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Full Length Research Paper

Synthesis, characterization and antimicrobial potential of transition metal complexes of triacetic lactone

Samina Shahid[1], Muhammad Asam Raza[2]* and Shafiq-Ur-Rehman[2]

[1]Institute of Chemistry University of the Punjab, Pakistan.
[2]Department of Chemistry, Government College University Lahore-54000 Pakistan.

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Seven novel and biological active transition metal complexes were prepared using 3-nitro- 4-hydroxy -6-methyl-pyran-2-one derivative of triacetic lactone as ligand. These complexes were characterized by elemental analysis, magnetic susceptibility measurement, UV/Vis, FTIR and NMR spectroscopy. These data showed that solid complexes of Ni (II), Co (II), Mn (II), Zn (II) and Fe (II) indicated octahedral geometries while Cu (II) complex was assigned square planar geometry. The antimicrobial activities of all above mentioned complexes along with ligand were tested against eleven different bacterial strains and ten fungal strains using agar well diffusion assay. Cu (II) complex showed highest inhibition zone against *Shigella dysentriae* (22.3 mm), greater than standard drug, while the Zn (II) complex showed maximum antifungal activity against *Trichophyton simii* (18.7 mm). The antimicrobial activities indicated that metal complexes showed enhanced activity as compared to the free ligand.

Key words: Transition metals complexes, antimicrobial activity, agar well diffusion assay, spectroscopy.

INTRODUCTION

Transition metals have varying utility and interesting chemistry. Coordination compounds are important due to their role in biological and chemical systems in various ways. It has been observed that metal complexes with appropriate ligands are chemically more significant and specific than the metal ions and original (Steinhardt and Beychok, 1964; Mildvan, 1970). Currently the significance of metal ions in various biological systems has become important, as they are more powerful inhibitor of an enzyme as compared to uncomplexed biological active compounds. Moreover, the evidences supporting the use of metal complexes in the fight against cancer, tumor, viruses and bacteria have further made this subject a matter of great research interest. There are a large number of metal complexes that are anticancer, anti-tumor and antibacterial (Rosenberg, 1971). The complexation of metallic elements with biologically inactive compounds renders them active; and in case the compounds are already active, it makes them more active. The mechanism involved in enhancing this biological activity upon complexation is still needed to be further investigated (Dioxon and Webb, 1964; Sender et al., 1965; Mahler and Cordes, 1966; Williams, 1972).

Much more attention has been devoted by bioinorganic as well as by medicinal chemists to develop the relationship between the metal ions and their complexes to use as antitumor and antibacterial agents. *In vitro* studies have indicated that some biologically active compounds may become more carcinostatic and bacteriostatic upon chelation. Such interaction of transition metal ions with amino acids, peptides and pyrones, are of immense biological importance. Several reviews shows that the metallo-organic chemistry of such compounds greatly influence their biological action highlighting the catalytic function or metals in many biological processes (Chohan et al., 2001).

Evidences supporting the introduction of metallic elements in several biological processes are rapidly accumulating. Kirshener et al. (1966) have investigated the antibacterial, antiviral and anticancer activities of more than twenty five inorganic compounds which included the metal atom as potentially significant part of the molecule. They suggested that the transfer of metal ion from the ligand to the cancer-associated viruses was a mechanism for releasing the anticancer drug in the locality of

*Corresponding author. E -mail: asamgcu@yahoo.com. Tel.: +92-333-8070850.

the tumor. Due to significant nature of metallic ions, their metal complexes are now being included in the search for ideal anticancer drugs.

The main objective of the present project is synthesis, characterization and biological activities of novel transition metal complexes from triacetic lactone (Figure 1).

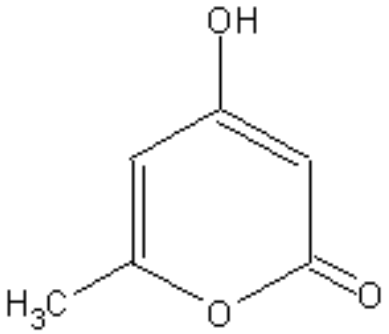

Figure 1. The structure of Triacetic lactone.

Experimental

Synthesis of (4-hydroxy-6-methyl-pyran-2-one) triacetic lactone

A mixture of dehydroacetic acid 15 ml (0.3 M) and 90% sulfuric acid (85 ml) was heated under nitrogen in an oil bath until the temperature of the solution reached upto 130°C. The temperature was maintained at 130-136°C for 2-3 h. The resulting solution was cooled in an ice-water mixture (350 ml), resulted in solid product and was recrystallized with ethyl acetate (Money et al., 1966).

Synthesis of 3-nitro 4-hydroxy-6-methyl -pyran-2-one

4-Hydroxy-6-methyl-pyran-2-one (0.04 M) was treated with concentrated sulphuric acid (5 ml) at room temperature. The reaction mixture was stirred followed by the addition of nitrating mixture (conc. H_2SO_4 and conc. HNO_3 in 1:1) to it. It was then maintained at the temperature 0 to -5°C. Then ice-water mixture was added in the reaction mixture. Fluffy precipitates appeared which were filtered, washed with cold water and dried.

Preparation of metal complexes

An ethanolic solution of 3-nitro-4-hydroxy-6-methyl-pyran-2-one (0.02 M, 3.42 g in 30 ml) was added to 20 ml ethanolic solution (0.02 M) of Copper (II) chloride, anhydrous Nickel (II) chloride, anhydrous Cobalt (II) chloride, Zinc (II) chloride, Mohr's salt, anhydrous Manganese (II) chloride, Silver nitrate. Then few drops of saturated solution of sodium carbonate were added to it. The reaction mixture was heated on a steam bath for one hour. After cooling, the resulting precipitate were filtered, washed with water, ethanol and then dried, which resulted in the formation of their respective metals complexes.

Characterization of ligand and complexes

The estimation of the metal in the metal complexes was obtained by using HITACHI Z-8000 Atomic Absorption Spectrophotometer. Amount of carbon in the complexes was estimated by using ELTRA (CS-800) Carbon-Sulphur Analyzer (Germany). The elemental analysis of H and N were determined on a Colemann Automatic Analyzer. The magnetic moments of the solid complexes were determined by the Gouy's method at room temperature. A double ended Gouy tube was calibrated using Hg [Co $(SCN)_4$] as a standard. The magnetic susceptibility of this compound was taken as 16.44+106 e.g. units at 20°C. the observed molar magnetic susceptibilities of the complexes were corrected for diamagnetism of the ligands and magnetic moments were calculated according to the procedure described by Figgs and Lewis (Fiffis et al., 1960). Ultra violet and visible absorption spectra of the complexes in the range 250-900 nm in DMF were obtained on a CECIL- C-7200 Spectrophotometer. The infra red spectra of the ligand and solid complexes in KBr Disc were recorded on a MIDAC 2000 Series Spectrophotometer. Proton NMR spectra of the pure ligand and their complexes were recorded on a Bruker (AM 300) spectrometer.

Antimicrobial activity

Antibacterial Activity was carried out at the Microbiology department, King Edward Medical University by using the agar well diffusion method (Naqvi et al., 1985) pathogenic bacteria were collected from different patients, admitted in Mayo Hospital Lahore.

All the synthesized ligand and corresponding metal complexes were screened *in-vitro* for their antimicrobial activities against bacterial strains (*Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhimurium, Shigella dysenteriae, Bacillus cereus, Corynebacterium diphtheria, Staphylococcus aureous*, *Streptococcus pyogenes* and *Tuberculi bacilli*) and fungal strains (*Trichophyto longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Aspergillus niger*, *Fusarium solani*, *Candida glaberta*, *Trichophyton simii*, *Rhiozopus oligosporus* and *Stachybotrys atra*) using agar well diffusion method. The samples were dissolved in DMSO to make a concentration of 100 µg/ml ligand and complexes and 2 mg/ml for standards. The inoculum (1 x 10^8 Cfu/mL) was added to molten agar and the media were shaken to disperse the microorganisms. Four millimeters diameter wells were punched in the agar with a sterile cork borer. 10 µl of the sample was introduced in the well. Imipenem was used as positive reference for bacterial strains while miconazole was used for fungal strains. Antimicrobial activity was evaluated by measuring the diameter of inhibition zone in mm. The experiments were conducted in triplicate.

RESULTS AND DISCUSSION

Analytical data of ligands and their metal complexes

Elemental analysis of the ligand and its metal complexes are reported in Table 1. The elemental analysis data agreed well with the proposed formulae for the ligand and metal complexes. The solubility of the complexes of ligand was studied in various solvents. Most of the complexes are soluble in DMSO and DMF while insoluble in common solvents such as methanol, ether, chloroform, n-hexane and carbon tetrachloride.

Characterization of the ligands and their complexes by spectroscopic measurements

FTIR

The FTIR spectrum (Table 2) of 3-nitro-4-hydroxy-6-

Table 1. Physico analytical data for ligand and its metal complexes.

Ligand/ Complex	Color	Yield (%age)	MP (^oC)	μ_{eff}* (B.M)	Found %age calculated			
					C%	H%	N%	M%
Ligand	Light yellow	-	165	Dia	42.10 (42.13)	5.00 (5.04)	8.15 (8.18)	-
Cu(L)2	Dark green	31	210	1.6	34.18 (34.17)	2.38 (2.39)	6.63 (6.64)	15.06 (15.07)
$Ni(L)_2H_2O$	Greenish yellow	33	270	3.5	33.14 (33.13)	2.76 (2.78)	6.44 (6.44)	13.50 (13.49)
$Mn(L)_22H_2O$	Light brown	23	245	3.8	33.44 (33.43)	2.81 (2.80)	6.48 (6.49)	12.73 (12.74)
$Fe(L)_22H_2O$	Rust Brown	32	285	5.1	33.35 (33.36)	2.79 (2.80)	6.45 (6.48)	12.95 (12.92)
$Co(L)_22H_2O$	Light Brown	27	190	3.8	33.12 (33.12)	2.77 (2.78)	6.42 (6.44)	13.53 (13.54)
$Zn(L)_22H_2O$	Off White	55	225	Dia	32.65 (32.64)	2.73 (2.74)	6.33 (6.34)	14.81 (14.80)
Ag(L)	Grey	46	160	2.4	25.82 (25.83)	1.80 (1.81)	5.02 (5.02)	38.65 (38.66)

*298 K.

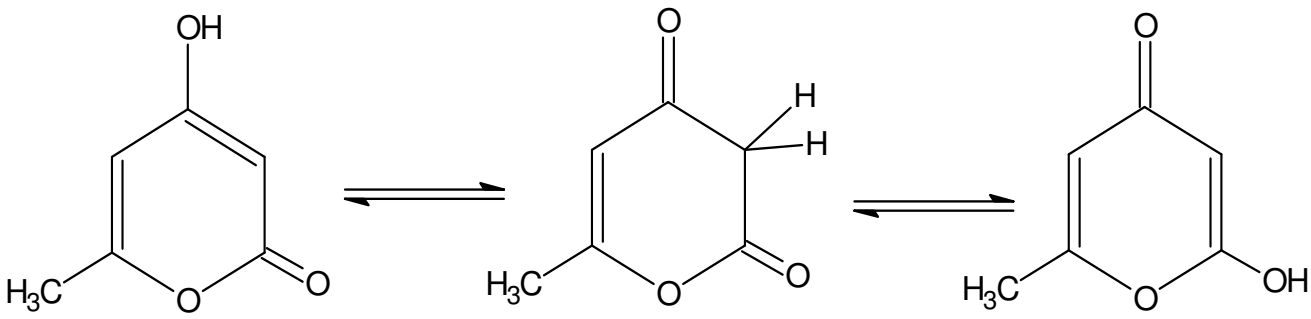

Figure 2. Tautomerism of triacetic lactone.

methyl-pyran-2-one gave a broad band which may be due to -OH group at frequency 3100 cm^{-1} and is at much lower frequency than the free -OH group (3600-3500 cm^{-1}). The pyrone ring showed C-H stretching at 3005 cm^{-1} while bending vibrations appeared at 720 cm^{-1}. The stretching frequency at 1600 cm^{-1} can be attributed to C=C bond. A strong band appearing at 1719 cm^{-1} can be assigned to ketonic carbonyl. Other bands at 1345 and 1250 cm^{-1} are due to stretching vibrations of the ketone, while a medium band at 1105 cm^{-1} may be assigned to C-N stretching vibrations (Abu-el-Wafa et al., 1989). As the spectrum was recorded in nujol therefore the asymmetric vibration of $-NO_2$ group cannot assigned due to presence of Nujol peak in the same region while symmetric vibration of $-NO_2$ group is observed at 1292 cm^{-1}which is lower than normal frequency (Rehman et al., 2008).

NMR

In the ^1H NMR spectrum the enolic -OH signals, observed at δ 16.2 due to deshielding effect of electronegative oxygen atom in the spectrum of ligands disappeared in complexes confirming that the bonding of metal ion to the ligands takes place through a proton displacement from the enolic -OH group. The new signals observed in the spectrum of all metal complexes except copper and silver salt at δ 3.3-3.6 (Khandar et al., 2002) and are not present in the spectrum of free ligand which can be assigned to H_2O molecules associated with complex formation. This fact is also supported by infrared spectroscopy.

Electronic spectra

The electronic spectra (Table 2) of nickel (II) complex

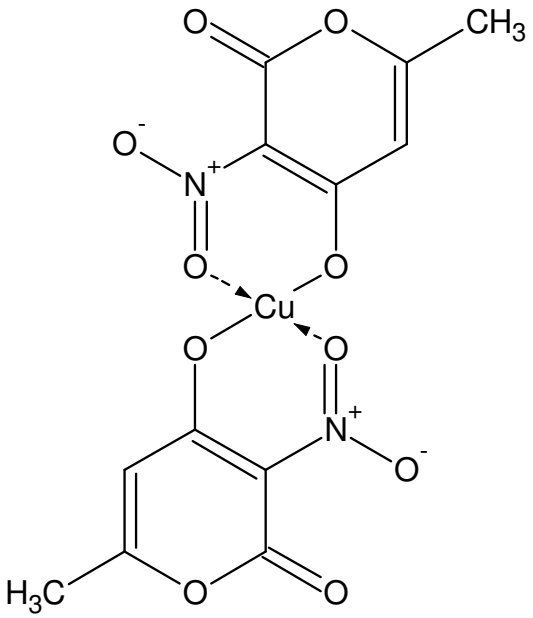

Figure 3. Proposed Structure of Cu $(L)_2.2H_2O$.

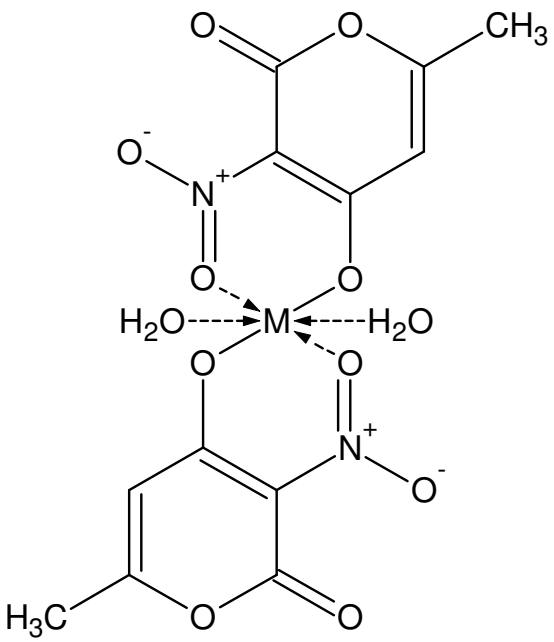

Figure 4. Proposed structure of of $M(L)_2.2H_2O$. Where M = Ni (II), Mn(II), Ni(II),Zn(II) and Co(II).

with ligand showed d–d transition in the energy regions 975 to 970, 640 to 636 and 375 to 370 nm. These are assigned to the transitions $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow {}^3T_{tg}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{2g}(P)$ respectively, consistent with their well defined octahedral configuration. In $[Co(L_4)_2.2H_2O]$ these peaks are observed at 965, 575, 484 and 365 nm low energy 970 - 965 and strong high energy peaks at 365-352 nm, which are assigned to the tran-

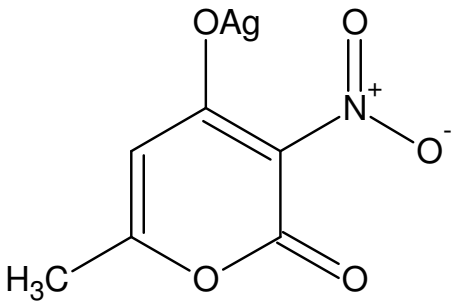

Figure 5. Proposed structure of Ag(L).

sitions $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ respectively, where the last one which is almost in between the UV-Visible and is assigned as metal –charge transfer spectra (Pasto, 1969).

Antimicrobial activities

The zone of inhibition was measured in mm and ANOVA (software) was applied on the results of antimicrobial assay. The results are shown in Tables 3 and 4. The maximum antibacterial activity was show by the Cu (II) against *S. dysentriae* which is 22.3 mm which is greater than standard drug. Similarly the maximum antifungal activity was shown by the Zn (II) complex against *T. simii* which is 18.7 mm. It was observed that metal complex have high antimicrobial activities than their parent ligands. It was also concluded that ligand and complexes are more active against bacterial strains than fungal strains. By using these complexes, the inhibition of growth of fungal and bacterial strains indicated that complexes have dynamic activity against these strains. Results showed that mechanism of inhibition of different complexes vary from species to species.

Conclusion

Modern research on the subject of medicinal substances is a hot issue today. Traditional way of treating any infection and diseases was the use of botanical extracts that was so costly, laborious and time consuming. Due to increasing rate of human population as well as infections and diseases caused by a number of microorganisms including both fungal and bacterial strains, it is almost impossible for the chemist of present era to use the traditional way of manufacturing drugs like antibacterial and antifungal.

These were the facts that ultimately become the motivation to plan the presented work on “Characterization of ligand and their metal complexes”. In this study, synthesized ligand and its corresponding metal complexes (Cu(II), Ni(II), Mn(II), Fe(II), Zn(II), Co (II) and Ag (I)) were used against different bacterial strains. Imipe-

Table 2. Electronic spectroscopic data of metal complexes.

Compound	λ max (nm)	Solvent	Assignments	FTIR peaks
Ligand	285	DMF	-	3050(s),3005(b,s),1719(s),
				600(s),1460(m), 1292(m), 720(m)
Cu(L)2	670, 515, 330	DMF	$^2B_{1g} \rightarrow {}^2A_{1g}$, $^2B_{1g} \rightarrow {}^2E_g$,	3050(s), 2910(m,s), 1720(s),
			Charge Transfer	1600(s),1450(m),1280(m), 720(m)
$Ni(L)_2H_2O$	873, 630, 375, 331	DMF	$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$. $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$	3400(b,s), 3050(s), 1720(s),
			metal charge transfer	1600(s), 1451(m), 1280(m), 721(m)
$Mn(L)_2 2H_2O$	508, 410, 345	DMF	$^6A_{1g}(F) \rightarrow {}^4T_{1g}(G)$, $^6A_{1g} \rightarrow {}^4T_{2g}(G)$	3569(b,s), 3050(s), 1722(s), 600(s),
			Charge Transfer	1451(s), 1280(m), 720(m)
$Co(L)_2 2H_2O$	870, 575, 485, 352	DMF	$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$, $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$	3400(b,s), 3050(s), 1720(s),
			Charge Transfer	1600(s), 1456(s), 1280(m), 721(m)

s = Sharp, m = medium, b = broad, w = weak.

Table 3. Antibacterial activity of ligand and its transition metal complexes.

Compound	Zone of Inhibition (mm)										
	A	B	C	D	E	F	G	H	I	J	K
Control*	20.2 ± 0.2	20.5 ± 0.7	20.7 ± 0.8	19.5 ± 0.8	18.2 ± 0.6	21.4 ± 1.2	21.3±0.8	20.1±1.0	18.8±0.5	18.3±0.5	20.3±0.4
Ligand	10.5 ± 0.5	11.3 ± 1.0	10.4 ± 0.5	12.3 ± 0.8	11.3 ± 1.2	12.0 ± 0.5	12.2±0.7	11.8±0.7	10.3±0.5	11.3±1.2	13.5±0.9
Cu(L)2	18.6 ± 0.6	17.6 ± 0.8	19.2 ± 1.0	18.5 ± 0.8	17.8 ± 0.7	22.3 ± 0.5	20.5±1.3	19.3±1.0	17.4±1.3	18.0±1.2	19.4±0.8
$Ni(L)_2H_2O$	16.8 ± 0.5	16.4 ± 0.5	18.4 ± 0.7	17.3 ± 0.6	18.2 ± 1.3	18.4 ± 1.0	19.2±1.1	17.3±0.7	17.5±1.2	16.9±0.6	15.8±1.0
$Mn(L)_2 2H_2O$	18.2 ± 0.7	17.6 ± 0.8	14.9 ± 0.7	18.2 ± 0.8	15.8 ± 0.6	17.0 ± 0.9	16.5±1.4	18.2±0.8	18.0±1.0	17.6±0.7	18.2±0.8
$Fe(L)_2 2H_2O$	18.3 ± 0.6	18.7 ± 0.5	18.0 ± 0.5	17.2 ± 1.2	16.9 ± 0.8	18.5 ± 1.1	18.7±0.8	17.3±0.5	18.5±0.5	16.0±0.5	17.2±1.3
$Co(L)_2 2H_2O$	15.6 ± 0.9	18.3 ± 0.6	17.8 ± 0.9	18.3 ± 1.0	17.6 ± 0.5	17.4 ± 0.8	16.7±0.8	18.4±0.8	19.8±0.6	16.2±0.8	18.5±1.1
$Zn(L)_2 2H_2O$	17.6 ± 1.2	18.2 ± 0.7	19.3 ± 0.7	17.9 ± 0.8	18.6 ± 0.7	17.3 ± 1.3	20.4±0.6	18.6±0.7	17.9±0.4	18.0±0.8	18.5±0.8
Ag(L)	19.3 ± 1.0	18.4 ± 0.7	17.6 ± 0.8	18.2 ± 1.2	19.3 ± 0.5	18.2 ± 1.2	20.0±0.9	21.0±1.2	18.1±0.9	17.8±1.0	18.6±0.7

* Imipenem.
A = *Escherichia coli*, B = *Klebsiella pneumonae*, C = *Proteus mirabilism*, D = *Pseudomonas aeruginosa*,E = *Salmonella typhi*, F = *Shigella dysentriae*, G = *Basillus cereus*, H = *Corynebacterium diphtheriae*, I = *Streptococcus pyogenes*, J = *Staph aureous* , K = *Staph aureous*, and L = *Tuberculi bacilli.*

num was used as control for antibacterial activity, to compare the results obtained from the ligand and their metal complexes. The activity of various metal ions, organic compounds and metal complexes in biological systems has been explained on the basis of different complex species formed in the living organisms. Metal ions are also found in several bacterial species and reported to play an important role in different enzymatic and phy-

Table 4. Antifungal activity of ligand and its transition metal complexes.

Compound	Zone of inhibition (mm)									
	A	B	C	D	E	F	G	H	I	J
Control*	17.6 ± 1.2	18.2 ± 0.7	19.3 ± 0.7	17.9 ± 0.8	18.6 ± 0.7	17.3 ± 1.3	20.4 ± 0.6	18.6 ± 0.7	17.9 ± 0.4	18.0 ± 0.8
Ligand	12.4 ± 0.4	11.4 ± 1.2	10.8 ± 0.8	10.0 ± 0.5	11.3 ± 1.2	12.0 ± 0.5	12.2 ± 0.7	11.8 ± 0.7	10.3 ± 0.5	11.3 ± 1.2
Cu(L)2	17.4 ± 0.6	17.6 ± 0.8	16.2 ± 1.0	16.5 ± 0.8	17.8 ± 0.7	19.3 ± 0.5	20.5 ± 1.3	18.3 ± 1.0	17.4 ± 1.3	18.0 ± 1.2
$Ni(L)_2H_2O$	16.8 ± 0.5	16.4 ± 0.5	18.4 ± 0.7	17.3 ± 0.6	18.2 ± 1.3	17.4 ± 1.0	16.2 ± 1.1	17.3 ± 0.7	17.5 ± 1.2	16.9 ± 0.6
$Mn(L)\ 2H_2O$	15.6 ± 0.9	18.3 ± 0.6	17.8 ± 0.9	18.3 ± 1.0	17.6 ± 0.5	17.4 ± 0.8	16.7 ± 0.8	17.4 ± 0.8	17.8 ± 0.6	16.2 ± 0.8
$Fe(L)_2 2H_2O$	16.3 ± 0.6	18.7 ± 0.5	18.0 ± 0.5	17.2 ± 1.2	16.9 ± 0.8	17.5 ± 1.1	15.7 ± 0.8	17.3 ± 0.5	16.5 ± 0.5	16.0 ± 0.5
$Co(L)_2 2H_2O$	15.6 ± 0.9	16.3 ± 0.6	16.8 ± 0.9	15.3 ± 1.0	17.6 ± 0.5	17.4 ± 0.8	16.7 ± 0.8	18.4 ± 0.8	17.8 ± 0.6	16.2 ± 0.8
$Zn(L)_2 2H_2O$	17.6 ± 1.2	18.2 ± 0.7	18.3 ± 0.7	17.1 ± 0.6	18.6 ± 0.7	15.3 ± 1.3	18.2 ± 0.6	18.7 ± 0.7	16.3 ± 0.4	17.0 ± 0.8
Ag(L)	16.3 ± 1.0	18.4 ± 0.7	17.6 ± 0.8	17.2 ± 1.2	17.5 ± 0.5	18.2 ± 1.2	18.5 ± 0.9	16.0 ± 1.2	15.8 ± 0.9	17.8 ± 1.0

*Micanozole.
A = *Trichophyto longifusus*, B = *Candida albicans*, C = *Aspergillus flavus*, E = *Microsporum canis*, D = *Aspergillus niger*, F = *Fusarium solani*, G = *Candida glaberta*, H = *Trichophyton simii*, I = *Rhiozopus oligosporus*, and J = *Stachybotrys.*

siological reactions. It has been reported that pyrone derivatives act as antimicrobial (toxic) effect (Rehman et al., 2008). However the biological activity of Cu (II), Ni (II), Mn (II), Fe (II), Zn (II), Co (II) and Ag (I), with derivatives of triacetic lactone, has not been studied earlier.

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