SYNTHESIS, CHARACTERIZATION, ANTIOXIDANT, LARVICIDAL, AND IN-VITRO ANTI-TUMOUR ACTIVITIES OF SOME NOVEL SCHIFF BASE TRANSITION METAL COMPLEXES DERIVED FROM CURCUMIN AND METHYL-3-AMINO-2-THIOPHENE CARBOXYLATE

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Abstract **:** In this paper, a new Schiff base ligand (L), was prepared by condensation of 1,7-bis-(4-hydroxy3-methoxyphenyl)-1,6-heptadiene-3,5-dione (Curcumin) with methyl-3-amino-2-thiophene carboxylate. The transition metal complexes of Ligand, (M = Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) were also successfully synthesized and characterized by various spectroscopic techniques. The ligand and their complexes were characterized by powder XRD and SEM-EDAX analysis. All the synthesized compounds were screened for their anticancer activities against breast cancer cell line MCF-7 and Leukemia cancer cell line K562 by sulforhodamine-B(SRB) assay. Interestingly, the Schiff base (L) and its Mn(II), Co(II) and Zn(II) metal complexes showed superactive anticancer activity against MCF-7 and K562 cell lines. In addition, the antioxidant and larvicidal activities were also done for all the metal complexes. The percentage of antioxidant scavenging activity and mortality of the larvicidal activity were also determined.

Keywords **- Curcumin, transition metal complexes, XRD, SEM-EDAX, antioxidant, larvicidal activities.**

1.INTRODUCTION

Schiff bases form a significant class of compounds in medicinal and pharmacological chemistry due to their varied biological applications such as antibacterial, antifungal (Pandeya et.al., 1999) and antitumor (Mladenova et.al., 2002) agents. Generation of different heterocyclic derivatives of Schiff bases has created a boom in the field of medicinal chemistry. Curcumin [1,7-bis(4-hydroxy-3methoxyphenyl)- 1,6-heptadiene- 3,5-dione], which is a naturally occurring yellow pigment obtainable from the rhizomes of turmeric (Curcuma longa Linn.), is a common ingredient used in spices, cosmetics and traditional Chinese medicine. The medicinal activity of curcumin has been known since ancient times and this molecule has been the object of several investigations in the field of biology, medicine and pharmacology over the last decades, such as antioxygenation, antibiosis and antitumor activities (Zhang et.al.,2002). Curcumin exhibits potential therapeutic application against several chronic diseases including cancer, inflammatory, neurological, cardio-vascular and skin diseases (Indira Priyadarsini, 2009). Recently, it has been considered as a key molecule for development of novel therapeutics for Alzheimer's disease (Shirai et.al., 2010). Schiff bases derived from 2-aminothiophene derivatives are extensively studied due to their broad spectrum of biological properties and anti-HIV PR inhibitor activities (Souza et.al., 2012). The Schiff base ligands with sulphur, oxygen and nitrogen donor atoms act as good chelating agents forming stable chelates with transition metal ions. Such metal complex on coordination shown to exhibit increased potential biological activity (Bootwala et.al., 2012) Based on the above facts, the prime aim of the present work was to synthesize a series of Schiff base transition metal complexes derived from Curcumin and Methyl-3-Amino-2-Thiophene Carboxylate, evaluate the antitumour activity of the synthesized metal complexes. Also, the synthesized metal complexes were screened for larvicidal and in-vitro antioxidant activity.

II. EXPERIMENTAL

Materials and methods

All the chemicals and solvents used in the preparation of ligands and their metal complexes were of A.R grade. Curcumin and Methyl-3-Amino-2-Thiophene Carboxylate were purchased from Sigma-Aldrich. Metal salts like Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) chlorides and the solvents were purchased from Merck.

2.1 Synthesis of Schiff base ligand Methyl-3-[5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-triene-3-ylidene)amino]thiophene-2-carboxylate [L]

Curcumin (0.01 mol, 3.6839g) was dissolved in 20 ml methanol and stirred well at room temperature. Then methanolic solution of curcumin was added to a hot aqueous solution of methyl-3-amino-2-thiophene carboxylate. The obtained orange coloured mixture was stirred and refluxed at 80^0C for about 6 hrs. After cooling, the resulting orange fine precipitate was filtered and washed well with distilled ethanol repeatedly to remove any unreacted chemicals. The obtained orange crystals were then dried at room temperature.

2.2 Synthesis of Schiff base metal complexes

To the hot solution of schiff base ligand (0.01 mol) in methanol (20ml) was added a hot methanolic solution (10ml) of respective metal chlorides (0.01 mol) drop by drop in 1:1 (ligand: metal) molar ratio. pH of the solution was maintained just below the value of hydrolysis of the metal ion using alcoholic ammonia. The reaction mixture was magnetically stirred and refluxed for 4 hrs at 80^0C. The coloured precipitate was filtered and washed by cold ethanol to remove the residue reactants. Finally the obtained powder was dried to get the complex.

2.3 Antioxidant assay (DPPH scavenging activity)

The antioxidant activity of the synthesized curcumin derivatives was evaluated using the DPPH (1,1-Diphenyl-2-picryl-hydrazyl) free radical scavenging assay (Larrauy et.al.,1998). It is a rapid technique for screening the radical scavenging activity of specific compounds (Sreejayan et.al., 1996). 100 g/ml of the test sample solution was added to 4ml of 0.01 M methanolic DPPH at various concentrations (20, 40, 60, 80 g). After stirring, the mixture was incubated for 20 min at room temperature and the absorbance at 517 nm was measured. Ascorbic acid (100 g/ml) was used as the standard. A blank was prepared without adding standard or test compound (95% methanol). Lower the absorbance of the reaction indicates higher the free radical scavenging activity. The capability to scavenge the DPPH radical were calculated using the equation,

$$\% \text{ of inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \qquad (1)$$

where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of test compounds (Manmohan singhal et.al., 2011).

2.4 Larvicidal activity

The mosquito larvae were collected from water habitats of Nagercoil, Kanyakumari District using a wide mouth container. The mosquito samples were brought to the laboratory, morphologically identified using standard manual and used for larvicidal activity studies. Cleaned sterile Erlenmeyer flasks were taken and 10 early instar larvae of *Culex* were taken in 100 mL of tap water. To that 100 ppm of synthesized complexes was added. The negative control was set up with sterile distilled water without metal complex while the positive control was the commercial larvicide with test solution. Percentage of mortality was assessed after 24 h of incubation. A number of dead larvae in each batch were counted every hour for 24 h exposure period. The treated larvae was mounted on a slide and examined under a microscope for image capture. The LC50 and LC90 of Cu(II) complex were determined in mosquito larvae (*Culex*). The mortality of the larvae was also tested by substituting 25,50 and 100 ppm complex. It was incubated for 24 h and the percentage mortality was obtained (Arruda et.al., 2010).

2.5 In vitro analysis of Anticancer activity:

The anticancer activity was performed at Tata Memorial Centre Advanced for Treatment, Research and Education in Cancer (ACTREC), Khar, Navi Mumbai – 410210, (MCF-7 and K562 cell line) by SRB assay. The principle behind SRB assay is, under acidic conditions, a bright pink aminoxanthine dye SRB binds dye to basic amino acid residues in TCA (Trichloro acetic acid) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of visible at least two order of magnitude. The cell lines were cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum (FBS) and 2 millimolar L- glutamine at 37^0C in a humidified atmosphere of 5% CO_2. About 5x103 cells/well were seeded in 96-well micro titer plate using a culture medium. After 24 hours, Schiff base (L) and its Mn(II), Co(II), Cu(II) and Zn(II) metal complexes at the concentrations of 10,20,40 and 80 µg/ml were added to respective wells at a single concentration and incubated for 48 hours. After incubation the sulforhodamine-B assay was performed (Skehan et.al.,1989).

The percentage growth inhibition was calculated using following formula,

% cell inhibition= 100-{(At-Ab)/ (Ar-Ab)} x100 (2)

Where, At = Absorbance value of test compound, Ab = Absorbance value of blank,

Ar = Absorbance value of reference

III. RESULTS AND DISCUSSIONS

The condensation of Curcumin with Methyl-3-Amino-2-Thiophene Carboxylate gives the Schiff base ligand, Methyl-3-[5-hydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-triene-3-ylidene)amino]thiophene-2-carboxylate [L]. The ligand which coordinated with Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} ions separately to give coloured complexes. The Schiff base ligand (L) and its metal complexes are stable at room temperature and soluble in almost all organic solvents like DMSO and DMF.

3.1 X-Ray Diffraction analysis

X-ray diffraction studies of compounds were investigated from the angle of 10^0 to 80^0. The powder XRD patterns of L and its Cu(II) complex are recorded in the range $2\theta = 0\text{-}80$ A^0 were shown in figure:1.

The average crystalline size d_{XRD} of the complexes was calculated using Scherrer's formula,

$d = 0.89\lambda/\beta\cos\theta$),

where'd' is the average crystalline size of the phase under investigation. 'λ' is the wavelength of X-ray beam used. 'β' is the full width at half maximum of diffraction and 'θ' is the Bragg's angle (Cullity,1978) From the observed XRD patterns, the average crystalline size for the ligand, L and Cu(II) complex are found to be 53.22 nm and 52.84 nm respectively. After complexation, the particle size decreases. This suggests that the ligand and the complexes are nanocrystalline in nature.

After complexation, the particle size decreases. This suggests that the ligand and the complexes are nanocrystalline in nature. Comparing the XRD pattern of the ligand and Cu(II) complex, additional peaks at 32.760, 34.086, 40.932 and 57.498 which clearly indicate that copper is coordinated to the ligand during complexation. (Turkoglu et.al., 2003). The average crystallite size obtained from XRD also shows that the particles were agglomerated that these complexes have polycrystalline with nanosized grains.

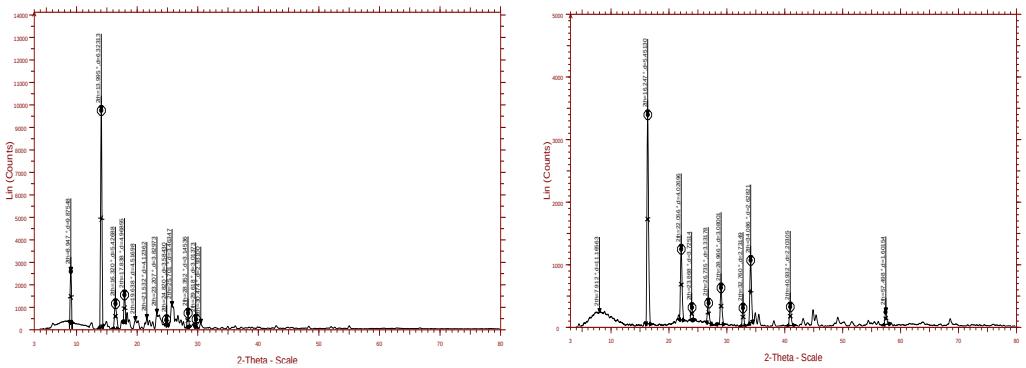


Figure No.1: Powder XRD pattern of ligand (L) and its Cu(II) complex

3.2 SEM – EDAX Analysis

Morphology of synthesized ligand and complexes were characterized by SEM analysis. SEM images of ligand L and its Cu(II) complex were shown in figure:2. SEM picture of the metal complexes shows that the particles are agglomerated with controlled morphological structure and the presence of small grains in non-uniform size. After agglomeration, SEM image of compounds shows irregular shaped grains with elongated morphology and increased particle size. The results of Energy Dispersive X-ray analysis (EDAX) data reveals the purity of the complex which indicates that there is no elemental contamination present in the complex. The % content of elements in the complex is C (51.04), O (20.36), S (5.09), Cl (5.48) and Cu (10.20) respectively.

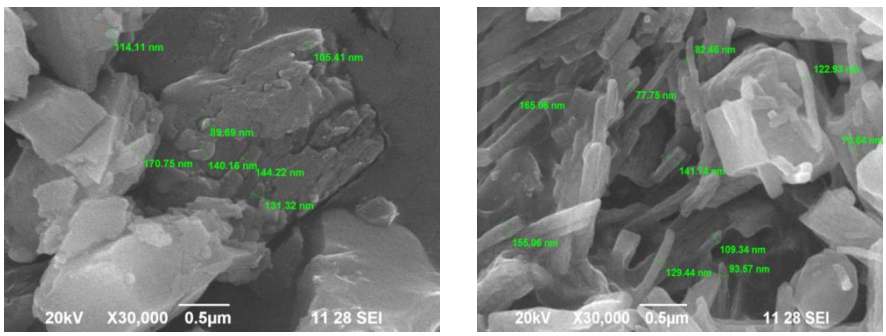


Figure No.2: SEM images of Ligand and its Cu(II) complex

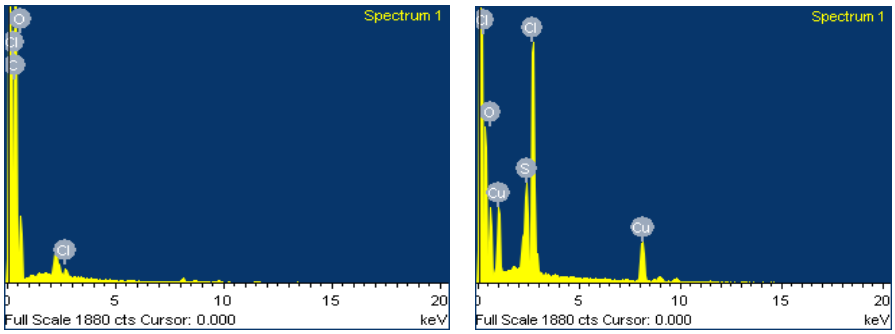


Figure No.3: EDAX images of Ligand (L) and its Cu(II) complex

3.3 Antioxidant activity

Antioxidant activity evaluation of ligand and its complexes was measured in terms of decreases in absorbance at 517 nm of DPPH methanolic solution (0.1 mmol) produced by the effect of each compound as a result of their ability to donate a hydrogen giving to the reduced form of DPPH radical. The reducing abilities of the synthesized compounds were determined by their interaction with the free radical DPPH at 20 mg concentrations for 15 min. This investigation indicates that there is a greatest possibility of finding potent antioxidants. The examined changes in the free radical scavenging ability of the test samples on the basis of percent inhibition are presented in Table:1.

Table No.1: DPPH assay of L and its complexes

S.No.	Compounds	% of Inhibition (mg/ml)
Control	-	100
1	L	90.4
2	L-Mn	92.8
3	L-Co	92.0
4	L-Ni	88.9
5	L-Cu	31.5
6	L-Zn	71.5

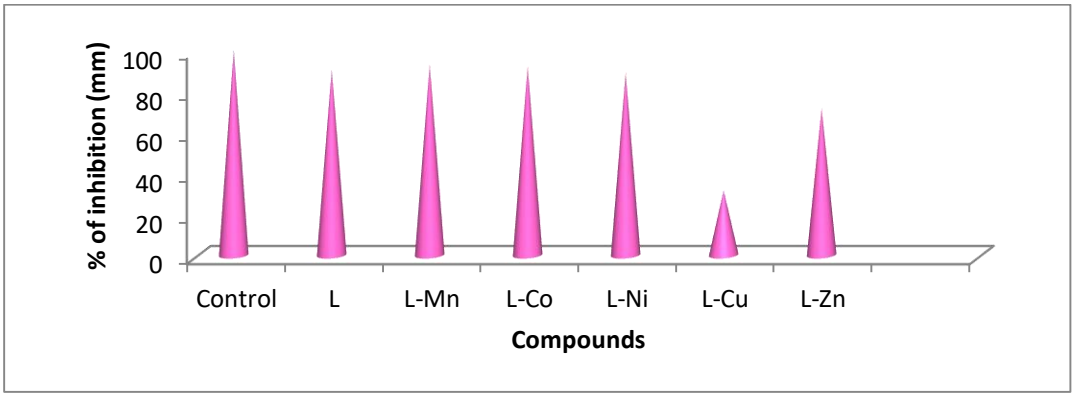


Figure No.4: Bar diagram representation of antioxidant activity

Result reveals that L-Mn and L-Co have exhibited very good free radical scavenging activity. Ligand (L), L-Ni and L-Zn complex shows moderate activity. L-Cu showed less activity compared to standard. Radical scavenging activity of metal complexes as well as the standards was increased in a dose-dependent manner, antioxidant ability of Schiff bases increased significantly after chelation of transition metal ions. The oxidizing potentials of the samples are associated with the presence of compounds to exert actions by breaking the free radical chain via hydrogen atom donation (Hernandez et.al.,2000). Therefore, the results obtained from this study provide linkage to the use of the synthesized compounds in the treatment of pathological diseases arising from oxidative stress. The bar graph representation of percentage of free radical scavenging activity is shown in figure:4.

3.4 Larvicidal activity

The larvicidal activity of the Schiff base ligand and the copper complex was performed against the larvae of culex and the result of mortality values are listed in table :2.The *Culex* mosquito larva mortality was calculated of various concentrations of copper complexes in the range of 25 to 100 ppm. Among the tested concentration, larva mortality was recorded from 25 to 100 ppm concentration for the period of 60h, whereas the percentage of mortality was significantly increased from the 25 to 100 ppm. The Cu(II) complex showed highly significant larvicidal activity than the ligand. Thus the larvicidal effect of these synthesized complexes may make these compounds to serve as potential insecticidal substances in mosquito control.

Table No.2: Larvicidal activity of ligand and their Copper(II) complex in various concentrations

Compounds	Concentrations (ppm)	Mortality rate at different time intervals (%)				
		12 hr	24 hr	36 hr	48 hr	60 hr
L	25	0	5	10	15	20
	50	0	15	20	25	35
	100	10	20	35	55	80
L-Cu	25	5	20	25	35	50
	50	10	25	45	55	70
	100	20	45	55	80	90

The average larval mortality data were subjected to statistical analysis for calculating LC_{50} and LC_{90} for synthesized Cu(II) complex. Minimum lethal concentration of the complexes indicates the more toxicity of the complex towards larvae.

LC50 value of copper complex = 50ppm

LC90 value of copper complex = 100ppm.

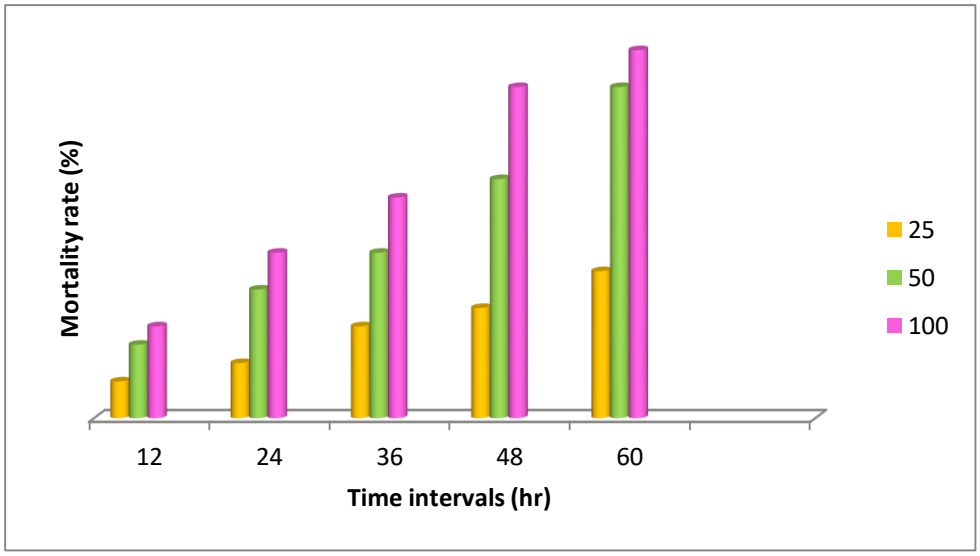


Figure No.5: Larvicidal activity of ligand and their Copper(II) complex in various concentrations.

The metal complex showed enhanced larvicidal activity than the Schiff base. The increased mortality rate observed for cu complex can be attributed to the increase in lipophilicity on complexation (Choudary et.al., 1994). Chelation increases the lipophilic nature of the central metal atom, which in turn, favours the molecules in crossing the cell membrane of the microorganism and enhancing larvicidal activity of complex.

3.5 Evaluation of Anti-tumour activity

The in-vitro anticancer activity of the ligand (L) and its Zn(II), Cu(II), Co(II) and Mn(II) complexes was determined by sulforhodamine -B assay using ADR (Adriamycin) taken as a reference on human breast cancer cell line MCF-7 and human Leukemia cell line K-562. The test compounds were examined at various concentrations and the LC50, TGI and GI50 values obtained for each compounds are summarized in Table:3. The good results however were obtained for all the complexes. Interestingly, all compounds were found to be active against Breast cancer cell line MCF-7. The good results however were obtained for all the complexes. Interestingly, all compounds were found to be active against Breast cancer cell line MCF-7. At the concentration of 80 mg/ml, compounds L-Mn, L-Zn and Ligand (L) showed 3.7, 4.4 and 6.2% inhibition respectively, in compared to Adriamycin 20.3 and 2.8 inhibition indicating that these compounds possess anticancer activity to a greater extent.

Table No.3: Cytotoxicity of Schiff base (L) and its Zn(II), Cu(II), Co(II) and Mn(II) metal complexes on MCF-7 and K-562 cancer cell lines.

	% Control Growth							
	Drug Concentrations (µg/ml)							
	Human Breast Cancer Cell Line MCF-7				Human Leukemia Cell Line K-562			
	10	20	40	80	10	20	40	80
L	21.2	15.2	0.3	6.2	42.3	50.5	77.2	28.0
L-Zn	18.7	15.1	12.6	4.4	18.2	19.6	32.3	27.9
L-Cu	50.3	62.2	57.2	73.8	11.3	28.9	56.5	69.3
L-Co	29.2	21.9	18.3	23.4	14.5	19.3	32.7	19.7
L-Mn	55.2	31.2	23.9	3.7	-2.5	-8.6	20.9	26.5
ADR	20.3	5.9	3.5	2.8	-22.3	-16.0	-7.4	15.0

Biological evaluation of Leukemia cancer cell line K-562 indicates, concentration of drug that produces total inhibition of the cells (TGI) shows that L-Co, L-Mn and L-Zn exhibited more potent. Moderate activity was found for ligand(L) and no activity was found forL-Cu. Among four dose level of compounds, maximum inhibitory activity was found at 80 µg/ml. Percentage control growth results of all the compound are in line with reference ADR. Results shows that Ligand (L) and its Zn(II),Co(II) and Mn(II) complexes are super active on human cancer cell lines MCF-7 in the assay system used with GI_{50} near or less than 10 µg/ml which is comparable to that of Adriamycin (ADR), a standard positive control drug with GI_{50} value less than 10 µg/ml. Therefore ligand and complexes may prove as lead compounds for in-vivo screening of anticancer activity.

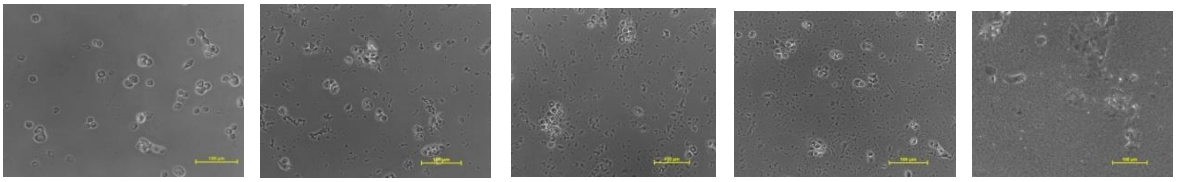
Table No.4: Cytotoxicity of Schiff base ligand, L and its Zn(II), Cu(II), Co(II) and Mn(II) metal complexes on MCF-7 and K-562 cancer cell lines

	Human Breast Cancer Cell Line MCF-7			Human Leukemia Cell Line K-562		
	LC_{50}	TGI	GI_{50}*	LC_{50}	TGI	GI_{50}*
L	NE	NE	<10	NE	NE	35.4
L-Zn	NE	NE	<10	NE	NE	<10
L-Cu	NE	NE	NE	NE	NE	48.0
L-Co	NE	NE	<10	NE	NE	<10
L-Mn	NE	NE	<10	NE	NE	<10
ADR	NE	NE	<10	NE	NE	<10

Value GI50* of <10 µg/ml-superactive, 10-15 µg/ml- moderately active, 15-30 µg/ml- Weakly active, 30-80 µg/ml-Resistant, >80 µg/ml- inactive

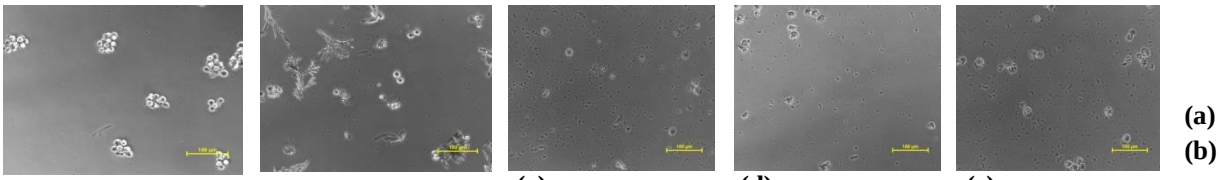
GI50= Concentration of drug causing 50% inhibition of cell growth, TGI= Concentration of drug causing total inhibition of cell growth, LC 50=Concentration of drug that 50% of the cells, NE-Non evaluable data, ADR= Adriamycin (Doxorubicin, Positive control drug).

The morphological observation showed that cells had normal spindle shaped morphology at all (10, 20, 40 and 80 µg/ml) concentrations of various samples are shown in Figure:6-7.



(a) (b) (c) (d) (e)

Figure No.6: Anticancer activity; Images of Ligand & metal complexes (a) Reference (b) L (c) L-Zn (d) L-Co and (e) L-Mn on human breast cancer cell line MCF-7



(a) (b) (c) (d) (e)

Figure No.7: Anticancer activity; Images of Ligand & metal complexes (a) Reference (b) L (c) L-Zn (d) L-Co and (e) L-Mn on Leukemia cancer cell line K562

IV. CONCLUSION

In this study, a Schiff base ligand (Curcumin and methyl-3-amino-2-thiophene carboxylate) was synthesized. They formed stable complexes , 1:1(L:M ratio) with transition metal ions such as Zn(II), Cu(II), Ni(II), Co(II) and Mn(II). The synthesized compounds were characterized by XRD and SEM-EDAX analysis. They are also tested for antioxidant and larvcidal activity. The XRD and SEM analysis explains the nanocrystalline structure of the compounds. EDAX studies gives information about metal purity and elemental composition. Antioxidant studies reveal that most of the synthesized compounds have potential antioxidant activity. The larvicidal activity of copper complex showed increased mortality rate than the ligand. Finally the anticancer activity of the ligand (L) and its Mn(II), Co(II), Cu(II) and Zn(II) Complexes was determined by sulforhodamine -B assay on human breast cancer cell line MCF-7 and human Leukemia cell line K-562. Results revealed that the ligand (L) and its Mn(II), Co(II) and Zn(II) complexes are super active on human cancer cell lines MCF-7 and K-562 when compared with reference ADR.

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