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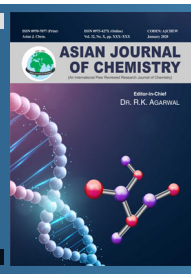

Synthesis, Characterization and Antituberculosis Activity of Biologically Nanostructured Zinc and Titanium Metal Compounds

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Green chemistry was used to obtain nano-range sized titanium and zinc compounds from their macro-sizes by using an aqueous extract of horse gram (*Macrotyloma uniflorum*). Ultraviolet-visible (UV-vis) and Fourier-transform infrared (FTIR) spectrophotometers were employed for characterizing the nanoparticles of biosynthesized metal nanoparticles. Transmission electron microscopy (TEM) was used to analyse the reduced nanoparticles of Ti and Zn metals. Microdilution was employed to determine *in vitro* properties, such as effects of nanocomplex antimicrobials on *Mycobacterium tuberculosis* (MTB) H37RV strain. MTB strains isolated from patients with multidrug-resistant tuberculosis (MDR-TB) were resistant to first-line drugs. Novel synthesized nano-complexes exhibited potential antituberculosis activities. Titanium nanocomplexes exhibited the highest minimal inhibitory concentration (MIC) in comparison to zinc nanocomplex. In a cytotoxic study, an IC_{50} of 1000 µg/mL, for both Ti and Zn nanocomplexes, was reported, and thus, these complexes were non-toxic when compared to isoniazid.

Keywords: *Macrotyloma uniflorum*, Biosynthesis, Antituberculosis strains.

INTRODUCTION

Currently, in advanced biotechnology, a combination of biotechnology and nanotechnology is being used for the formulation, development and application of nanomaterials [1]. The novel properties of nanomaterials, such as their physicochemical, optoelectronic and magnetic characteristics, make them highly advantageous. These properties exist excellent applications due to their surface morphology, shape and size [2-4].

The chemical and physical properties of nanomaterials, such as catalytic activity, thermal conductivity, electrical conductivity, mechanical properties and light absorption, are considerably different from those of macro-sized materials of the same elements, because of a large ratio of surface area to volume and extraordinarily small dimensions [5]. Nanometals, including iron [6,7], copper [8], zinc oxides [9], selenium [10], *etc*. are widely used in drug delivery applications, medication, cosmetics formulation and anticancer and antibacterial activities.

The use of harmful chemicals to prevent colloidal aggregation in the conventional technique employed for nanoparticle fabrication leads to toxicity risk [11]. Non-conservative methods are more beneficial than conservative methods for nanoparticle fabrication [12,13], because green chemistry provides a clean, eco-friendly and various sizes, shapes, and compositions with improved chemical and physical properties [14].

Environmental friendly, safe and inexpensive methods with the use of plants and microorganisms for nanoparticle fabrication have been reported in literature [15,16]. Green technology allows the transformation of metal ions into metal nanoparticles through inherent biochemical processes [17]. Thus, scientists have explored and investigation green routes for nanosynthesis. Plants can hyperaccumulate and biologically reduce metal ions [18]. Plants with hyperaccumulating property have been employed in the green nanosynthesis of metals and detoxification [19]. Generally, specific culture conditions are required for incubation and isolation in other biological-eco-friendly methods that employ microorganisms, *i.e*. fungi and bacteria, and these methods are complicated and expensive.

The use of plants and plant extracts provides the benefits of easy, safe, eco-friendly, inexpensive biosynthesis of nanoparticles in a short time [20]. Furthermore, biosynthesis with

plant extract provides relatively simple and uncomplicated nanoparticle synthesis that can easily be scaled up, which is beneficial for large-scale production [21-23]. Growth conditions, such as light, pH medium, growth supplements, temperature and buffer strength can be optimized to substantially enhance the enzyme activities of microorganisms [24,25]. Active biomolecules, such as phytoproteins, alkaloids, sugars, polyphenolic compounds, phenolic acids and terpenoids, play a crucial role in the stabilization and reduction of nanoparticles biosynthesis [26,27]. An interaction between bioactive molecules and aqueous metal ions provides a diversity of nanoparticle forms and sizes [28,29].

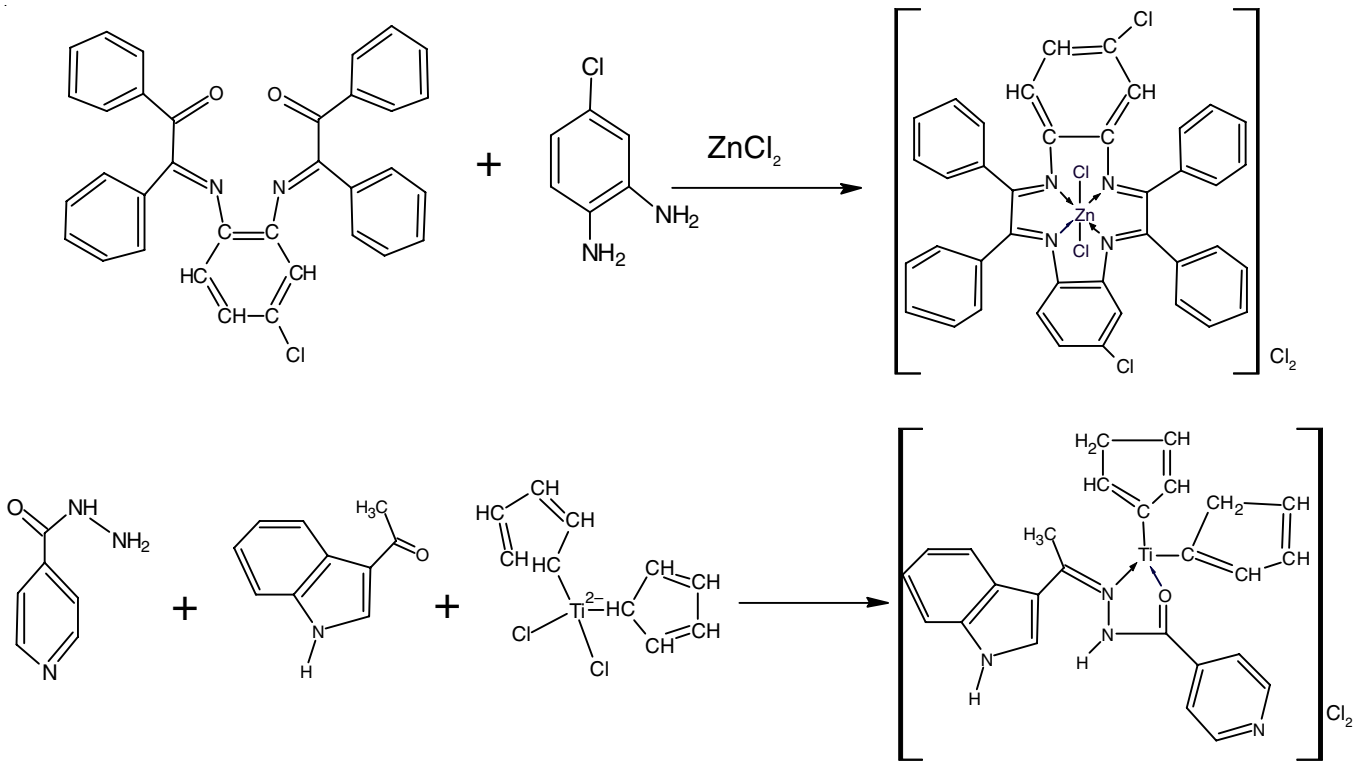
A study on TiO_2 suspensions reported that the suspension functioned as both insecticide and larvicide for *Hippobosca maculate* (hematophagous fly) and *Bovicola ovis* (sheep lice) [30]. TiO_2 nanoparticles fabricated using *Psidium guajava* extract provided antioxidant and antibacterial properties against several pathogens [31-35]. An extract of aloe vera and latex of *Calotropis procera* have been used as stabilizing and reducing agent, respectively, in the spherical ZnO nanoparticle synthesis [36,37]. ZnO nanoparticles have been extensively used in wastewater treatments and food packaging because of their excellent antimicrobial activity [38,39].

EXPERIMENTAL

Template condensation was used for the preparation of Zn(II) macrocyclic complex of *bis*(benzil)-4-chloro-1,2-phenylenediamine by using suitable diamine, *i.e*. two moles of both 4-chloro-1,2-phenylenediamine and benzil in the presence of $ZnCl_2$ to obtain $[Zn(C_{40}H_{32}N_4Cl_2)]Cl_2$ according to the reported procedure [40]. Titanium complex was prepared by condensing *bis*(cyclopentadienyl)titanium(IV) dichloride by using isoniazid (isonicotinylhydrazide) and 3-acetylindole to obtain the macrocyclic compound $[Ti(C_{26}H_{22}N_4O)]Cl_2$.

Synthesis of Zn and Ti nanocomplexes: The mature seeds of black-coated *Macrotyloma uniflorum* (horse gram) were used for the preparation of Zn and Ti nanocomplexes. After germination, $KMnO_4$ solution was used to wash seeds, which were then soaked in distilled water for 12 h approximately. The germinated seeds were ground in acetone for removing lipids present and filtered using a double layer-Whatman filter paper. The obtained mixture was dried overnight at 35 ºC. The dried mixture was triturated to obtain a powder and subsequently, the powder was mixed with a buffer solution. This mixture was stirred after an addition of NaCl and finally was incubated for 24 h at 37 ºC. The incubated mixture was centrifuged for 8-10 min at 10000-12000 rpm and at < 4 ºC, and the obtained supernatant containing enzyme protease was collected. A total of 3-4 mL of collected supernatant was added to 40 mL of 0.01 to 0.02 M metal solution. The resulting solution was stirred and stored at room temperature for approximately 2-3 days (**Scheme-I**). The colour change of solution indicated the transformation of metal complex into nanoparticles [41].

Antimycobacterial activity: The drug activity against two strains of tuberculosis bacteria of nanoparticles of Zn and Ti was tested. *M. tuberculosis* strain used in this study were MTB H37Rv (control strain, which was cultured in Lowenstein Jensen (LJ) medium and incubated for 4 weeks at 37 ºC), MDR-TB (strain isolated from patients with TB) and MTB (strain isolated from patients with TB). To determine the MIC, these strains were cultured in the Middlebrook 7H9 broth liquid and Lowenstein Jensen solid media. Standard drugs used in this study were rifampin, isoniazid, ethambutol and streptomycin. Different concentrations of two aliquots obtained from test



Scheme-I

samples with various dilutions were used to test the growth of MTB strains. Tests were conducted on the 3rd, 5th, 7th, 9th, 15th and 21st day of culture. After incubation, microbial growth in the samples was analyzed through Ziehl-Neelsen (ZN) smear staining and light-emitting diode fluorescence microscopy (LED-FM). Subsequently, biochemical tests including catalase activity were performed.

RESULTS AND DISCUSSION

To characterize the nanometallic compounds of Zn and Ti, various analyses were conducted, such as UV-visible spectroscopy (UV1800S, Shimadzu, Japan), TEM (JEM2100, JEOL, Japan), FTIR (Alpha-T, Bruker) and X-ray diffraction (X'Pert PRO XRD PW 3040 system).

UV-visible analysis: The UV-visible spectra of the synthesized nanocomplexes exhibited a band at 305 nm caused by n-π* transition (>C=N chromophore linkage), which moved to a higher wavelength, because nitrogen donated an electron lone pair (Fig. 1). Moreover, two peaks corresponding to the benzenoid ring were obtained at 210 and 250 nm because of π-π* transitions. In the K-band, an increase in conjugation caused a hypochromic shift, and in the B-band, a decrease in conjugation caused the hypsochromic shift.

FT-IR analysis:. The spectrum (Fig. 2) exhibited three major peaks in both synthesized nanocomplexes. The peaks corresponding to C-H stretching of aromatic moieties and N-H stretching were obtained at 2340 and 3400 cm^{-1}. The peak corresponding C=C aromatic stretching was observed at 1600 cm^{-1}.

TEM analysis: The TEM results revealed a uniform distribution of the nanoparticles of various sizes. The TEM images of Ti and Zn nanocomplexes revealed that nanoparticles in a size range of 50-100 nm were formed (Fig. 3).

The antituberculosis effects of Ti and Zn nanocomplexes on MTB H37RV strain (control strain), MTB strain (isolated from patient with TB), and MDR-TB strain (first-lined drug-resistant strain isolated from patient with TB) were determined using LED-FM, MIC assay and biochemical tests.

The effectiveness of each synthesized nanocomplexes with different concentrations was different on MTB, because of factors such as the complexity and cell wall composition of MTB. These two factors actively blocked the entry of an external substance, such as test compounds used in this study.

On the 3rd and 5th day, all concentrations of the prepared nanocomplexes were active against the three strains. However, on the 7th , 9th, 15th, and 21st day only high concentration (1000 µg/mL) of the synthesized nanocomplexes was active. The results (Table-1) indicated that the drug activity of synthesized nanocomplexes against H37RV and MTB was higher than that against MDR-TB. However, Zn nanocomplexes exhibited higher antimicrobial activity than Ti nanocomplexes. Table-2 indicates that Zn nanocomplexes with an MIC value of 1000 µg/mL exhibited a high antituberculosis activity against MTB strains (*i.e.* MTB and H37RV).

All concentrations of each the nanocomplexes inhibited the bacterial growth till the 5th incubation day. After the 21st day of incubation, Zn nanocomplexes only with a concentration of 1000 µg/mL inhibited the growth of MTB strains. The antituberculosis activity of Zn and Ti nanocomplexes against MTB,

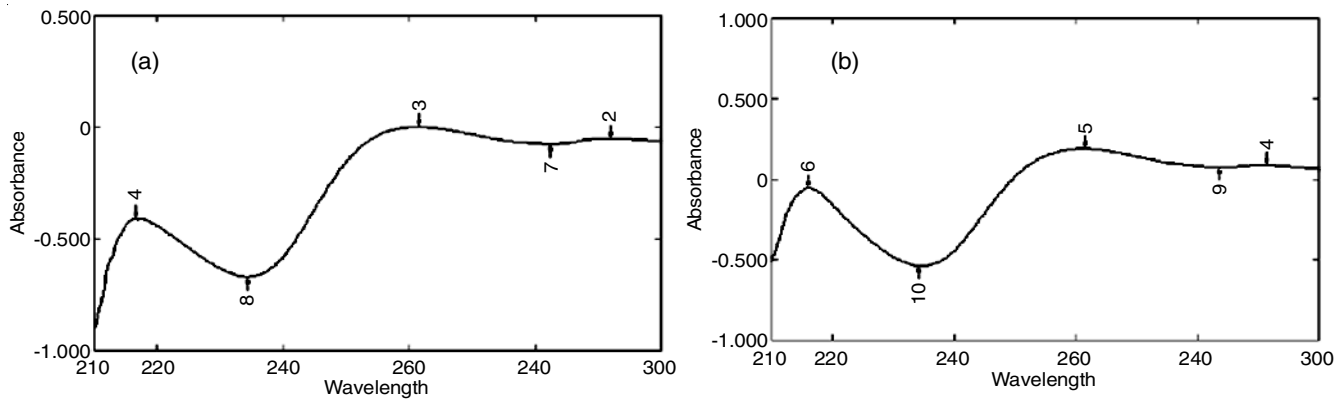


Fig. 1. UV-visible spectra of Zn nanoicomplex (a) and Ti nanocomplex (b)

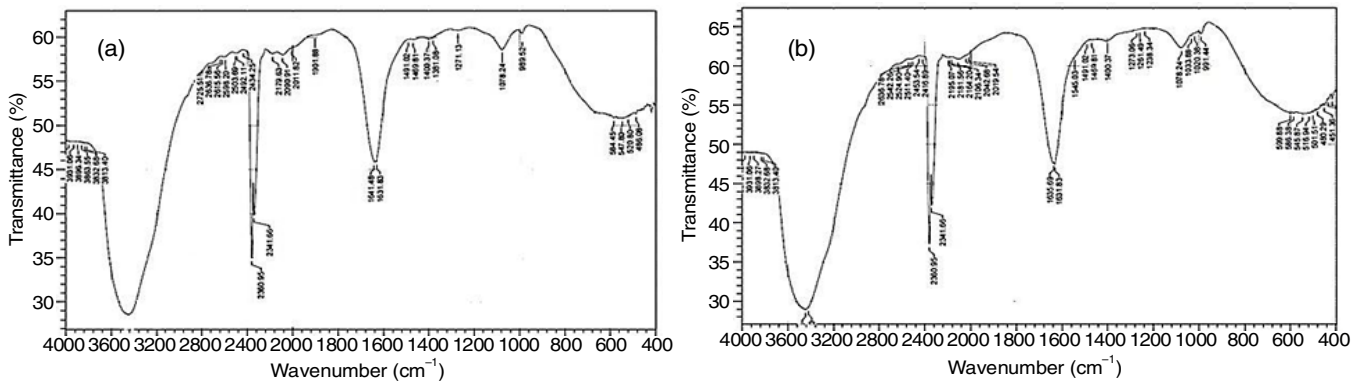


Fig. 2. IR spectra of Zn nanoicomplex (a) and Ti nanocomplex (b)

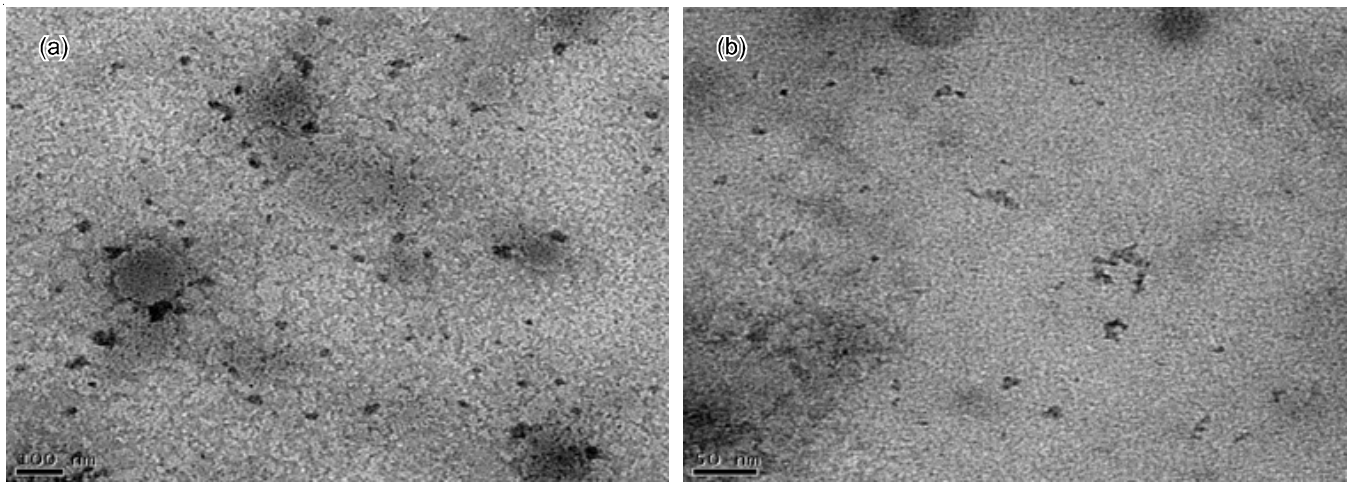


Fig. 3. HRTEM micrographs of Zn nanoicomplex (a) and Ti nanocomplex (b)

TABLE-1
ANTITUBERCULOSIS ACTIVITY OF ZINC AND TITANIUM METAL NANOCOMPLEXES FOR DAY 7 TO 21

Sample		7th day (µg/mL)		9th day (µg/mL)		15th day (µg/mL)		21st day (µg/mL)		7th day/9th Day/15th Day/21st day (µg/mL)						
Metal	Strain	1000	500	1000	500	1000	500	1000	500	250	125	64	32	16	8	4
Zn nanocomplex	H37RV	-ve	Tur	-ve	Tur	-ve	Tur	-ve	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur
	MDRTB	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur
	MTB	-ve	Tur	-ve	Tur	-ve	Tur	-ve	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur
Ti nanocomplex	H37RV	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur
	MDRTB	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur
	MTB	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur	Tur

-ve = No turbidity, Tur = Turbidity

TABLE-2
RESULTS OF ANTITUBERCULOSIS ACTIVITY OF ZINC AND TITANIUM METAL NANOCOMPLEXES

Compound	Activity	Concentrations	Smear microscopy ZN (LED)	Biochemical test (Catalase)	Result
Zn nanocomplex	Active	1000 µg/mL	Negative at 1000 µg/mL	Negative	Drug active at 1000 µg/mL for both the strains H37RV and for MTB
Ti nanocomplex	Active	All concentrations till 5th day	Positive	Positive	Drug Active

H37RV and MDR-TB strains was evaluated through AFB, MIC, LED-FM, ZN staining and biochemical tests (catalase). Zn nanocomplex was more active against MTB than Ti nanocomplex.

Conclusion

In this study, metal nanocomplexes were produced using an eco-friendly, inexpensive, adaptable and reproducible method. The biochemical properties and toxicity of Zn and Ti nanocomplexes were fine-tuned to obtain a novel and versatile antituberculosis agents, which are yet to clinically proven competent.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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