

Green synthesis of cobalt sulphide nanoparticles using synthesised cobalt (II) complex as a single route intermediate

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Nanotechnology is increasing at a very fast rate due to its many possible applications in the biomedical, industry, pharmaceuticals, commercial and their areas. In this paper, we have reported the biosynthesis of chalcogenide nanostructured pharmacologically active cobalt sulphide nanoparticles (CoS) using 3-ethylidene oxopropanamide thiosemicarbazone Co(II) chloride complex. The synthesised cobalt (II) complex of schiff base ligand was reacted with freshly prepared aqueous leaves extract of *Nyctanthes arbour tristis* (harsingar leaves) for synthesis of CoS nanoparticles. The biofabrication of CoS nanoparticles is a very simple, efficient, fast, eco-friendly and inexpensive method. In this method we use the aqueous environment for green synthesis of CoS nanoparticles. The use of aqueous medium plays a very important role in reducing reaction time, reducing minimum possibilities of side reactions and proper execution in conversion of very good quality of nanoparticles in a very less time. The synthesized compounds like schiff base, Co (II) complex and CoS nanoparticles were analyzed through various structure, morphological, electronic, vibrational and pharmacological characterizations. Powdered x-ray diffraction studies confirm the formation of well defined equispaced crystalline nanoparticles. Transmission electron microscopy and FESEM microscopy exhibit rod like structures of CoS nanoparticles with an average particle size of 56 nm. Sharp electronic absorption band at 280 nm indicates the synthesis of good quality CoS nanoparticles. The FT-IR spectral studies confirmed the presence of Co –S stretching, N-H bending and C=N stretching vibrations in Cobalt complex of Schiff base ligand. The thermal analysis of cobalt complex was performed to investigate the thermal stability of complex. The cobalt complex was stable up to 300°C. The effective results of all pharmacological activities like *in vitro* antimicrobial, antioxidant and anti inflammatory activities explained the presence of strong electron withdrawing and electron releasing functional groups are present in schiff base ligand and its Co (II) complex. The biofabrication of CoS nanoparticles via aqueous extract of fresh leaves of *Nyctanthes arbour tristis* in proper stoichiometric ratio is a good method for synthesizing highly efficient bioactive agents which can be considered as a good drug candidate for various biological applications in future for mankind.

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Keywords: Cobalt complex, Cobalt sulphide nanoparticles, Bio fabrication, Structural and morphological characterization, Pharmacological activities

1. Introduction

In material Science bio fabrication of nanoparticles from plant parts has gained great attention because this is a reliable, sustainable and eco friendly protocol for synthesizing a wide range of nanomaterials including metal / metal oxides / metal sulphides nanoparticles, hybrid materials and bioinspired materials because green synthesis is regarded as an important tool to decrease the toxic effects associated with the classical methods of synthesis of nanoparticles in laboratory and industry. Transition metal Chalcogenides have received great attention during the last two decades because of their specific physical, chemical and biological properties including their optical, magnetic and catalytic properties.

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Different stoichiometric composition of cobalt sulphide nanoparticles have received great attention due to their physical chemical, biological, electronic and optical properties and their wide application in catalyst (1-3), electro chemical super capacitors (4-6), and Li ion batteries (7-10). Better quality, uniformly dispensed, highly crystalline nanoparticles of cobalt sulphide were prepared by using various physical and chemical pathways (11-14) such as sol-gel, dipping, electro deposition process, chemical vapour deposition method (CVD), spray pyrolysis, inert gas condensation process and solvo thermal process such as nanotubes (15,16), nanowires (17), hollow sphere (18,19). The study has shown that the reaction time, temperature and concentration of the reactants have a major effect on the morphology and size distribution of nanoparticles (20). However, there are quite a less reports on the synthesis of cobalt sulphide nanoparticles by the bio fabrication process via green synthesis of nanoparticles. Top down classical methods for synthesis of nanoparticles required for large scale production of nanomaterials for industries have an extremely harmful environmental impact. (21). As a solution to environmental pollution problem, the use of aqueous extract of plant parts is being reported by various researchers. It involves the bio fabrication of coordinated transition metal(II) complexes containing both the metal ions in +2 oxidation state and chalcogen atoms such as N, S and halogen. In this method, metal ions and heteroatoms both are present within the same macromolecular complex. The use of plant extracts has a various number of eco friendly advantages over classical methods like simple green synthesis of nanoparticles is very efficient, fast, less time consuming reducing minimum possibilities of side reaction and conversion of very good quality and high yield of nanoparticles in a very less time. Therefore, there are very less possibility of impurities being interfered in the synthesis of CoS nanoparticles. The template synthesis of metal complexes to develop metallic nanoparticles as an intermediate reaction precursor is a very novel concept for synthesis of CoS nanoparticles because of coordination behaviours of transition metal complexes in +2 oxidation state. Transition metal oxides have excelled in a range of applications, including optical sensors, energy storage, photovoltaic, resistive memory device, capacitor, Resistive RAM and piezoelectricity. [22-25]. Cobalt aluminate nanoparticles have been created for a variety of applications, including sol gel, hydrothermal, co-precipitation, combustion, chemical vapour deposition, and polymer precursors [26-33].

Now-a-day biofabrication process of synthesis of nanoparticles has been reported as a novel way to produce highly efficient, good quality large scale nano compounds. In the present work we have first time reported the synthesis of CoS nanoparticles using macrocyclic cobalt (II) complex and freshly prepared aqueous extract of *Nyctanthes arbor tristis* leaves as an intermediate source and evaluation of their structural, morphological, electronic, vibrational and biological characterizations.

2. Experimental

2.1. Materials and instruments

Cobalt sulphide nanoparticles were synthesised in the presence of aqueous extract of fresh leaves of *Nyctanthes aubor tristis* plant by the liquid phase synthesis via biofabrication method. Analytical grade chemicals and reagents Cobalt chloride, p- toluidene , diethylmalonate, hydrazine hydrate, p-chloroacetophenone, DMSO, and thiosemicarbazide hydrochloride are obtained from Merck and used as received without further purification . Distilled water was used for all the experiment.

UV-Vis absorption spectra were recorded on a Perkin Elmer UV/Vis Lambda 25 using a 1 cm path length cell with dichloromethane solvent. . The Fourier- transform infrared spectroscopy (FT-IR) spectra (KBr pellets) were recorded using a Perkin Elmer FT-IR spectrometer with the wavelength range from 400 to 4000 cm^{-1} . Melting points of compounds were obtained by an electro thermal melting point apparatus and were not corrected. The elemental analysis was conducted using CHN Analyser, Thermo –Flash EA-1112 Series at the temperature up to 900° C and vanadium penta oxide (V_2O_5) was used as an oxidizer to prevent inhibition caused by sulphur element. Thin Layer Chromatography (TLC) was performed using n-hexane/EtOAc (1:3) as an

eluent. X-ray measurements for the ligands and their Cobalt (II) complexes were performed at room temperature using a Bruker axis D8 using CuK α radiation.

2.2. Synthesis of CoS nanoparticles

The whole synthesis process was divided into 6 steps

- Synthesis of Schiff base ligand
- Synthesis of Co (II) complex
- Preparation of aqueous extract of fresh leaves of *Nyctanthes arbour tristis* plant.
- Synthesis of CoS nanoparticles via green synthesis

2.2.1. Synthesis of Schiff base ligand

Schiff base ligand was prepared by the condensation reaction between para-toluidine and diethylmalonate in 1:1 molar ratio. The process of synthesis of Schiff base ligand can further be divided into steps.

Step –I Synthesis of Ester

An equimolar amount of para-toluidine and diethylmalonate were taken in round bottom flask in 1:1 molar ratio. Shake the solution vigorously and heat the mixture for 30 minutes at 70°C. Cool the solution and add 30 ml ethanol in the mixture. After continuous stirring of mixture for 30 minutes, colourless suspensions is obtained then pour this solution into 100 g ice with continuous stirring. After vigorous stirring of contents for 2 hour, a white crystalline precipitate is obtained.

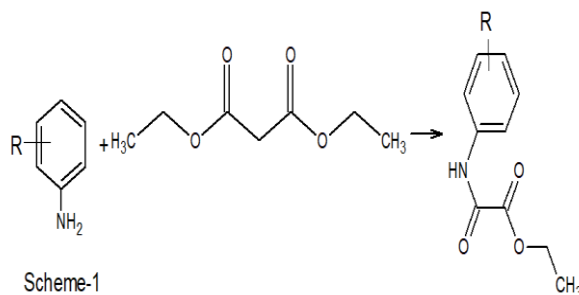


Fig. 1. Synthesis of Ethylmalonilate.

Step-II Synthesis of malonanilic acid hydrazide

Hydrazine hydrate (10ml) was added slowly in the mixture of Ethylmalonilate and ethanol with continuous mechanical stirring for about 1 hour. After vigorous stirring of mixture off white precipitate was separated, the contents were left overnight. After 24 hours ageing process, white crystalline product separated out. The scheme for the synthesis of malonanilic acid hydride is shown in figure.

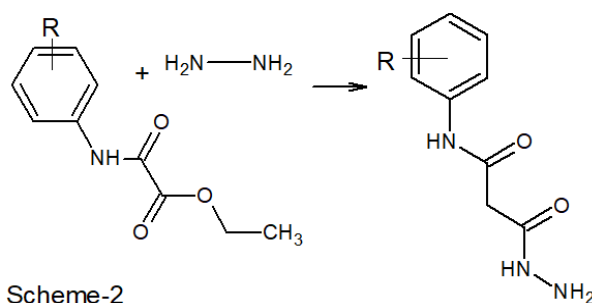


Fig. 2. Synthesis of malonanilic acid hydrazide.

The crystalline product was filtered off, washed with ethanol and then air dried. The crude product was recrystallized from absolute ethanol and dried in vacuum desiccators over P_4O_{10} . The yield was calculated by simple weighing method and found to be 68%. The melting point was calculated using melting point apparatus is 186°C .

Step – III Synthesis of Malonanilic acid hydrazone

Firstly, malonanilic acid hydroxide (0.01m, 2.96 g) was dissolved in 50 ml ethanol at temperature of 50°C then, an equimolar amount (0.01m, 1.26g) of *parachloroacetophenone* was added in hot absolute ethanol (30ml) in separate round bottom flask. Finally, both the solution are mixed with continuous mechanical stirring at 60°C for $\frac{1}{2}$ hr then mixture was refluxed at 70°C for 6 hr. After 6 hrs of refluxing the Product was cooled at room temperature. After 24 hours ageing product was filtered, washed with absolute ethanol, recrystallized from absolute ethanol and dried over silica gel.

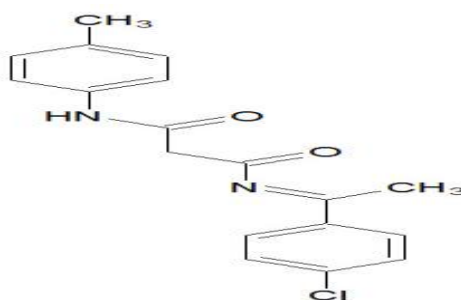


Fig.3. Synthesis of malonanilic acid hydrazone via acid hydrazone

Step –IV Synthesis of Co (II) Complex

Synthesised hydrazone (1mM) was dissolved in ethanol (40 ml) and this mixture was added gradually in a ethanolic mixture of thiosemicarbazide hydrochloride and Cobalt (II) chloride salt which was prepared by dissolving (0.1mM) of the Co (II) salt and thiosemicarbazide hydrochloride in 50ml of ethanol with continuous mechanical stirring at 80°C for 8 hrs. Brown solid precipitate formed which was filtered off and washed throughly with absolute ethanol.

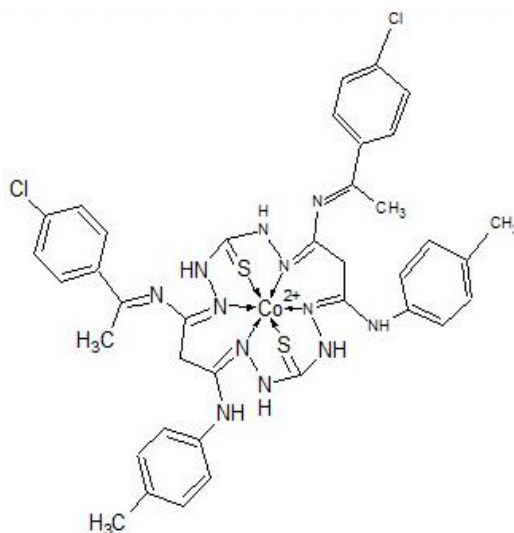


Fig.4. Synthesis of Co (II) Complex from malonanilic acid hydrazone

Step V -Biofabrication of CoS nanoparticles

For obtaining CoS nanoparticles, 1 g of Co (II) complex of Schiff base ligand was dissolved in 30 ml of DMSO in a round bottom flask then add 100 ml of aqueous extract of fresh leaves of *Nyctanthes arbour tristis* plant with continuous stirring at room temperature for 30 minutes and then was kept for aging for another 30 minutes. The final solution was heated in microwave oven for 5 minutes with 800W Power microwave resulted immediate formation of CoS nanoparticles. The resulting solution containing CoS nanoparticles was then cooled, centrifuged, and washed with absolute methanol several times.

2.3. Structural Morphological Characterization of Compounds

Electronic characterization CoS nanoparticles were collected and characterized by various spectroscopic, structural and morphological characterization techniques like FTIR, UV- Visible, XRD, Particle size analysis, TEM and SEM. The Structure and Particle size of CoS nanoparticles were characterized by a Bruka axis D8 Phase X-ray diffractometer (XRD) using Cu K α radiation ($\lambda = 1.540 \text{ \AA}$). In the 2θ ranging from 20 to 80°. TEM and HRSEM micro graphs were obtained by TECHAI G2F20 operated at 300 KV using a drop of Suspension of the sample in ethanol on carbon coated copper grid. Electronic spectra of CoS nanoparticles on quartz in the range of 200-900 nm were obtained using Parkin Elmer Lambda 25 spectrophotometer.

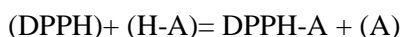
2.4. Evaluation of invitro antibacterial efficiency of synthesized compounds

Procurement of MTCC cultures of bacteria from PGI Chandigarh which are *E.Coli* (MTCC-1687), *E.faecalis* (MTCC-439) and *S.aureus* (MTCC-737) and indigenous Methicillin Resistant *S.aureus* isolates was used. Stocks of experimental compound of concentration of 10mg/ml were prepared in DMSO passed through 0.22 mm dissociable syringe filter aseptically for sterilization followed by preparation of successive 5 dilutions using sterile distilled water. For antibacterial activity nutrient agar media plates were prepared for working with bacteria. Bacterial inoculum was taken from broth of revived cultures using sterile swab and seeded onto the nutrient agar media followed by punching of 5 wells of 6mm diameter. 10 microliter of different dilution of each compound were poured into each 5 different wells of pre-inoculated culture plates separately with different microbial species. The culture plates were incubated at $37 \pm 1^\circ\text{C}$ for 24hrs respectively. Observations were taken in the form of zone of inhibition (in mm) after incubation.

2.5. Evaluation of In-vitro Antioxidant Activities of compounds

The radical scavenging activity of different samples was determined by using DPPH assay according to Chang et al., (2001). The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO) was used as reference.

Principle: 1,1Diphenyl 2- Picryl Hydrazyl is a stable (in powder form) free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,



Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Reagent preparation: 0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

Working procedure: Different volumes (2 - 20 μl) of plant extracts were made up to 40 μl with DMSO and 2.96 ml DPPH (0.1 mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 min. After 20 min, the absorbance of the mixture was

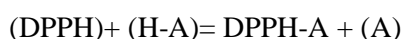
read at 517 nm. 3ml of DPPH was taken as control. The % radical scavenging activity of the plant extracts was calculated using the following formula,

$$\%RSA = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

where; RSA is the Radical Scavenging Activity; Abs control is the absorbance of DPPH radical +ethanol; Abs sample is the absorbance of DPPH radical +sample drug

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Working procedure: Different volumes (2 - 20 μ l) of plant extracts were made up to 40 μ l with DMSO and 2.96 ml DPPH (0.1 mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. The % radical scavenging activity of the plant extracts was calculated using the following formula,

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2.6. Evaluation of In-vitro Anti-Inflammatory Activities of compounds

Following are the two methods utilized for evaluation of anti-inflammatory activity of given samples Inhibition of albumin denaturation The anti-inflammatory activity of samples are was studied by using inhibition of albumin denaturation according technique described by Mizushima et al., (1968) and Sakat et al., (2010) with suitable modifications. The reaction mixture consists of test sample or extracts and 1% aqueous solution of bovine albumin fraction, maintaining the neutral pH of the reaction mixture. This is followed by incubation at 37°C for 20 min and then heated to 50° C for 20 min, after cooling the samples; the turbidity was measured at 660nm antiproteinase action. The activity was performed refereeing to the methods suggested by Oyedepo & Femurewa (1995); Sakat et al., (2010) with suitable modifications. 2 ml of reaction mixture containing 0.06 mg trypsin, 1 ml 20 mM Tris-HCl buffer (pH 7.4) and 1 ml test sample of different concentrations (100 - 500 μ g/ml). The mixture was incubated for 5 min at 37°C followed by addition of 1 ml of 0.8% (w/v) casein and again followed by second incubation of 20 minutes. Then the reaction was stopped by adding 2 ml of 70% perchloric acid. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. Both the experiment were performed in triplicate. The percentage inhibition of protein denaturation in both experiments was calculated as following formulae.

$$\text{Percentage inhibition} = \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs control}} \times 100$$

3. Results and discussion

This research work reports the bio fabrication of cobalt sulphide nanoparticles from synthesized Cobalt (II) complex using aqueous extract of *Nyctanthes arbor tristis* linn leaves which was free from any impurity and does not require continuous stirring of reaction mixture. The appearance of colored particles indicates the formation of crystalline cobalt sulphide nanoparticles.

3.1. Phytochemical screening of aqueous extract of *Nyctanthes arbor tristis* linn leaves

In order to understand the presence of various phytochemical constituent in aqueous extract of *Nyctanthes arbor tristis* linn (harsingar) leaves, the extract was subjected to qualitative phytochemical screening. For this, some specific functional group tests were performed to check the availability of bio molecules like carbohydrates, proteins, flavonoids, alkaloids, glycosides, steroids etc.

Table 1. The qualitative estimation of phytoconstituents present in *Nyctanthes arbor tristis* linn leaf extract.

Serial No.	Phytoconstituents	Availability in Aqueous extract
1.	Flavonoids	+
2.	Alkaloids	+
3.	Glycosides	+
4.	Steroids	+
5.	Phenols	+
6.	Terpenoids	+
7.	Saponins	-
8.	Resins	+
9.	Tannins	+
10.	Cardiac Glycosides	-
11.	Phytosterolsand	+
12.	Carbohydrates	+
13.	Fixedoiland fats	-

3.2. Visual characterization

Synthesized Cobalt (II) complex solution has turned green in color, when freshly prepared aqueous extract of *Nyctanthes arbor tristis* linn leaves was added in the solution with constant stirring which indicated the formation of cobalt sulphide nanoparticles. According to literature, there are three phases of synthesis of metallic nanoparticles via green approach. The first phase is activation phase which indicates metal ion reduction and then their nucleation process starts. The second phase is considered as the growth phase, which involves the aggregation of biosynthesized small metallic nanoparticles. The last phase is the termination phase which facilitates the final shape and geometry of biosynthesized nanoparticles. In the bio-reduction process of cobalt sulphide nanoparticles, crystalline cobalt complex was dissolved in ethanol. Due to ionic nature of cobalt complex, it immediately dissociates into Co^{2+} ions and Cl^{-} ions, when freshly prepared aqueous leaf extract of *Nyctanthes arbor tristis* linn (harsingar) mixed with ethanolic solution of cobalt complex. The various functional groups are present in leaf extract immediately interact with Co^{2+} ions and reduces it to its zero valent state i.e. Co^0 . Which leading to the formation of cobalt sulphide nanoparticles followed by the growth phase, leaving behind the remaining components as by product.²⁸

Green coloured CoS nanoparticles were obtained using aqueous extract of fresh leaves of *Nyctanthes arbor tristis* plant via ultracentrifugation method. Bio fabrication of CoS nanoparticles provides selective and high quality, uniform size, high reaction rate and ecofriendly method. Green Synthesis of CoS helps to deliver the hydration energy into the reaction contents and increases the

speed of reaction and efficiency of conversion of reactants into products. Green Synthesis of nanoparticles reduces the crystallization time of nanoparticles and improves the crystalline pattern or behaviour of the final end product in a very specific route. In our work, biosynthesis of CoS nanoparticles helps to obtain good quality spherical nanoparticles. The short reaction time in biosynthesis of nanoparticles results in increasing the nanoparticles purity by decreasing the unwanted side reactions compared to classical thermal methods.

3.3. Optical Properties

3.3.1. UV-Vis Spectroscopy Study

The optical Properties of synthesised nanoparticles were measured at room temperature using UV-Vis and FT-IR spectrophotometer. Very sharp absorption occurs in the range of 200-700 nm. The formation of sharp absorption spectra of nanoparticles at 260-780 nm indicates the formation of good quality of nanoparticles (51) formed in the process of bio fabrication of CoS nanoparticles using aqueous extract of fresh leaves of *Nyctanthes arbor tristis* plant . A broad absorption peak appeared at 590 nm due to the surface plasma on resonance phenomenon occurred when CoS nanoparticles were prepared from synthesised Co(II) complex via malonanilic acid hydrazone Schiff base ligand. The electronic spectra of Schiff base ligand was shifted from 325 nm to 340 nm after coordination with Co 2+ ion in divalent oxidation state. Due to this shifting in electronic spectra of Schiff base ligand bathochromic shifts was aroused in coordinated Co(II) complex due to back bonding of d electrons from Co 2+ ion to C=N group of Schiff base ligand. This back bonding of d electrons decreased the bond energy of azomethine group of Schiff base ligand.

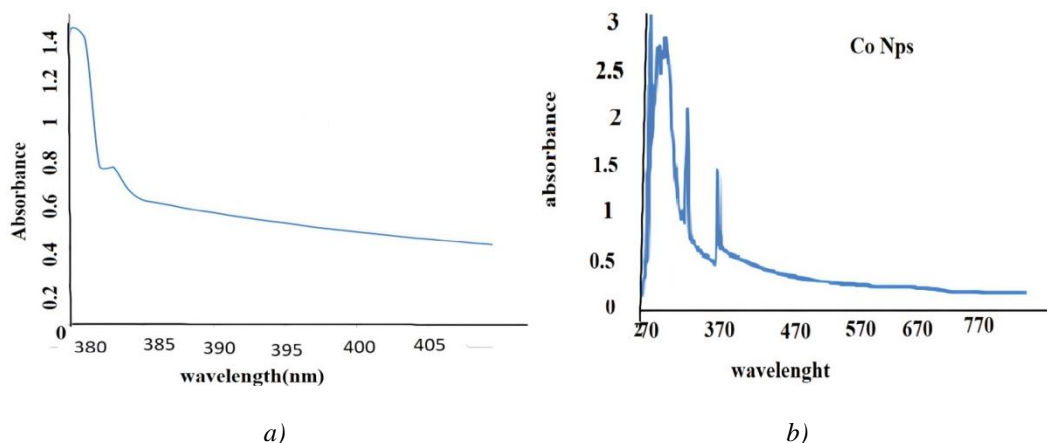


Fig. 5. UV Spectra of Schiff base ligand of a) Cobalt (II) complex and b) CoS nanoparticles.

3.3.2. FTIR Spectroscopy Study

FTIR spectroscopic study of synthesized compounds is a very reliable and accurate spectroscopic technique to identifying the presence of different donor and acceptor functional groups in synthesised compounds. According to FT-IR spectra of Schiff base ligand it is proved that the ligand showed keto-enol isomerism. In the keto form ligand coordinated with Co 2+ ion via azomethine nitrogen atoms. Due to coordination behaviour of ligand, the IR frequency of Co (II) complex shifts from 1620 to 1680 cm⁻¹ after coordination of Co (II) ion with Schiff base ligand which contains electron donor and electron acceptor functional groups.

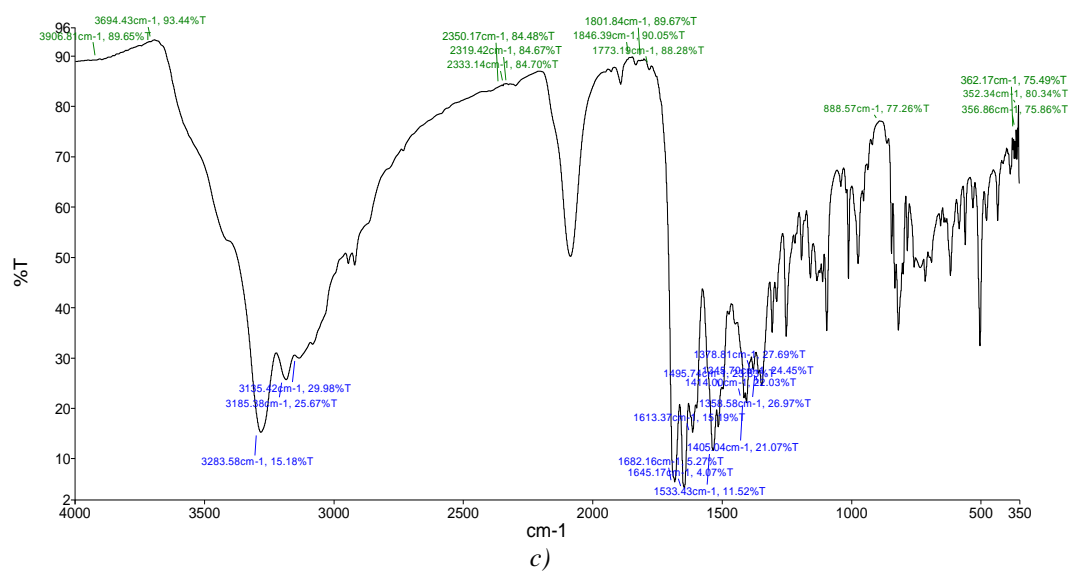
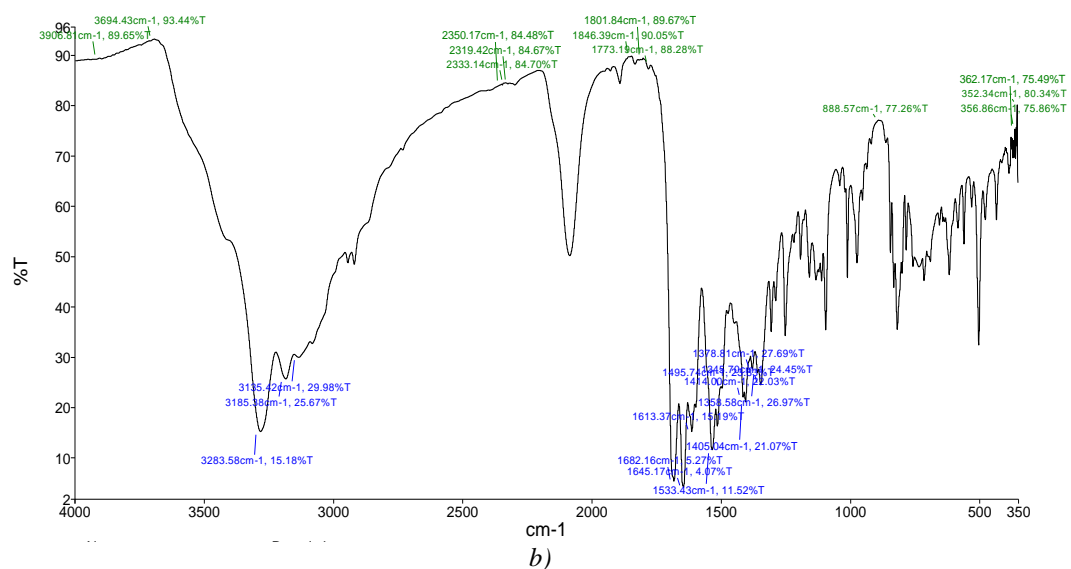
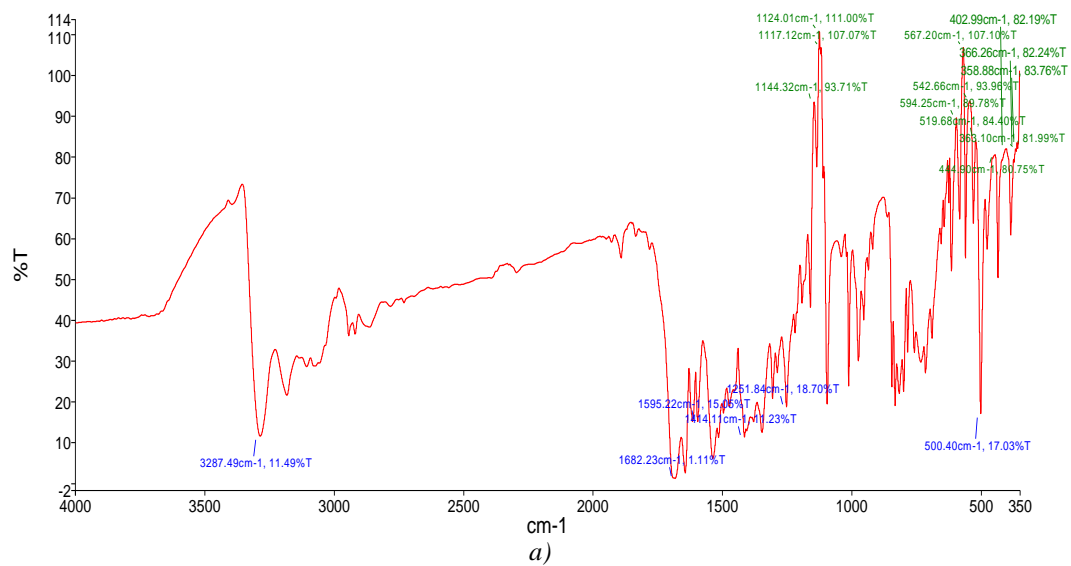


Fig. 6. FT-IR Spectra of Schiff base ligand, Cobalt (II) Complex and CoS nano particles.

3.3.3. Structural studies

The XRD technique is a very important crystallographic technique for the identification of Crystalline nature of materials synthesized via classical method or modern method like Ultrasonication, biosynthesis and microwave assisted synthesis. The structure and particles size of the synthesised compounds were characterized by a Bruker axis D8 phase X-ray diffractometer (XRD). X ray diffractogram for CoS nanoparticles obtained from Co(II) Complex of Schiff base ligand is shown in fig. 5. All peaks are matched with CoS nano structures JCPDS – 00 – 002- 1459 card. Using Cuk radiation ($\lambda = 1.540 \text{ \AA}$) in the 2 θ ranging from 20 to 80. According to JCP DS 00 - 002 – 1459, CoS nanoparticles in spherical phase has been identified. Eight high intensity diffraction at $2\theta = 16.9, 18.7, 28.2, 30.75, 33.4, 38.13, 43.2,$ and 48.1 from 111, 200, 311, 222, 400, 331, 420, 511 planes indicates the nanocrystalline nature of synthesized CoS nanoparticles. The average particle size of CoS nanoparticles was calculated by the Debye- Scherer's Equation (1) is 56 nm

$$D = \frac{0.9 \lambda}{B \cos \theta} \quad (1)$$

where D is the diameter of the nanoparticles λ is the X- ray wavelength is equal to 1.54 \AA and B is the half width of the diffraction peak.

Reflection of XRD Peaks are small in size and broad and indicates the nanosize of CoS nanoparticles.

Because the nanoparticles were synthesized through aqueous extract of fresh leaves of *Nyctanthes arbor tristis* plant are pure therefore no additional peaks are observed. Synthesis of nanocrystalline spherical shape of CoS nanoparticles may be due to the green synthesis of formation of CoS nanoparticles.

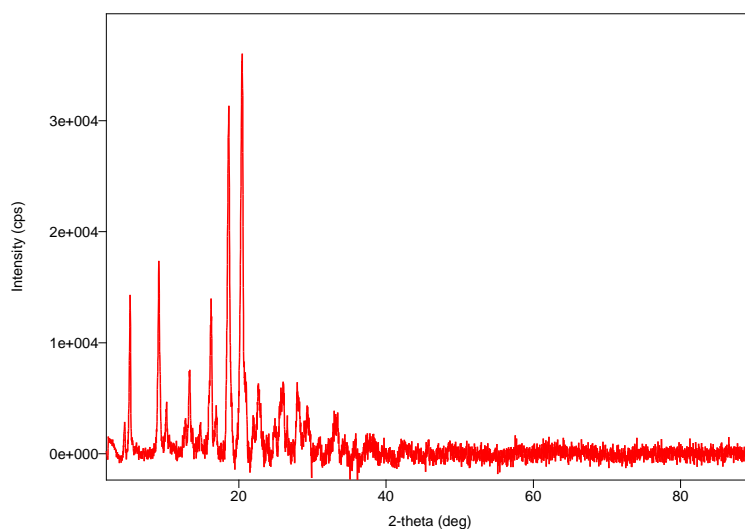


Fig. 7. XRD Spectra of Cobalt Sulphide nano particles.

3.3.4. Morphological studies

Transmission electron micrograph of CoS nanoparticles prepared from cobalt (II) complex which was obtained by Schiff base ligand is shown in figure 6. Spherical shape nanoparticles are formed from cobalt complex. The thickness and radius of nanoparticles are estimated using Gatan Software. Reduced FFT shown in figure indicates high crystalline behaviour of synthesised CoS nano particle and clear occurrence of lattice fringes in CoS nano particles. The presence of dark and bright fringes confirms the formation of very good crystalline structure of nanoparticles.

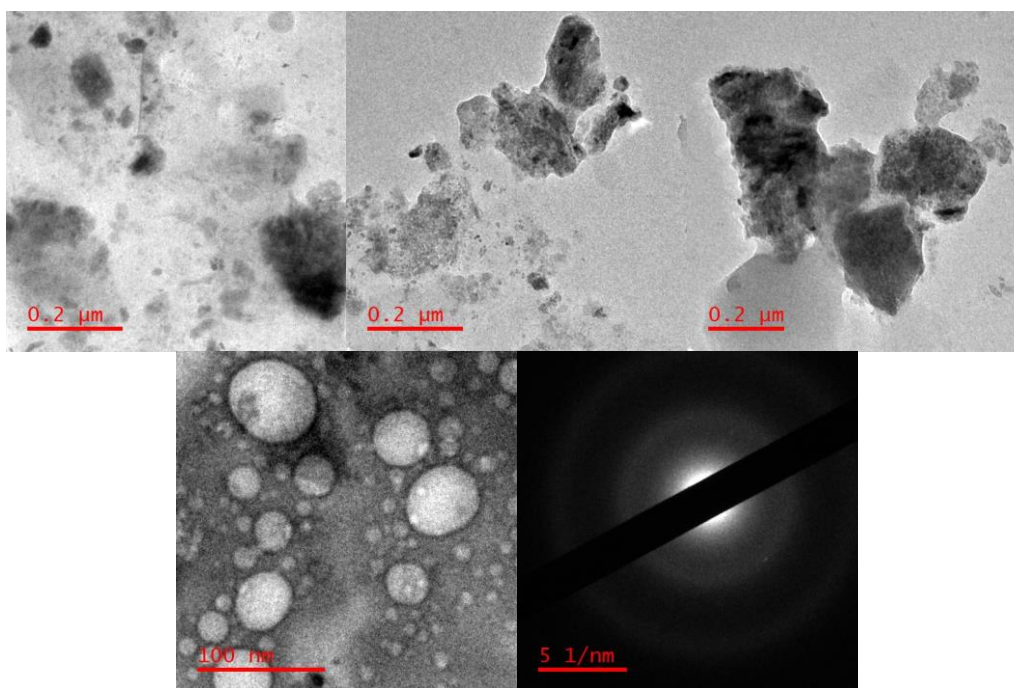


Fig. 8. TEM images of CoS nanoparticles.

EDS studies of Co (II) complex confirms the presence of C,N,S and Co elements in the compound. The other impurities like Cl, Ca elements were also found due to interaction of complex with solvents during refluxing and recrystallization process for purification of compound for further utilized in the synthesis of CoS nanoparticles via green synthesis method. TEM micrographs showed that CoS nanoparticles were not accumulated in clusters but they were separated by equal space which was proved by microscopy visualizing under the high resolution microscope.

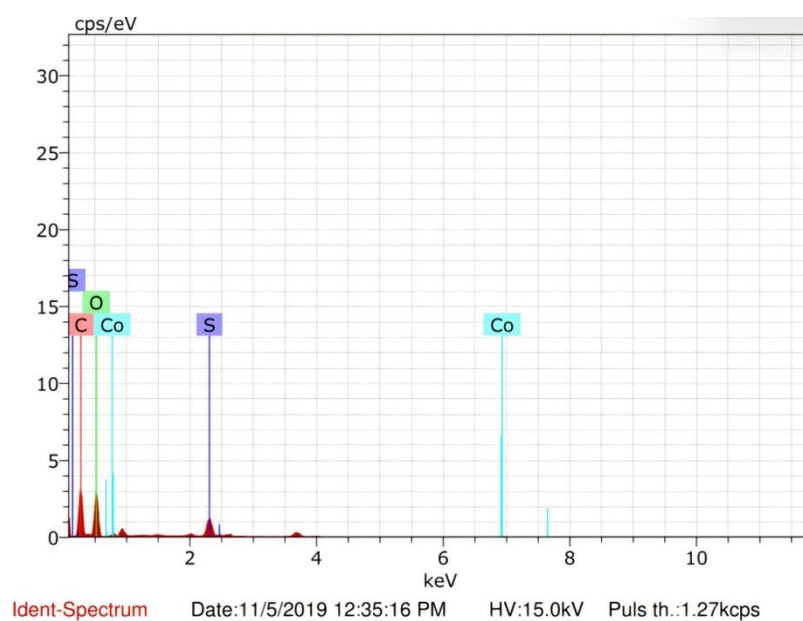


Fig. 9 EDX Spectra of Cobalt (II) complex.

Table 2. EDX Spectra.

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom.C [at.%]	Error (3 Sigma)
Carbon	K-series	45.92	59.96	73.48	27.73
Oxygen	K-series	16.74	21.86	20.11	14.29
Sulphur	K-series	2.46	3.21	1.47	0.54
Calcium	K-series	4.37	5.71	2.10	0.85
Chlorine	K-series	3.04	3.98	1.65	0.63
Cobalt	K-series	4.04	5.28	1.19	2.00
Total		76.58	100.00	100.00e	

Scanning Electron Microscopy

Scanning electron microscope micrographs explains well dispersed, versatile and oval shape of biosynthesized CoS nanoparticles when fresh leaf extract of *Nyctanthes arbor tristis* plant added to the ethanolic solution of Co(II) complex which was synthesised from Schiff base ligand in the Presence of thiosemicarbazide hydrochloride solution . The presence of solvent does not change the shape of nanoparticles but its presences initiate the process of nucleation process of synthesis of CoS nanoparticles. Mostly in case of higher concentrations of synthesised Co (II) complex. SEM micrographs revealed that the synthesised CoS nanoparticles were assembled in a very open and quasi linear structure as compared to dense closely packed assembly.



Fig. 10. SEM micrographs of CoS nanoparticles.

3.3.5. TGA Analysis

The TGA analysis indicates that the graphs of Cobalt (II) complex begin to decompose at 240.16^oC, 251.21^oC & 279.30^oC respectively. Comparison of the decomposition temperature of the compounds shows that Cobalt complex decompose at higher temperature than its ligand The TGA curve for Cobalt complex displays 2.758 mg weight loss within the temperature range of 240-280^oC and exhibit a man loss of 117.26 % .The TGA curve of ligand displays 7.26 mg 6.68 mg & 7.36 mg weight loss within the temperature range.

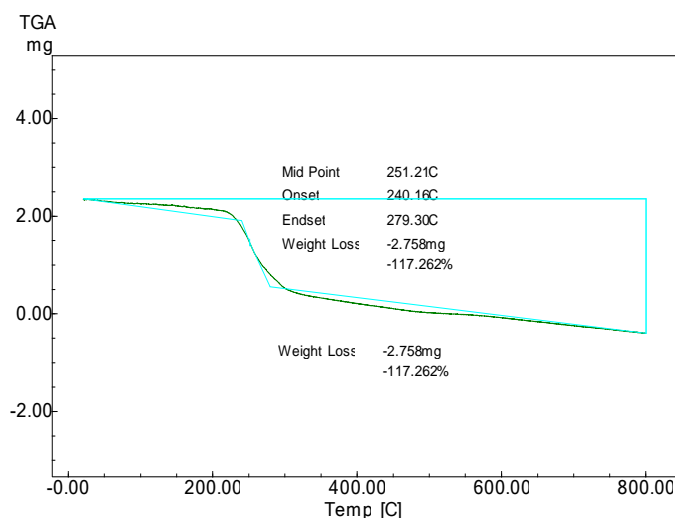


Fig. 11. TGA curve of Cobalt (II) Complex.

3.3.6. Antibacterial activity

In vitro antibacterial efficacy of synthesised compounds were studied by incorporating various concentration of compounds on agar plate that was incubated with 6 $\mu\text{g}/\text{ml}$ 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 same concentrations of control antibiotic kanamycin was used to compare the antibacterial activities of synthesised compounds. The results of antibacterial activities of synthesised compounds showed that the growth of bacteria significantly decreased in case of CoS nanoparticles as compared to Co (II) complex and Schiff base ligand. The results of antibacterial activities of compounds also revealed that all compounds are nontoxic in nature to broad spectrums bacterial species.

Table 3. Inhibition of standard drug Kanamycin against all test microbes.

S. No.	Test Microbes	Diameter of Zone of Inhibition(in mm) at different drug concentration				
		50 $\mu\text{g}/\mu\text{l}$	25 $\mu\text{g}/\mu\text{l}$	12.5 $\mu\text{g}/\mu\text{l}$	6.25 $\mu\text{g}/\mu\text{l}$	3.125 $\mu\text{g}/\mu\text{l}$
1.	E. Coli (MTCC-1687)	11 mm	10 mm	Nil	Nil	Nil
2.	E. Faecalis (MTCC-439)	30 mm	28 mm	25 mm	23 mm	20 mm
3.	S.aureus (MTCC-737)	27 mm	26 mm	24 mm	22 mm	21 mm
4.	M.R.S.aureus (Indigenous)	22 mm	21 mm	19 mm	19 mm	18 mm

Table 4. Results of antibacterial activity of Schiff base ligand, Cobalt (II) complex & CoS nanoparticles.

S. No.	Concentration ($\mu\text{g}/\mu\text{l}$) in Dichloromethane	E. Coli (MTCC-1687)			S.aureus (MTCC-737)			E. Faecalis (MTCC-439)		
		Ligand	Co(II) complex	CoS nanoparticles	Ligand	Co(II) complex	CoS nanoparticles	Ligand	Co(II) complex	CoS nanoparticles
1.	100	10	22	20	15	28	24	09	14	12
2.	50	12	20	18	12	22	20	10	15	16
3.	25	11	21	19	12	16	18	14	20	22
4.	12.5	6.25	14	12	08	18	16	10	12	14
5.	6.25	7.5	17	15	06	12	11	nil	nil	nil

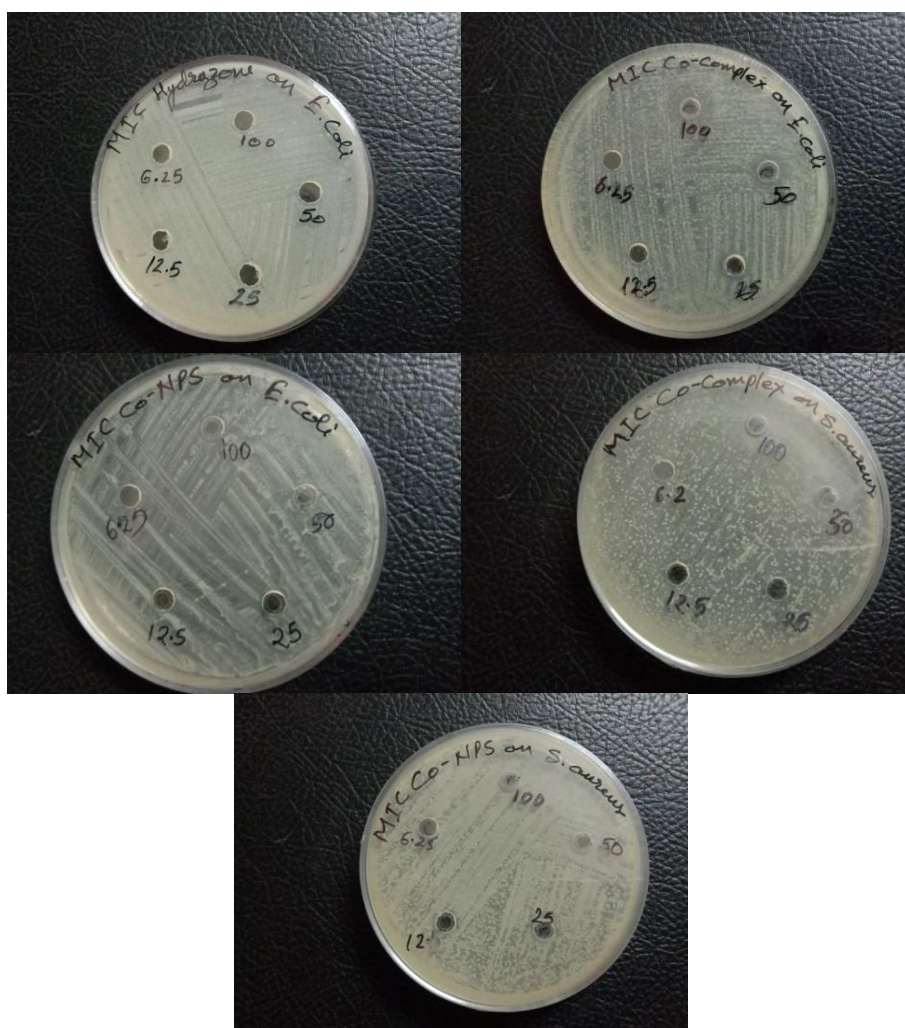


Fig. 12 Antibacterial activity of Schiff base ligand, Cobalt (II) complex & CoS nanoparticles.

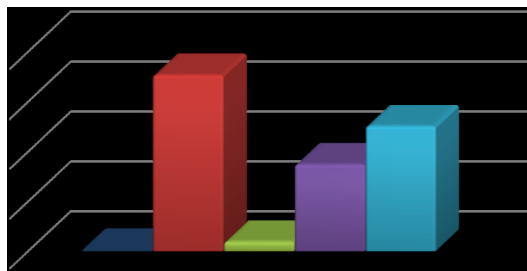


Fig. 13. Graphical representation for Invitro antibacterial activity potential of Schiff base ligand, Cobalt (II) Complex & CoS nanoparticles in comparison to the activity of standard antibiotic Kanamycin

3.3.7. Anti oxidant Activity

With the pairing of electrons in stable radical DPPH after reacting with the reducing agent, it loses its pink colour stoichiometrically depending on the number of electrons accepted by DPPH radical by the antioxidant or reducing agent. The loss of color due to the reaction with sample drug and standard antioxidant ascorbic acid is the indicator of their antioxidant potential which could be measured using UV-Vis Spectrophotometer.

- As a fact bases on the early experiments it is said that the samples with the low IC₅₀ are potent scavenger of free radicles than the samples with high IC₅₀ value which is true for the case of antioxidant potential too. (31). Compound considered in present investigation which was designated as Co(II) Complex is reported to impart free radical scavenging activity by DPPH method thus bears antioxidant potential.

- Results showed that Co(II) Complex was reported to have least IC₅₀ value as 26.83 µg/ml Thus having good anti oxidant potential.

- Although, the antioxidant potential of compound was lower than that of the standard antioxidant Ascorbic acid with IC₅₀ value 21.64 µg/ml with reference to present experiments.

Table 5. Percentage DPPH free radical inhibition activity by standard ascorbic acid at various concentrations.

S.No.	Concentration in µg/ ml	Absorption at 517nm	Percentage inhibition	IC ₅₀
1.	0	0	0	21.63823
2.	20	0.552	42.01681	
3.	40	0.321	66.28151	
4.	60	0.225	76.36555	
5.	80	0.112	88.23529	
6.	100	0.098	89.70588	

Table 6. Percentage DPPH free radical inhibition activity by Schiff base at various concentrations.

S.No.	Concentration in µg/ml	Absorption at 517nm	Percentage inhibition	IC ₅₀
1.	0	0	0	57.83
2.	20	0.568	30.88235	
3.	40	0.489	40.33613	
4.	60	0.325	48.63445	
5.	80	0.223	65.86134	
6.	100	0.658	76.57563	

Table 7. Percentages DPPH free radical inhibition activity by biofabricated Cobalt sulphide nanoparticles at various concentrations.

S.No.	Concentration in µg/ml	Absorption at 517nm	Percentage inhibition	IC ₅₀
1.	0	0	0	54.25
2.	20	0.573	32.882	
3.	40	0.492	43.3362	
4.	60	0.345	49.63446	
5.	80	0.253	68.86134	
6.	100	0.668	77.57683	

3.3.8. Anti-inflammatory activity

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation.

- The samples studied were effective in inhibiting heat induced albumin denaturation up to certain extent.

- The inhibition of cobalt complex was 68.9 % inhibition of albumin protein denaturation at t 100 µg/ml compared to Aspirin drug as standard anti-inflammation drug at same concentration with maximum inhibition 70.7%. in relation to control.

Table 8. Percentage inhibition of proteinase activity of Schiff base ligand against standard aspirin drug.

S.No.	Compounds	Concentration in µg/ml	Absorption at 210 nm	Percentage inhibition
1.	Control	100	0.733	0 %
2.	Aspirin	100	0.216	70.53 %
3.	Schiff base ligand	100	0.406	55 %
4.	Cobalt Complex	100	0.706	68.9 %
5.	Cobalt nanoparticles	100	0.726	59.1 %

4. Conclusion

Highly biologically effective CoS nano particles were synthesized using aqueous extract of fresh green leaves of – *Nyctanthes arbor tristis* plant via Co (II) complex which was obtained from Schiff base ligand of carbohydrazoe. From various structural and morphological studies it has been observed that the shape and size of the CoS nanoparticles depends upon the concentration of synthesized Co(II) complex which was used as Precursor . The use of fresh leave extract of *Nyctanthes arbor tristis* helps to decompose cobalt (II) complex very fast in the spherical shapes CoS nanoparticles within a very short time. This is simple, very efficient, fast, ecofriendly and inexpensive method for the preparation of CoS nano particles. The use of aqueous extract of leaves play a very important role in reducing reaction time, reducing minimum possibilities of side reactions and executed in conversion of very fine quality of CoS nanoparticles in a very less time. This ecofriendly method has very strong potential to be utilized for large scale industrial production of CoS nanoparticles. Form the results of biological activities of compounds it has been observed that the CoS nanoparticle can be consider as a good drug candidate for various biological applications like antibacterial agent, antioxidant agent and anti inflammatory agent in future for mankind.

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