



Cu(II), Zn(II), Cd(II), La(III), AND Sn(IV) COMPLEXES OF BIDENTATE 3- TRIAZINYL THIO ACETIC ACID: SYNTHESES, CHARACTERIZATION AND THEIR IN VITRO ANTIBACTERIAL EVALUATION

Manoj Kumar¹, Tanuja Kumari², Jyoti Joshi³, Sunil Chhimpa⁴, P.J. John⁵, Bidya S. Joshi⁶
E-Mail Id: kmanoj.ru@gmail.com, bsj_jaipur@yahoo.in

^{1,6}Department of Chemistry, University of Rajasthan, Jaipur, Rajasthan, India

^{2,3}Department of Chemistry, Malaviya National Institute of Technology, Jaipur, Rajasthan, India

^{4,5}Department of Zoology, Centre for Advanced Studies, University of Rajasthan Jaipur, Rajasthan, India

Abstract- Present work portray the synthesis of a new series of Cu(II), Zn(II), Cd(II), La(III), and Sn(IV) complexes from a potential bidentate 3-Carboxymethylthio-5-phenyl-1,2,4-triazine (ligand, CMTPT), with metal salts/oxide in an alcoholic medium. Complexes were characterized quantitatively as well as qualitatively, and credible structure of the synthesized compounds was investigated using different physicochemical techniques namely elemental analysis, Fourier transform infrared spectroscopy (FT-IR), mass spectra (LC-MS) and ¹H NMR spectroscopy. The spectral studies reveal that the complexes possess monomeric composition and the metal centre moiety is six-coordinated with octahedral geometry in lanthanum (III) and dibutyltin(IV) complexes whereas Zn(II) and Cd(II) complexes were found to possess a tetra-coordinated make-up with the ligand coordinated as a uninegatively charged bidentate chelating agent via the oxygen atoms of acetic acid of the ligand. Thereafter, FT-IR and LC-MS spectral data validated the coordinated water molecule in the outer sphere of Cu(II) complex. The ligand (CMTPT) and its metal complexes were screened for in vitro antimicrobial activity against the Gram positive bacteria, *Bacillus subtilis* (MTCC-121) as well Gram negative bacteria, *Escherichia coli* (MTCC-40) and it was observed that metal complexes show enhanced biological activity as compared to pure ligand. In the present study, dibutyltin(IV) complex showed antibacterial activity with 40 mm diameter of highest spectrum zone of inhibition.

Keywords: 1, 2, 4-triazine, lanthanum, dibutyltin, transition metal, Antimicrobial activity, inhibition zone.

1. INTRODUCTION

Presently one of the most extensive persuasive areas of research interests includes the development of 1,2,4-Triazines and their condensed derivatives, play an essential task and occupy a pivotal attitude in modern medicinal chemistry due to their high potential pharmacological activities; the most biological activities are antimicrobial^[1-3], anti-inflammatory^[4-5], antihypertensive, diuretic^[6], acaricidal^[7], antifungal^[8], antitumor^[9], antiproliferative^[10], anticonvulsant^[11], anticancer^[12], antioxidant^[13], antihelminthic^[14], anti-AIDS^[15], antidepressant^[16], analgesic^[17], antimalarial^[18], hypnotic^[19], and antiviral^[20-21] activities. In addition, the prominent heterocyclic 1,2,4-Triazine motif, coupled with polyfunctional donors (N, O and S) also gained enormous attention resulting from the interest of structural diversities as well as the biological activities and it is not surprising that numerous 1,2,4-triazinyl compounds have been prepared and characterized in recent years^[22-23]. The preparation and the structural study of metal complexes of oxygen donor (contained 1,2,4-triazinyl) moiety have received considerable attention due to the physiological importance of oxygen donor and the biological synergistic effect of certain metal ions^[24-27]. Moreover, organotin carboxylates particularly continues to grow because of their considerable biological activity and its being introduced there compounds as potential anti-neoplastic and anti-tuberculosis agents, PVC stabilizers and anti-tumour drugs as well a polymer catalysts^[28-29]. In the present study the Cu(II), Zn(II), Cd(II), La(III), and dibutyltin(IV), complexes with 3-Carboxymethylthio-5-phenyl-1,2,4-triazine (ligand, CMTPT) are synthesized and their components and properties are characterized by elemental analysis, ¹H NMR, LC-MS and FT-IR spectra. In addition, we have reported that, complexes of the title ligand (CMTPT) acting as potentially bidentate giving monomeric complexes with said organotin and transition metal ions. It will help in understanding the chelated behavior of oxygen donor of the 1,2,4-triazinyl containing system with metal ions and enlighten more on the biological application of chelated complexes, and it has been observed that the overall value of nitrogen, oxygen and sulfur containing organic fragment is improved when they are binding with metal ion. These compounds were carried out to evaluate the antibacterial activities against Gram (+) and Gram (-) bacteria, they exhibited high degree of inhibition zone towards both tested Human pathogenic microorganisms, and the results are described herein.

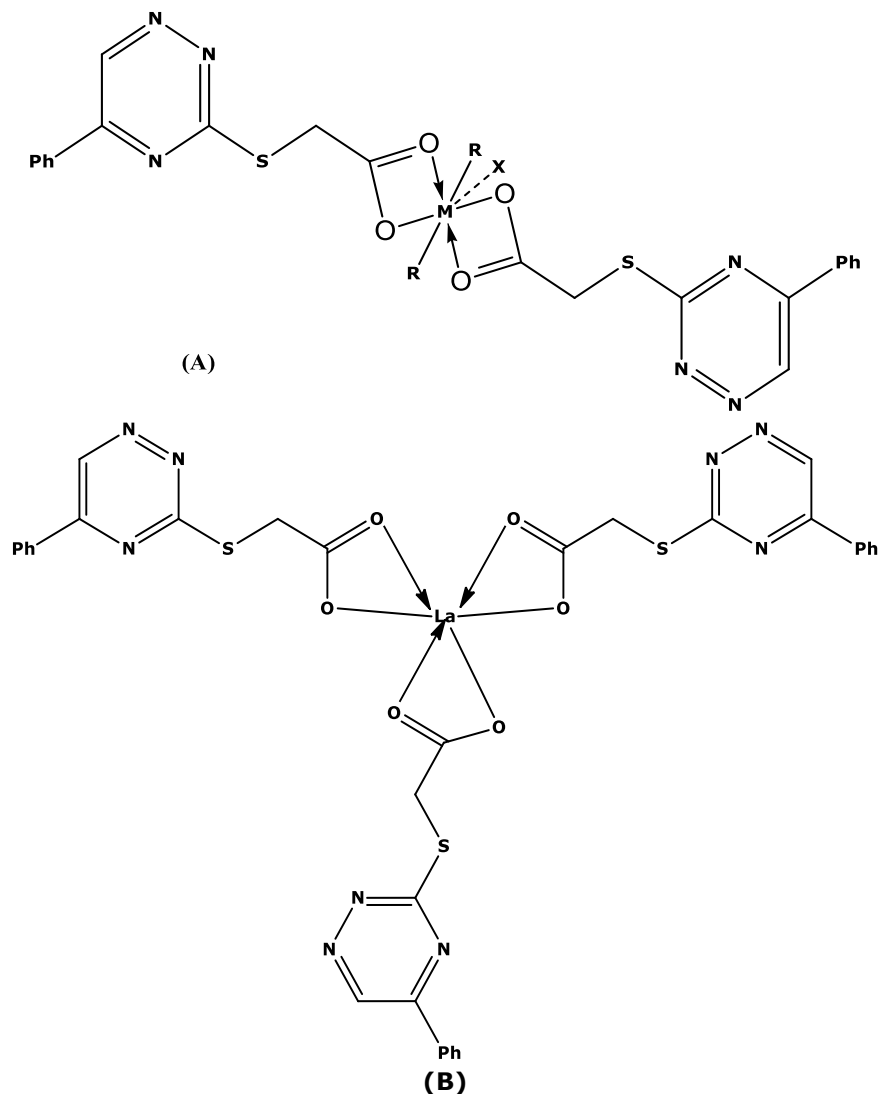


Fig. 1.1 The Proposed structures of metal complexes ((Fig 1.1(A): M= Sn, Cu, Zn, Cd; when only M=Sn, R=CH₂CH₂CH₂CH₃; and X=H₂O for Cu(II))

2. EXPERIMENTAL SECTION

2.1 Materials and Methods

Thiosemicarbazide (98%) was purchased commercially source from Sigma- Aldrich. The starting material phenylglyoxal hydrate was prepared as standard method^[30]. Solvents were dried before using according to standard procedure^[31]. The metal salts were used in their hydrated form as received and double distilled water was used throughout the study. The melting points were recorded in (°C) on melting point apparatus/device using air atmosphere. Infrared Spectra of ligands and their metal complexes were taken in dry KBr pellets using in the range 400–4000 cm⁻¹ with a SHIMADZU model 8400 FT-IR spectrophotometer. ¹H (300MHz, 400 MHz) NMR spectra were collected on a spectrometer in DMSO-d₆ and chloroform-d (for dibutyltin complex) solution using Tetramethylsilane as an internal reference. Mass spectra of all the compounds were recorded with a Waters Q-TOF micro mass (LC-MS). Standard Gravimetric methods were used to determine the metal contents^[31]. All the synthesized compounds (1-6) have been screened against pathogenic bacterial strains of *Bacillus subtilis* (MTCC-121) and *Escherichia coli* (MTCC-40) by using disc diffusion assay method.

2.2 Syntheses

2.2.1 3-Carboxymethylthio-5-phenyl-1,2,4-triazine (ligand; CMTPT):-

The ligand (CMTPT) was synthesized by reported method (M. Tisler et al., 1960) with some modifications^[32]. A mixture of 5-Phenyl-1,2,4-triazine-3(2H)-thione (0.5 g) and 10 ml of 20% aqueous monochloroacetic acid (ClCH₂CO₂H) was refluxed at 30-35°C temperature for 2 h. The completion of the reaction was monitored through TLC using ethyl acetate and petroleum ether (1:2 v/v) as the eluent, and colourless crystals were filtered, washed with ethanol, and then left to dried. N:CPh.CH:N.N:CSC₂H₄COOH; M.P. 192-194°C. Yield: 0.57 g. (88%); C₁₁H₉O₂N₃S, m/z= 247.27. FT-IR (KBr, cm⁻¹): 1590 (C=N), 1544 (N=N), 753 (C-S). ¹H NMR δ (ppm): 9.7 (1H, N=CH), 7.4–8.2 (5H, Ph) 4.1 (2H, CH₂), 10.6 (-OH).

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2.2.2 Synthesis of 1:2 metal Complexes

The complexes were prepared by treating the ethanolic solution of chlorides of Cu(II) ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (0.13g; 0.75 mmol)), Zn(II) (ZnCl_2 (0.1g; 0.75 mmol)), and Cd(II) ($\text{CdCl}_2 \cdot \text{H}_2\text{O}$ (0.15g; 0.75 mmol)) with ethanolic solution of ligand (CMTPT; 0.37 g, 1.5 mmol), stirred under reflux for 4 hr at 35-40°C temperature. The resulting products were filtered, washed with distilled water several times and finally recrystallized from ethanol. The purity of the said synthesized complexes was ensured by TLC.

Cu(CMTPT)₂·H₂O: Brown solid; M.P. 177-179 °C; Yield 58%; $m/z = 573$; $\text{C}_{22}\text{H}_{18}\text{CuN}_6\text{O}_5\text{S}_2$; Calcd. C, 46.03% (found 46.09%); H, 3.16% (found 3.13%); N, 14.64% (found 14.71%); O, 13.93% (found 13.95%); S, 11.17% (found 11.14%); Cu, 11.07% (found 11.05%). FT-IR (KBr, cm^{-1}): 1575 (C=N), 1542 (N=N), 752 (C-S), 480 (Cu-O). m/z (LC-MS, %relative abundance): 573 (79%), 411 (28%), 409 (57%), 314 (8%), 298 (32%), 276 (35%), 247 (49%), 245 (100%), 217 (17%).

Zn(CMTPT)₂: Pale yellow; M.P. 211-213 °C; Yield 51%; $m/z = 557$; $\text{C}_{22}\text{H}_{16}\text{ZnN}_6\text{O}_4\text{S}_2$; Calcd. C, 47.36% (found 47.42%); H, 2.89% (found 2.83%); N, 15.06% (found 15.11%); O, 11.47% (found 11.50%); S, 11.49% (found 11.47%); Zn, 11.72% (found 11.69%). FT-IR (KBr, cm^{-1}): 1570 (C=N), 1542 (N=N), 751 (C-S), 499 (Zn-O). ^1H NMR δ (ppm): 9.2 (1H, N=CH), 7.2–8.2 (5H, Ph) 3.9 (2H, CH₂). m/z (LC-MS, %relative abundance): 557 (27%), 485 (86%), 305 (19%), 261 (87%), 247 (74%), 217(100%), 185 (24%).

Cd(CMTPT)₂: Pale yellow; M.P. 170-172 °C; Yield 49%; $m/z = 605$; $\text{C}_{22}\text{H}_{16}\text{CdN}_6\text{O}_4\text{S}_2$, Calcd. C, 43.68% (found 43.61%); H, 2.67% (found 2.65%); N, 13.89% (found 13.92%); O, 10.58% (found 10.63%); S, 10.60% (found 10.57%); Cd, 18.58% (found 18.51%). FT-IR (KBr, cm^{-1}): 1582 (C=N), 1541 (N=N), 750 (C-S), 502 (Cd-O). ^1H NMR δ (ppm): 9.3 (1H, N=CH), 7.3–8.2 (5H, Ph) 3.8 (2H, CH₂). m/z (LC-MS, %relative abundance): 605 (8%), 528 (11%), 359 (17%), 248 (5%), 174 (100%), 172 (87%).

Bu₂Sn(CMTPT)₂: The dibutyltin (IV) complex was prepared by treating the ethanolic solution of dibutyltin oxide (Bu_2SnO (0.186g; 0.75 mmol)), with ethanolic solution of ligand (CMTPT; 0.37 g, 1.5 mmol), stirred under reflux for 3-4 hr. at 35-40°C temperature. The white crystalline product was filtered, washed with distilled water several times and finally recrystallized from ethanol, and further purity of the said synthesized complex was ensured by TLC. M.P. 180-182 °C; Yield 73%; $m/z = 726$; $\text{C}_{30}\text{H}_{34}\text{SnN}_6\text{O}_4\text{S}_2$, Calcd. C, 49.67% (found 49.76%); H, 4.72% (found 4.76%); N, 11.58% (found 11.52%); O, 8.82% (found 8.80%); S, 8.84% (found 8.85%); Sn, 16.36% (found 16.34%). FT-IR (KBr, cm^{-1}): 1577 (C=N), 1540 (N=N), 483 (Sn-O), 573 (Sn-C). ^1H NMR δ (ppm): 9.37 (1H, N=CH), 7.2–8.2 (5H, Ph) 3.9 (2H, CH₂). m/z (LC-MS, %relative abundance): 726 (13%), 480 (100%), 478 (65%), 436 (13%), 378 (9%), 322(6%), 151 (57%).

2.2.3 Synthesis of 1:3 Metal Complex

An ethanolic solution of $\text{LaCl}_3 \cdot 6\text{H}_2\text{O}$ (0.166g; 0.47 mmol), was added to an ethanolic solution of 3-Carboxymethylthio-5-phenyl-1,2,4-triazine, (CMTPT; 0.35g, 1.40 mmol) in 1:3 (metal : ligand) molar ratio. Then the mixture was stirred under reflux for 4 hr at 35-40°C temperature and a pale-yellow precipitate was obtained. The precipitate was filtered out, washed with distilled water several times and finally recrystallized from ethanol. Yield 53%; mp 209-211°C, $m/z = 877$; $\text{La}(\text{C}_{11}\text{H}_8\text{N}_3\text{O}_2\text{S})_3$.

La(CMTPT)₃: $\text{C}_{33}\text{H}_{24}\text{LaN}_9\text{O}_6\text{S}_3$, Calcd. C, 45.16% (found 45.25%); H, 2.76% (found 2.73%); N, 14.36% (found 14.41%); O, 10.94% (found 10.97%); S, 10.96% (found 10.91%); La, 15.83% (found 15.81%). FT-IR (KBr, cm^{-1}): 1578 (C=N), 1538 (N=N), 751 (C-S), 501 (La-O). ^1H NMR δ (ppm): 9.2 (1H, N=CH), 7.2–8.1 (5H, Ph) 4.0 (2H, CH₂). m/z (LC-MS, %relative abundance): 877 (9%), 787 (7%), 709 (100%), 670 (41%), 631 (55%), 508 (7%), 480 (7%), 329 (22%), 270 (64%), 172 (7%).

3. PHARMACOLOGY

3.1 In Vitro Antibacterial Assay

The synthesized metal complexes and thin acetic acid ligand (CMTPT) were screened for their in vitro antibacterial activities against human bacteria's namely the Gram (+) and Gram (-) bacteria i.e. *Bacillus subtilis* (MTCC-121) and *Escherichia coli* (MTCC-40) respectively, using the nutrient agar medium (constituents-0.5% peptone, 0.3% yeast extract, 1.5% agar, 0.5% NaCl, 1 litre distilled water) with pH adjusted to the nearly neutral (6.8) at 25°C and sub cultured in nutrient broth. Overnight suspension culture of each microbial strain mentioned above was spread over the surface of separate nutrient agar plate with a sterile glass spreader. Filter paper discs (6 mm in diameter) were dipped in DMSO solution containing 3-triazinyl thio acetic acid ligand and its complexes of varying concentration and placed on the agar plates. Each plate had discs of two different concentrations which are used for screening; the concentrations used for the study were 1mg/ml and 2mg/ml. The plates were incubated at 37°C (for *E. coli*) and 30°C (for *B. subtilis*) for 24-48 hours. Three types of control plates were incubated for the same run time along with the experimental plates. First one with inoculated microbial culture without the disc; second one with microbial culture with the disc dipped in DMSO and the negative control plate without inoculum and the disc respectively, at the same time. All the testing were carried out in triplicates to minimize inaccuracy and measured values are presented. Similar experiments were administered with series of neat ligand (CMTPT) individually and its Cu(II), Zn(II), Cd(II), La(III), and Sn(IV) complexes in different combinations as disclosed in the present study.

4. RESULTS AND DISCUSSION

Analytical data of ligand and its metal complexes are in a good agreement with the proposed stoichiometry of the compounds are summarized in experimental section, which indicate the formation of 1:2 metal complexes of ligand CMTPT with Cu(II), Zn(II), Cd(II), Bu(IV); and 1:3 metal complexes of ligand CMTPT with La(III) metal ion. The mass spectrum of these newly synthesized derivatives indicates the monomeric structure for all the complexes. During the complexation of the ligand CMTPT an O-H bond is disappeared and formation of new coordination between the 'Metal-Oxygen' is takes place which is deduced by the FT-IR and ¹H-NMR spectral data. The ligand (CMTPT) was soluble in ethanol. All the solid metal complexes are stable in air, infusible at higher temperature, and were purified with several time wash.

4.1 IR Spectra

FT-IR spectral data of free ligand (CMTPT) and its metal complexes reveal the involvement of coordination sites in chelation are reported in Table 1.1. In free ligand, the infrared spectrum showed some characteristic stretching bands at 3460, 1647, 1320, and 1590, cm⁻¹ assigned to $\nu(\text{OH})$, $\nu(\text{COO})_{\text{as}}$, $\nu(\text{COO})_{\text{s}}$, and $\nu(\text{C}=\text{N})$ of the triazine ring, respectively. The $\nu(\text{O-H})$ band of the ligand appeared at 3460 cm⁻¹ was absent in the infrared spectra of complexes 2 to 6, indicating the deprotonation and coordination of the carboxylate anions to the central metal atom moiety. The infrared spectra of complexes 2 to 6 revealed that the $\nu(\text{COO})_{\text{as}}$ was shifted to a lower wave length number compared to the ligand signifying that the coordination took place via the oxygen atoms of the carboxylate anion. The COO stretching vibrations play an important role to predict the bonding mode of the ligand. The value of $\Delta\nu$ [$\Delta\nu = \nu(\text{COO})_{\text{as}} - \nu(\text{COO})_{\text{s}}$] is used to ascertain the bonding properties of carboxylate anion to the metal atom and could be divided into 3 groups; (a) when $\Delta\nu > 350$ cm⁻¹, the carboxylate anion binds in a monodentate fashion, however, other very weak intra- and intermolecular interactions cannot be excluded; (b) When $\Delta\nu < 200$ cm⁻¹ the carboxylate anion binds in a bidentate fashion, and (c) when 350 cm⁻¹ $> \Delta\nu > 200$ cm⁻¹ an intermediate state between monodentate and bidentate (anisobidentate) takes place.

Based on the FT-infrared data in Table 4.1, all the complexes showed that the $\Delta\nu$ fall in the range of 350 cm⁻¹ $> \Delta\nu > 200$ cm⁻¹ which thus indicated that the carboxylate anions bonded to metal atom moiety in anisobidentate manner resulting in the metal centre exhibiting six coordination in La(III) and Sn(IV) complexes whereas tetra coordinated in Cu(II), Zn(II) and Cd(II) complexes respectively. This is further confirmed by the appearance of new $\nu(\text{M-O})$ stretching at 470-510 cm⁻¹. The band corresponding to the COO⁻ moiety has shifted to lower frequencies due to decreasing of the bond order (and increasing of bond length) of carbon-oxygen bond result from chelation of the 'O' to central metal ions. A broad band observed in Cu(II) complex in the region 3650–3742 cm⁻¹ assigned for $\nu(\text{OH}/\text{H}_2\text{O})$ evidenced the presence of coordinated water molecule.

Table-4.1 FT-IR frequencies (cm⁻¹) of Ligand and its Metal Complexes

Compound	IR Frequency (cm ⁻¹)		
	$\nu(\text{COO})_{\text{as}}$	$\nu(\text{COO})_{\text{s}}$	$\Delta\nu = [\nu(\text{COO})_{\text{as}} - \nu(\text{COO})_{\text{s}}]$
CMTPT	1647	1320	327
$\text{Cu}(\text{C}_{11}\text{H}_8\text{N}_3\text{O}_2\text{S})_2 \cdot \text{H}_2\text{O}$	1590	1302	288
$\text{Zn}(\text{C}_{11}\text{H}_8\text{N}_3\text{O}_2\text{S})_2$	1600	1305	295
$\text{Cd}(\text{C}_{11}\text{H}_8\text{N}_3\text{O}_2\text{S})_2$	1590	1305	285
$\text{La}(\text{C}_{11}\text{H}_8\text{N}_3\text{O}_2\text{S})_3$	1600	1305	295
$\text{Bu}_2\text{Sn}(\text{C}_{11}\text{H}_8\text{N}_3\text{O}_2\text{S})_2$	1588	1306	282

4.2 ¹H-NMR Spectra

To elucidate the structure of ligand (CMTPT) and its Sn(IV), La(III), Zn(II), Cd(II) complexes, ¹H-NMR spectra were recorded in DMSO-d₆ and chloroform-D (for dibutyltin complex). The parent compound showed peaks at 9.7, 4.1, and 7.4-8.2 ppm which were attributed to N=CH, S-CH(2H), and phenylic protons (5H). In free ligand, signal that appeared at δ 10.6 ppm due to -OH proton was not observed in the spectra of Sn(IV), La(III), Zn(II) and Cd(II) complexes, further supporting the complexation through O atom of the carboxylate group of the ligand leading to formation of new M-O bond. Thus, binding modes discussed in FT-IR spectra are further supported in ¹H-NMR spectra. In dibutyltin(IV) complex the butyl protons appears as a multiple in the range of 1.562-0.746 ppm due to -CH₂CH₂CH₂CH₃ group.

4.3 Mass Spectra

The mass spectrum of the synthesized metal complexes was recorded; the results are in good agreement with the proposed structures, some relevant mass spectral peaks along with their relative abundance and m/z ratio are depicted in the fig. 4.1. In the mass spectrum of this pure ligand a molecular ion peak $[M]^+$ at $m/z=247.07$ (calculated $M. Wt=247.27$) with an intensity of 34% which is equal to its molecular weight. In addition, in the mass spectrum of other complexes molecular ion peak observed at $m/z = 573, 557, 605, 877, \text{ and } 726$ are attributed to $\text{Cu}(\text{CMTPT})_2 \cdot \text{H}_2\text{O}$, $\text{Zn}(\text{CMTPT})_2$, $\text{Cd}(\text{CMTPT})_2$, $\text{La}(\text{CMTPT})_3$, and $\text{Bu}_2\text{Sn}(\text{CMTPT})_2$ respectively, which are coincident with the formula weights and affirm the identity of structures as disclosed in the figure 1.1. The mass spectrum of $\text{Cu}(\text{II})$ complex displays the parental ion peak $[M]^+$ at $m/z = 573.18$ (79%) and weak peaks at $m/z = 575.18, 576.18$ due to ^{13}C and/or ^{15}N isotopes. The mass spectrum of $\text{Cu}(\text{II})$ complex exhibits a peak corresponding to the fragment at 573.18 with 79% abundance pointing out the chelation between one copper, two deprotonated ligands and one aqua molecule as $[\text{Cu}(\text{CMTPT})_2 \cdot \text{H}_2\text{O}]^+$, while in this particular case a base peak appears at $m/z = 245.00$ (100%). Moreover, the other positive ions give the peaks at 411 (28%), 409 (57%), 314 (8%), 298 (32%), 276 (35%), 247 (49%), 217 (17%) mass numbers are attributed to fragments of copper complex give the idea of the stabilities of fragments (figure 2.1).

Mass spectra of Zn , Cd and dibutyltin complexes show a peak corresponding to the fragment $[\text{Zn}(\text{CMTPT})_2]^+$, $[\text{Cd}(\text{CMTPT})_2]^+$ and $[\text{Bu}_2\text{Sn}(\text{CMTPT})_2]^+$ respectively, which indicates the presence of a species containing one metal ion and two deprotonated ligands, whereas in the case of Lanthanum complex shows a peak corresponding to the fragment $[\text{La}(\text{CMTPT})_3]^+$ indicates the species which contain one metal ion and three deprotonated ligands. Experimental isotopic distributions show the same patterns as the theoretical ones for all prepared compounds as described in the present article.

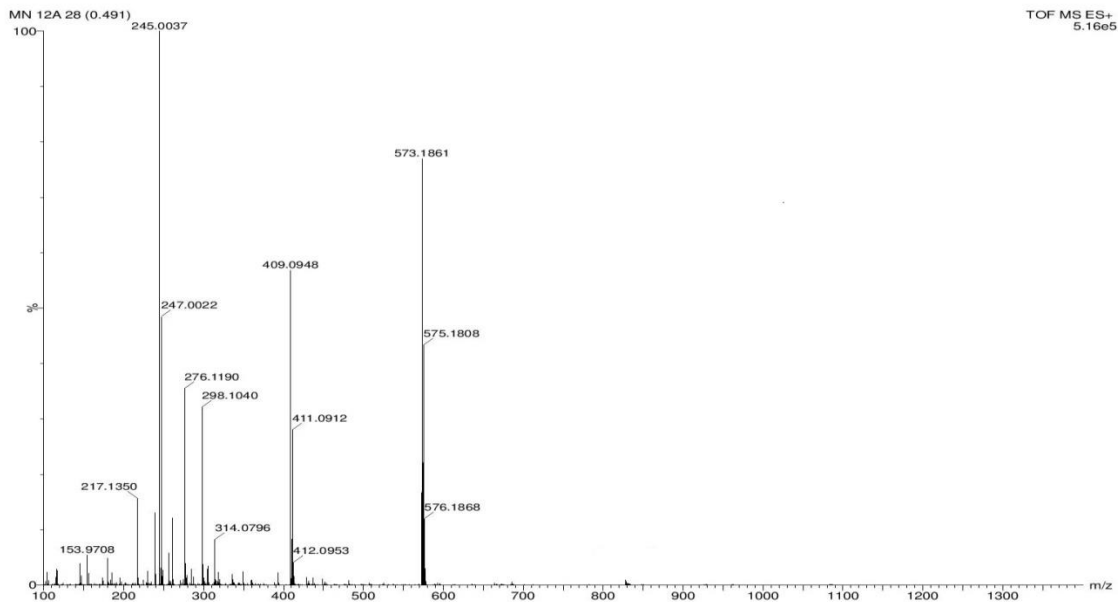


Fig. 4.1 LC-MS Spectrum of $\text{Cu}(\text{C}_{11}\text{H}_8\text{N}_3\text{O}_2\text{S})_2 \cdot \text{H}_2\text{O}$

4.4 Biological Evaluation

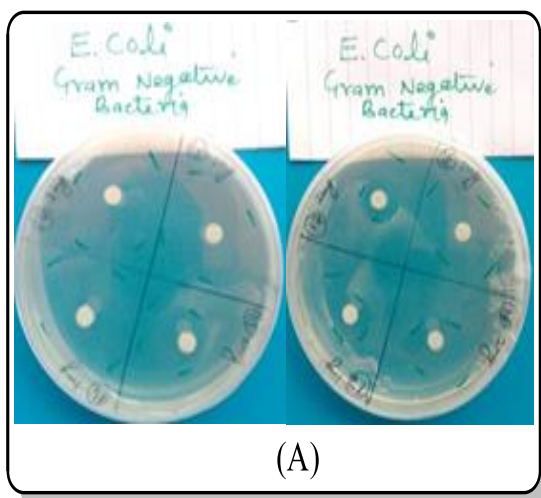
In vitro antimicrobial activity of ligand (3-Carboxymethylthio-5-phenyl-1,2,4-triazine; CMTPT) and its metal chelates has been examined against gram-positive bacteria (*B. subtilis*) and gram-negative bacteria (*E. coli*); and zone of inhibition tests were measured by using disc diffusion method (Table 2.1). Inhibition zone for gram-positive bacteria was found in the range of 07-14 mm and for gram-negative bacteria 16–40 mm. It has been observed that all the complexes i.e. $\text{La}(\text{CMTPT})_3$, $\text{Cu}(\text{CMTPT})_2$, $\text{Zn}(\text{CMTPT})_2$, $\text{Cd}(\text{CMTPT})_2$ and $\text{Bu}_2\text{Sn}(\text{CMTPT})_2$ were very effective against *E. coli* with zone of inhibition of 25 mm, 23 mm, 30 mm, 34 mm and 40 mm; and *B. subtilis* with zone of inhibition of 12 mm, 12 mm, 13 mm, 14 mm and 09 mm respectively. Thus, we found that, compounds 1-6, with both the stock concentration (1 mg/ml & 2 mg/ml), were active towards both the pathogenic bacterial strains. Among all the synthesized compounds, the highest antibacterial activity was performed by $\text{Bu}_2\text{Sn}(\text{CMTPT})_2$ against Gram-negative microorganism (2mg/ml) (*E. coli*) with zone of inhibition of 40 mm, followed by $\text{Bu}_2\text{Sn}(\text{CMTPT})_2$ itself (1mg/ml), and $\text{Cd}(\text{CMTPT})_2$ compound with the zone inhibition of 34 mm against *E. coli*. Thus, disc diffusion assay demonstrated that, $\text{La}(\text{III})$, $\text{Cu}(\text{II})$, $\text{Zn}(\text{II})$, $\text{Cd}(\text{II})$ and $\text{Sn}(\text{IV})$ complexes of CMTPT (ligand) showed more zone of inhibition as compared to the free ligand, therefore, it indicates that these complexes possess enhanced antibacterial activity against pathogenic strains of Gram-negative bacteria such as *E.coli*. We therefore, speculate that, this advancement in the antimicrobial activity may be due to an efficient diffusion of the metal complexes in to the bacterial cell and/or interaction with the bacterial cells and this can be better explained by the Overtone's concept of cell

permeability and Tweedy's chelation theory^[33]. According to Overtone's concept of cell permeability, that entry of any molecule into a cell is governed by its lipid solubility (liposolubility), therefore, liposolubility is an important factor that controls the antimicrobial activity. On chelation, polarity of the metal ion is reduced to a greater extent due to the overlapping of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. The chelation increases the delocalization of p-electrons over the whole chelate ring and enhances the lipophilicity of the complexes which in turn, increases the penetration of the complexes into lipid membranes, and results in blockage of metal sites in the enzymes of the microorganisms. Furthermore, complexes obstruct the respiration process of the bacterial cell and, block the synthesis of proteins and prevent further growth of the microorganism. Eventually, it has been observed that the results also showed dose-dependent effect for both types of bacterial strains: at lower concentration growth is lightened while at higher concentration more enzymes become inhibited. Hence, the zone of inhibition for $\text{Bu}_2\text{Sn}(\text{CMTPT})_2$ suggested that this compound had much stronger antibacterial activity compared to other synthesized compounds and, therefore, it could potentially be used for new therapeutic targets (Table-2.1 , fig.4.2- fig.3.3).

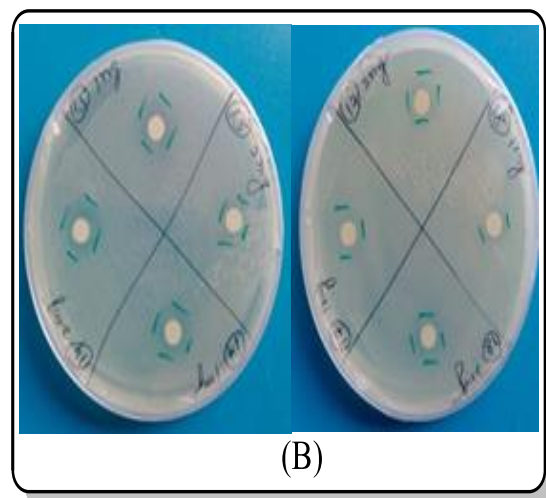
Table-2.1 Inhibition Zone (mm) of Ligand and its Metal Complexes against Pathogenic Bacteria's through Disc Diffusion Method

S. No.	Compound	Concentration of compound (mg/ml)	Diameter growth of inhibition zone (mm)		Disc diameter (in mm)	Antibacterial activity	DMSO (control)
			<i>E. coli</i>	<i>B. subtilis</i>			
1	CMTPT	1	16	13	6	+++	No activity found
		2	22	14		++++	
2	$\text{La}(\text{C}_{11}\text{H}_8\text{N}_3\text{O}_2\text{S})_3$	1	23	10	6	++++	
		2	25	12		++++	
3	$\text{Cu}(\text{C}_{11}\text{H}_8\text{N}_3\text{O}_2\text{S})_2 \cdot \text{H}_2\text{O}$	1	20	10	6	+++	
		2	23	12		++++	
4	$\text{Zn}(\text{C}_{11}\text{H}_8\text{N}_3\text{O}_2\text{S})_2$	1	20	13	6	+++	
		2	30	13		++++	
5	$\text{Cd}(\text{C}_{11}\text{H}_8\text{N}_3\text{O}_2\text{S})_2$	1	30	12	6	++++	
		2	34	14		++++	
6	$\text{Bu}_2\text{Sn}(\text{C}_{11}\text{H}_8\text{N}_3\text{O}_2\text{S})_2$	1	35	07	6	++++	
		2	40	09		++++	

(-)=no inhibition; (+)=zone size 6-9 mm; (++)=zone size 10-13 mm; (+++)=zone size 14-20 mm; (++++)=zone size >20 mm



(A)



(B)

Fig. 4.2(A): Zone of inhibition for metal complexes for Gram-negative bacterium (*E. coli*)

Fig. 4.3(B): Zone of inhibition for metal complexes for Gram-positive bacterium (*B. subtilis*)

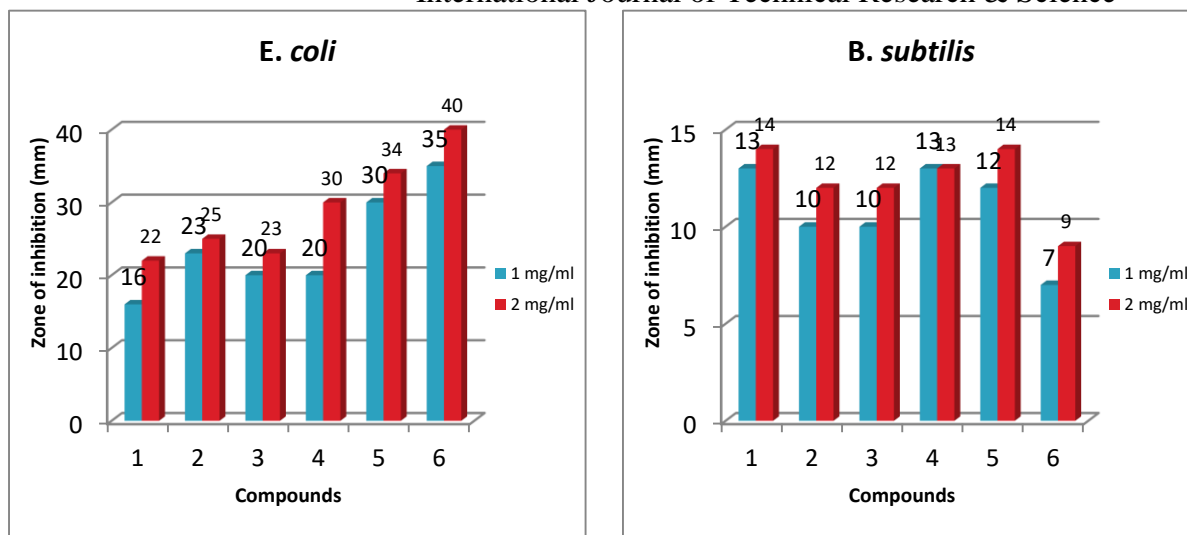


Fig. 4.4 Biological Evaluation: Comparison of Diameters of Zone of Inhibition for Compounds (1-6) against Gram (-) Bacterium (*E. coli*); and Gram (+) Bacterium (*B. subtilis*) at two different Concentrations

CONCLUSIONS

The 3-Carboxymethylthio-5-phenyl-1,2,4-triazine (ligand, CMTPT) and its Sn(IV), La(III), Cu(II), Zn(II), Cd(II) complexes were successfully synthesized and physico-chemically investigated using elemental analysis, ¹H NMR, FT-IR and LC-MS spectral studies. The results indicate that, ligand (CMTPT) acts as a mono-functional bidentate and it coordinates via carboxylate to the metal ion to afford the corresponding complexes 2 to 6 giving stable chelate ring. With the help of aforementioned techniques, octahedral geometry around the Sn(IV), and Ln(III) monomeric complexes and square planar geometry around the Zn(II) and Cd(II) monomeric complexes have been proposed. In vitro antibacterial activity towards two bacterial strains, namely *Bacillus subtilis* (MTCC-121) and *Escherichia coli* (MTCC-40) were screened, and the results revealed significantly more activity for these newly synthesized derivatives as compared to free ligand. For instance, $\text{Bu}_2\text{Sn}(\text{CMTPT})_2$ and $\text{Cd}(\text{CMTPT})_2$ complexes exhibit greatest activity against *E. coli*. as compared to free ligand. The synthesized compounds 1-6 were prepared in DMSO which had no antibacterial activity towards both the pathogens. Therefore, it may suggested that coordination of said ligand (CMTPT) with different metal ions makes them stronger bacteriostatic agents, thus inhibiting the growth of bacteria more than the parent ligand. The present study will provide more insightful information related to the nature of transition metal complexes and organotin(IV) complex along with sulphur, nitrogen and oxygen containing bioactive heterocyclic ligands.

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