complex of ligand and CuS nanoparticles have exhibited a $CTC_{50}$ value of Schiff base ligand are 11.5 ± 0.14,14.599 ±0.11, 12.49 ± 0.42µg/ml on A-431 human cancer cell clines respectively.

*Table 5. Cytotoxicity activity of Compounds against A431 skin cancer cell lines.*

| S. No. | Compounds | Test Concentration | %Cytotoxicity | $CTC_{50}$ (µgm/ml) |
|---|---|---|---|---|
| 1. | $[C_{36}H_{34}N_{14}S_2O_4]$ Schiff Base Ligand | 1000 | 80.20 ± 0.19 | 11. 5 ± 0.14 |
| | | 500 | 78.46 ± 0.14 | |
| | | 250 | 62.48 ± 0.10 | |
| | | 125 | 81.72 ± 0.04 | |
| | | 62.5 | 80.62 ± 0.40 | |
| | | 31.25 | 79.40 ± 0.34 | |
| | | 15.6 | 54.14 ± 0.80 | |
| | | 7.8 | 6.14 ± 0.16 | |
| 2. | $[Cu(C_{36}H_{34}N_{14}S_2O_4)]$ $Cl_2$ Coordinated Cu (II) Complex | 1000 | 91.19 ± 0.19 | 14.69 ± 0.12 |
| | | 500 | 89.40 ± 0.18 | |
| | | 250 | 88.26 ± 0.10 | |
| | | 125 | 87.62 ± 0.09 | |
| | | 62.5 | 87.04 ± 0.26 | |
| | | 31.25 | 83.82 ± 0.43 | |
| | | 15.6 | 57.12 ± 0.80 | |
| | | 7.8 | 9.10 ± 0.56 | |
| 3. | CuS Nanoparticles | 1000 | 81.92 ± 0.43 | 12.49 ± 0.42 |
| | | 500 | 82.6 ± 0.68 | |
| | | 250 | 77.86 ± 0.20 | |
| | | 125 | 70.34 ± 0.42 | |
| | | 62.5 | 32.64 ± 0.64 | |
| | | 31.25 | 19.06 ± 0.46 | |
| | | 15.6 | 6.78 ± 1.14 | |
| | | 7.8 | 1.42 ± 0.64 | |

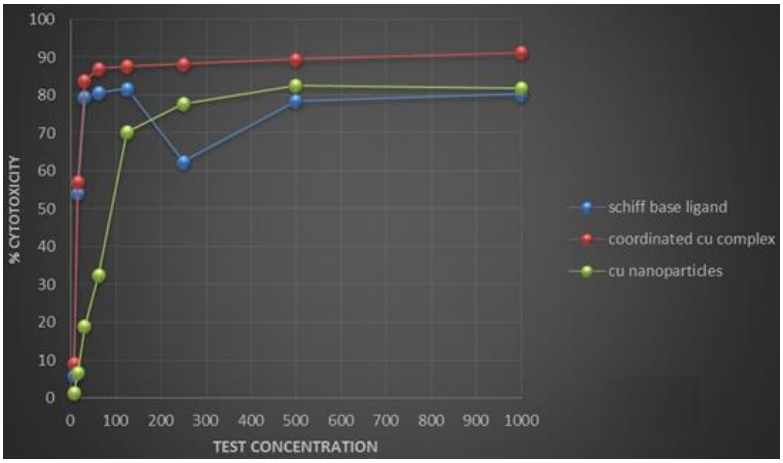



*Fig. 12Cytotoxic properties of compounds against A431 skin cancer cell lines.*

### 3.11. DNA Fragmentation study of synthesized Compounds

Recent research studies reported the significant applications of metal complexes and metal sulphide nanoparticles for DNA binding and DNA cleavage activities. So, we have investigated the DNA fragmentation study of compounds against human skin cancer cell lines A-431 via MTT assay in which the cell viability was measured based on the mitochondrial dehydrogenase enzyme activity. In this method, compounds were dissolved in dimethylsulphoxide solvent and blank samples containing the same volumes of DMSO were taken activity of solvent. In this cytotoxicity experiments, the results were analyzed by means of cell viability curve and expressed with CTC (µg/ml) values as shown in figure-13. A-431 cell lines were exposed to different concentrations of compounds ranging from 1000µg/ml to 7.8µg/ml in order to determine the percentage growth inhibition on A431 cells. Schiff base ligand, coordinated Cu(II) complex and CuS nanoparticles exhibited a $CTC_{50}$ value of 52 ± 0.82 µg/ml, 60.28 ± 0.94 µg/ml and 61.29 ± 0.96 µg/ml on A-431 cell lines respectively.

*Table. 6. DNA Fragmentation studies of synthesized compounds against human skin cancer cell line A-431.*

| S. No. | Compounds | Test Concentration (µg/ml) | % Cytotoxicity | $CTC_{50}$ (µg/ml) |
|---|---|---|---|---|
| | $[C_{36}H_{34}N_{14}S_2O_4]$ Schiff Base Ligand | 1000 | 83.24 ± 0.1 | 52 ± 0.82 |
| | | 500 | 73.86 ± 1.2 | |
| | | 250 | 60.59 ± 1.9 | |
| | | 125 | 54.27 ± 0.6 | |
| | | 62.5 | 50.64 ± 0.2 | |
| | | 32.25 | 26.60 ± 1.08 | |
| | $[Cu(C_{36}H_{34}N_{14}S_2O_4)]$ $Cl_2$ Coordinated Cu (II) Complex | 1000 | 94.24 ± 0.4 | 60.28 ± 0.94 |
| | | 500 | 84.98 ± 1.4 | |
| | | 250 | 66.60 ± 1.4 | |
| | | 125 | 56.28 ± 0.8 | |
| | | 62.5 | 52.62 ± 0.6 | |
| | | 32.25 | 27.60 ± 1.10 | |
| | CuS Nanoparticles | 1000 | 96.26 ± 0.6 | 61.29 ± 0.96 |
| | | 500 | 86.98 ± 1.2 | |
| | | 250 | 68.70 ± 1.6 | |
| | | 125 | 60.21 ± 0.9 | |
| | | 62.5 | 56.68 ± 0.8 | |
| | | 32.25 | 30.60 ± 1.12 | |

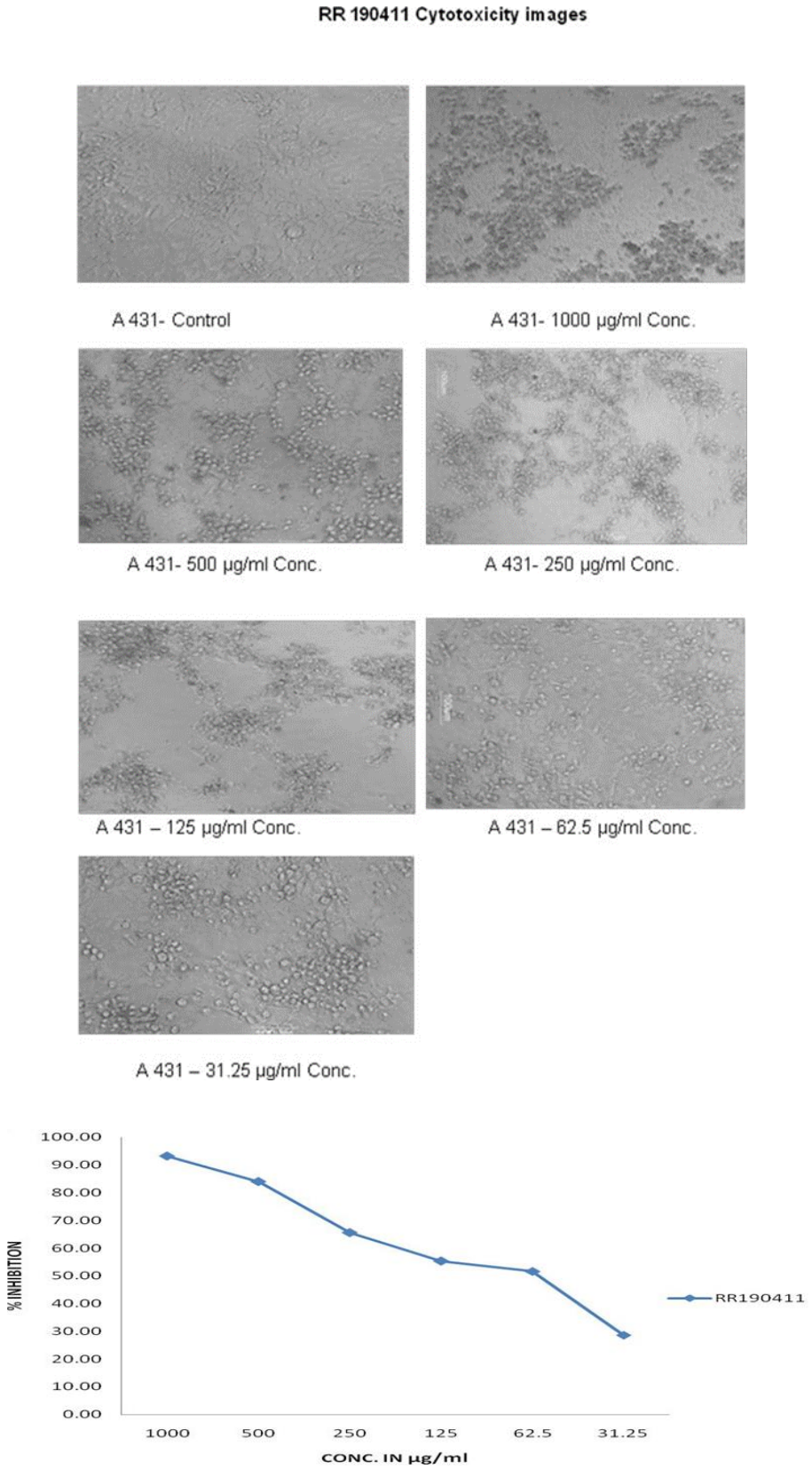



*Fig. 13. DNA Fragmentation studies of Compounds.*

## 4. Conclusion

Highly reactive Cu S nanoparticles were synthesized using hydrazinecarbothioamide Cu (II) complex through a single route molecular precursor source. From various spectroscopic, structural and morphological studies of synthesized compounds it has been observed that the shape and size of the CuS nanoparticles totally depends upon the concentration of schiff base ligand and its coordinated Cu(II) complex. The application of microwave radiations helps to decompose Cu (II) complex into spherical shaped CuS (II) nanoparticles very fast and reduces the reaction time.

This is a simple, very efficient, fast, ecofriendly and inexpensive method for the synthesis of CuS nanoparticles. The use of electromagnetic radiations play a very important role in the preparation of CuS nanoparticles through reduction in reaction time, reducing minimum possibilities of side reactions and properly executed the conversion of very fine quality of CuS nanoparticles in a very short time.

This ecofriendly procedure has very strong potential to be utilized for the large-scale production of CuS nanoparticles. From the results of pharmacological activity of compounds, it has been observed that the CuS nanoparticles can be consider as a good drug candidate for treating various diseases like anticancer, anti-inflammatory. We hope that the observed results clearly demonstrated that the CuS nanoparticles showed better results against human skin cancer cell lines as compared to precursors and in future they will become a novel antitumor agents for skin cancer treatment in human.

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