ANTITUBERCULOSIS ACTIVITY OF NEWLY GREEN SYNTHESIZED Zn NANO METAL COMPLEXES

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Abstract

Prediction of antituberculosis activity of newly synthesized zinc (Zn) metal nano complexes from aqueous extract of *Macrotyloma uniflorum* (horse gram). The synthesised nano particles were characterized by UV-visible spectrophotometer and FTIR. The reduced zinc nano particles were analysed with TEM analysis. FTIR spectra confirmed the involvement of diverse functional groups participated. The morphology and size of the zinc nanoparticles were examined by HRTEM analysis, which showed most of the nano particles were nearly spherical with nano range size. The zinc nanoparticles synthesized demonstrated potent antibacterial activity against *Mycobacterium tuberculosis*. The findings conclude that Macrotyloma *uniflorum* (horse gram) aqueous extract bio-reduced zinc micro particle to nano particles, possess potential antibacterial property. It has wide application in medicine and pharmaceutical fields.

Key Words: Anti tuberculosis, Green nano particles, UV & IR spectra, HRTEM, Macrotyloma uniflorum, Mycobacterium tuberculosis

INTRODUCTION:

Nanoparticles are particles between 1 and 100 nanometres (nm) in size with a surrounding interfacial layer. The interfacial layer is an integral part of nanoscale matter, fundamentally affecting all of its properties. The interfacial layer typically consists of ions, inorganic and organic molecules. Organic molecules coating inorganic nanoparticles are known as stabilizers, capping and surface ligands, or passivating agents[1].It is predominantly the nanoparticles extremely small size and large surface area to volume ratio that leads to the significant differences in properties (e.g., biological, catalytic activity, mechanical properties, melting point optical absorption, thermal and electrical conductivity) not seen in the same material at larger scales in their bulk form [2].Non-noble metallic nanoparticles such as iron [3,4],copper [5], zinc oxide [6], and selenium [7] have also been used in medical treatments, cosmetic formulations, and anti-bacterial applications.

Metal nano complexes:The term metal nanoparticle is used to describe nanosized metals with dimensions (length, width or thickness) within the size range 1-100 nm. The existence of metallic nanoparticles in solution was first recognized by Faraday in 1857 and a quantitative explanation of their colour was given by Mie in 1908. for example, silver(I) complexes commonly used as anti-microbial agents, bismuth(III) complexes for anti-ulcer treatments, gold(I) complexes as anti-arthritic agents, gadolinium(III), manganese(II) and iron(III) complexes as magnetic resonance imaging (MRI) contrast agents, technetium (99Tc) and scandium (47Sc) as radiopharmaceutical agents.[8]

Zinc: Zinc is a chemical element with symbol Zn and atomic number 30. It is the first element in group 12 of the periodic table.Zinc is the 24th most abundant element in Earth's crust .The most common zinc ore is Sphalerite (zinc blende), a zinc sulfide mineral. Zinc is a fairly reactive metal that will combine with oxygen and other non-metals. Zinc metal tarnishes in moist air and burns in air to form the white zinc oxide. It is a fair electrical conductor and will react with dilute acids to release hydrogen. Zinc does not react with water. Zinc is a bluish-white, lustrous, diamagnetic metal,though most common commercial grades of the metal have a dull finish. It is somewhat less dense than iron and

has a hexagonal crystal structure, with a distorted form of hexagonal close packing, in which each atom has six nearest neighbours (at 265.9 pm) in its own plane and six others at a greater distance of 290.6 pm[9].The metal is hard and brittle at most temperatures but becomes malleable between 100 and 150 °C.For a metal, zinc has relatively low melting (419.5 °C) and boiling points (907 °C).[10] The melting point is the lowest of all the d-block metals aside from mercury and cadmium; for this, among other reasons, zinc, cadmium, and mercury are often not considered to be transition metals like the rest of the d-block metals are.[10]Sphalerite Fig(1), which is a form of zinc sulfide, is the most heavily mined zinc-containing ore because its concentrate contains 60–62% zinc.

Fig 1: Sphalerite

Tuberculosis: Tuberculosis (TB) is an infectious disease usually caused by the bacterium Mycobacterium tuberculosis (MTB).Tuberculosis generally affects the lungs, but can also affect other parts of the body. The main cause of TB is Mycobacterium tuberculosis (MTB), a small, aerobic, non motile bacillus.

Some chelating agents have been developed for metal intoxication, e.g., water solublephosphine chelating agents are designed for chelating metals such as technetium, rhenium, platinum and gold. Many organic compounds used in medicine do not have a purely organic mode of action; some are activated or bio transformed by metal ions including metalloenzyme, others have a direct or indirect effect on metal ion metabolism. The pharmacological activities of these metal compounds depend on the metal ion, its ligands and the structure of the compounds. These factors are responsible for reaching them at the proper target site in the body. It is known that certain metal ions penetrate into bacteria and inactivate their enzymes, or some metal ions can generate hydrogen peroxide, thus killing bacteria.

MATERIALS AND METHODS:

Horse gram seeds: Horse gram scientifically known as *Macrotyloma uniflorum* is less known. It is widely consumed as staple diet in Southern parts of India.It is rich in iron, protein, calcium, gluten free with no sodium, cholesterol and fat.

SAMPLES:

1. Zn+Benzil+4Fl
2. $ZnCl_2$+4Cl+1,2 Phenyl
3. $ZnCl_2$+4Cl+4Fl

Procedure:

First black coated horse gram seeds were collected and the mature seeds were used in the synthesis.The next step is germination of seeds for which the seeds were washed with $KMnO_4$ solution.After washing the seeds were soaked in distilled water for about 12hours.The seeds were alllowed to germinate for 3 to 4 days at room temperture and they were moistened with distilled water regularly for every 12 hours.The germinated seeds are freeze dried and are grounded to 60 mesh size in acetone to eliminate lipid content.The mixture obtained was filtered through double layer whatmann filter paper number 1.Homogenate was dried in a tray drier overnight at 32˚C.The dry homogenate were further coarsely grounded using motar and pestle and the powder is obtained.The powder is then suspended in the buffer solution.The three buffer solution used are phosphate(PO_4) buffer which consist of [Na_2HPO_4 + NaH_2PO_4 of PH 8.0],Tris HCL(PH 8.0) and deionized water.The mixture (buffer and powder)is spined in 2M NaCl and incubated for 24 hours at room temperature.This

mixture was further filtered and centrifuged at 10000rpm for 8 minutes below 4˚C and the supernatent was collected.

RESULTS:

UV-Visible Spectroscopy:

UV/Visible spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules. Fig 2,3,4 shows the UV-Visible absorption spectra of different Zinc nano complexes used in this study. The UV-Visible absorption spectra of different nano particles are used in this study. A band due to the >C=N chromophore in the spectrum of the compound at 365 nm shifts to a higher wavelength. Such a shift in n- π * transition band is probably due to the donation of lone pair of electrons by the nitrogen. Further, two bands at 240 nm and 300 nm are due to π- π * transitions, these are assigned to the benzenoid ring and (>C=N) band of the azomethine group respectively. The K band π- π * showed a red shift due to the increase in conjugation and the B-band undergoes a hypochromic shift.

Fig 2: Sample 1

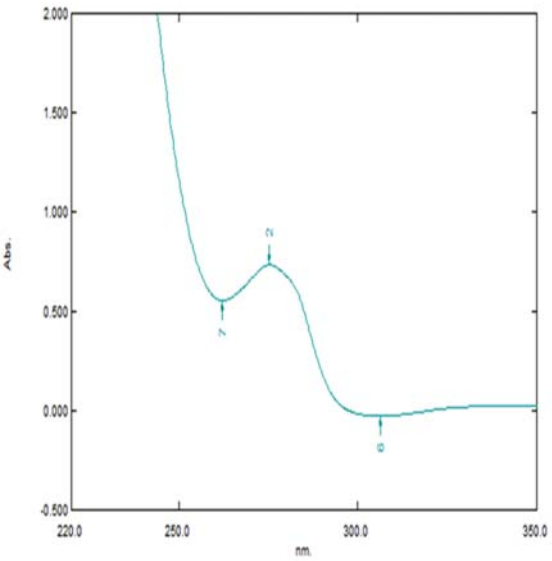


Fig 3: Sample 2

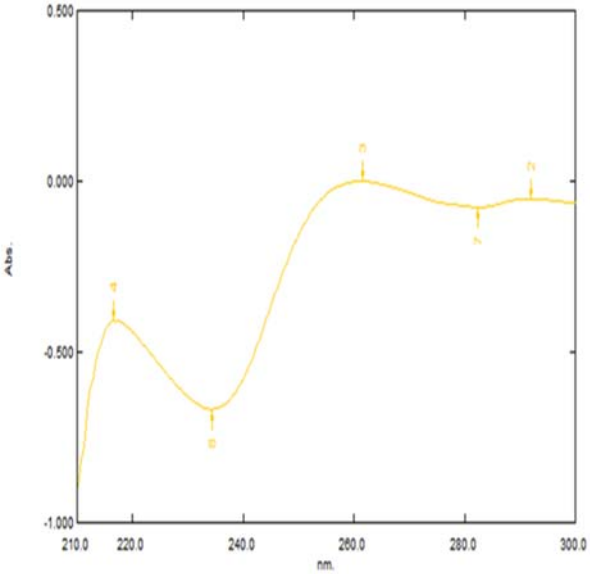
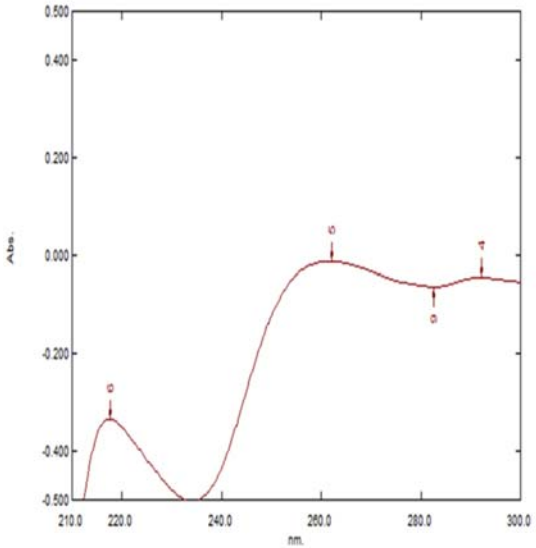


Fig 4: Sample 3



Fourier transforms infrared spectroscopy (FTIR):

Fourier-transform infrared spectroscopy (FTIR) is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range. The goal of any absorption spectroscopy is to measure how well a sample absorbs light at each wavelength. FTIR spectroscopy is conducted to identify the functional groups present on nanoparticles. FTIR Analysis, also known as FTIR Spectroscopy, is an infrared spectroscopy method used to identify organic, polymeric, and in some cases, inorganic materials. In the spectrum, as shown in Fig(5,6,7)there are 3 major peaks observed in which the 1st peak is at 3433cm-1 is for N-H stretch, 2nd peak at 2343cm-1 is for C-H stretch in the aromatic compounds and the 3rd peak at 1633 which is a very weak peak is for C=C aromatic stretch.

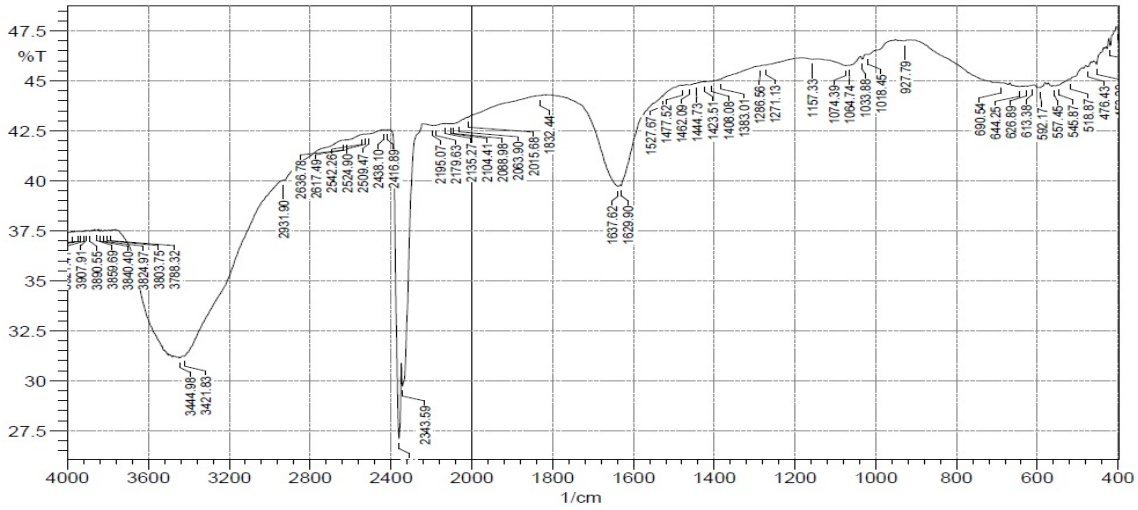


Fig 5: Sample 1

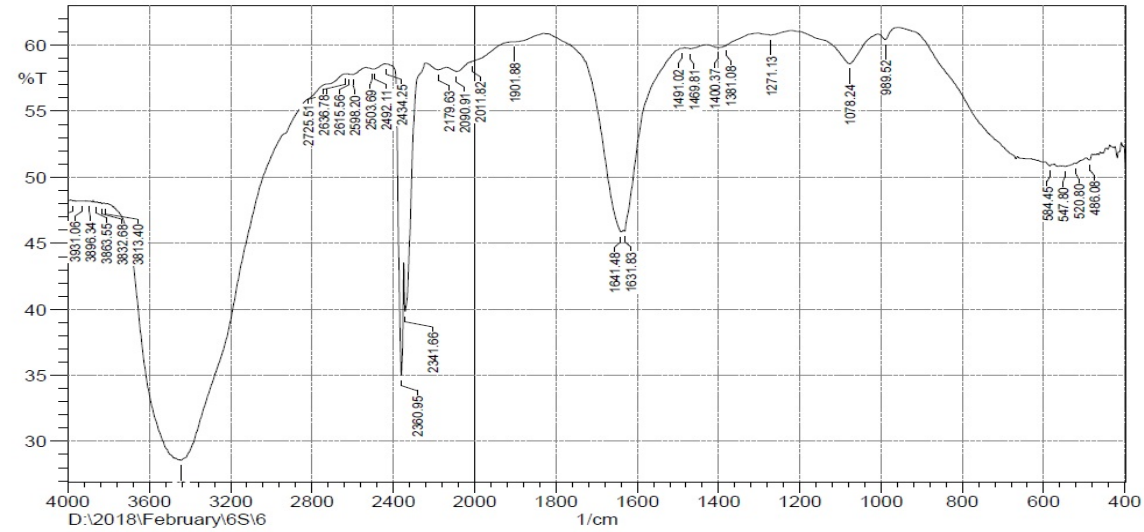


Fig 6: Sample 2

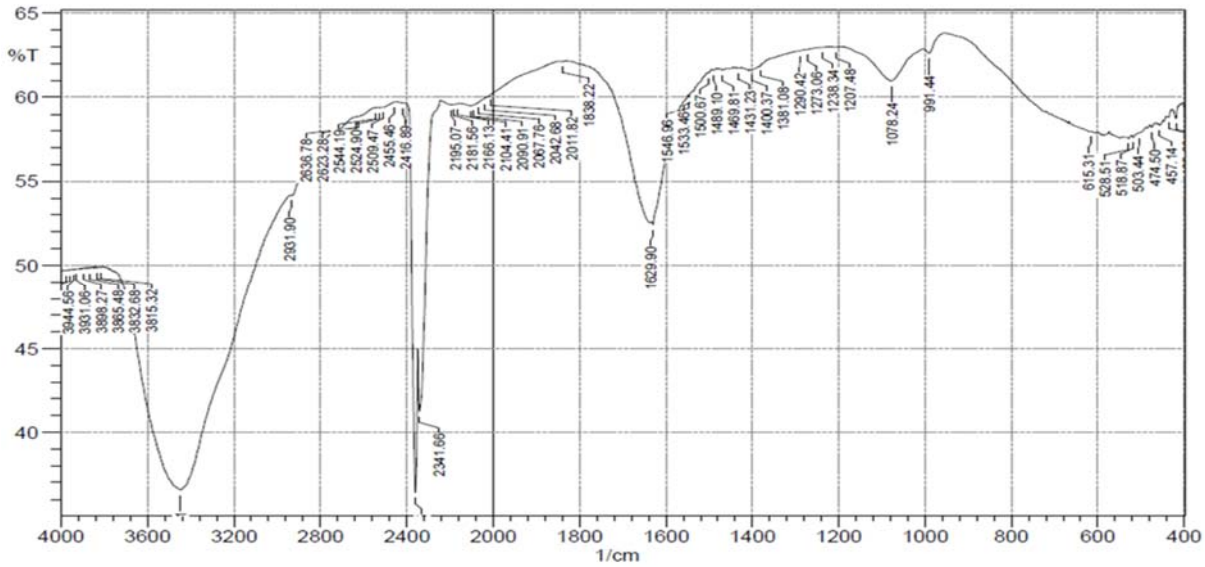


Fig 7: Sample 3

High Resolution Transmission Electron Micrographs(HRTEM):TEM is used primarily to determine particle size and shape. TEM characterization showed a uniform distribution of nanoparticles, with different sizes. Fig 8(A) is the transmission electron micrograph of Zn metal ligand nano complexes coverted by biological route of varying sizes.

The image confirms the nanoparticle formation . Fig 8(B) is the transmission electron micrograph of Zn metal ligand nanocomplexes and the image confirms the nano particle presence at 50nm.

8(A) **8(B)**

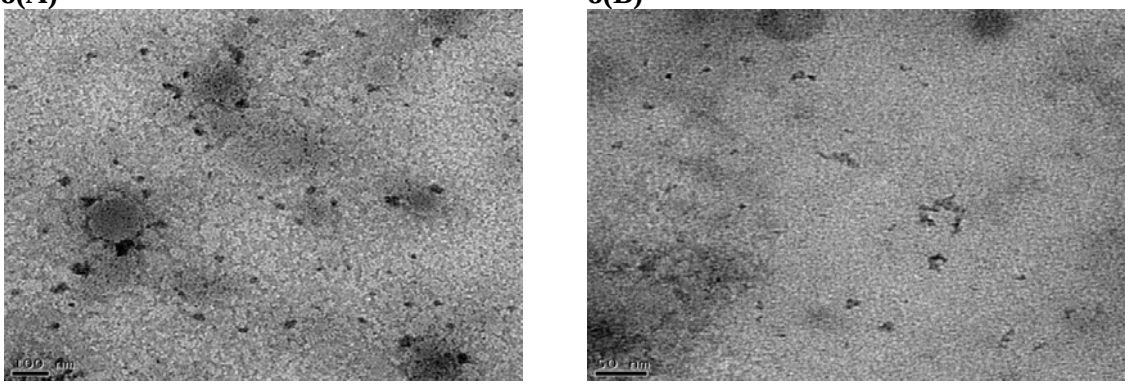


Fig8(A) & 8(B): High Resolution Transmission Electron micrographs of Zn metal nano complexes

ANTITUBERCULOSIS ACTIVITY OF METAL NANO COMPLEXES:

The prepared ligand and their respective metal complexes were tested against *Mycobacteriumtuberculosis*.

The anti-tuberculosis effects of Zinc and Titanium metal nano complexes against *Mycobacterium tuberculosis* H37RV strain (controlstrain),MTBstrain(*Mycobacterium tuberculosis* strain isolated from TB patient) and MDR-TB(The first lined drug resistant strain isolated from TB patient) is evaluated by Minimum inhibitory concentration assay, LED microscopy and biochemical tests like Catalase.

However, Zn metal nano ligands showed better activity than Ti metal nano complexes. As shown in Table 8, Zn metal nano complexes has unpredictable high anti-tuberculosis activity against Mycobacterium tuberculosis (MTB and H37RV strain) and their MIC value is 1000µg/ml .

S.NO	SAMPLE		3 DAYS			5 DAYS			7DAYS			9DAYS			12 DAYS			15 DAYS		
		STRAIN	1000	500	250	1000	500	250	1000	500	250	1000	500	250	1000	500	250	1000	500	250
1	2	H37RV	NT	NT	NT	NT	NT	NT	NT	G	G	NT	G	G	NT	G	G	NT	G	G
		MTB	NT	NT	NT	NT	NT	NT	NT	G	G	G	G	G	G	G	G	G	G	G
		MDR-TB	NT	NT	NT	NT	NT	NT	T	T	T	T	T	T	T	T	T	T	T	T

Fig 9: Activity on different strainsNT=No Turbidity, G=growth, T=Turbidity

COMPLETE REPORT						
S.NO	Drug name	Activity	Concentrations	Smear Microscopy	Biochemical Test	Final Report
1.	2	ACTIVITY	1000ug/ml	NEGATIVE AT 1000ug/ml	NEGATIVE	DRUG ACTIVE AT 1000ug/ml for both the strains H37RV and for MTB

Fig 10:Complete report

Conclusion:

The antituberculosis potential of biogenic Zn metal nano complexes was evaluated. The bio synthesised nano particles were characterized by UV,FTIR and TEM. The bio synthesised Zn metal nano complexes were potential at 1000µg/ml against *Mycobacterium tuberculosis* bacteria. These test compounds and their applications inthe formulation of novel antimicrobial therapeutic drugs seem promising.

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