Journal of Materials and Environmental Sciences
ISSN : 2028-2508
CODEN : JMESCN

Copyright © 2018,
University of Mohammed Premier
Oujda Morocco

J. Mater. Environ. Sci., 2018, Volume 9, Issue 7, Page 2153-2157

http://www.jmaterenvironsci.com

Antioxidant and Antimicrobial Activity of Some Transition Metal Complexes with Non-natural Amino Acids Used As Ligand

F. Hadjer [1], B. Tahar[1], A. Djallal. Eddine [2], D. Sofiane*[1]

[1]*Physical Chemistry Studies Laboratory, University of Saïda Dr. Moulay Tahar, Algeria.*
[2]*Laboratory of Biotoxicology, Pharmacognosy and Biological recovery of plants, Department of Biology, Faculty of Sciences, University of Dr. Moulay Tahar, Saida, Algeria.*

Received 31 Jul 2017,
Revised 07 Feb 2018,
Accepted 10 Feb 2018

Keywords
- ✓ Non-natural Amino Acids,
- ✓ Quaternary Ammonium Salts,
- ✓ Metal Complexes,
- ✓ Antimicrobial Activity,
- ✓ Antioxidant Activity.

daoudi_20@yahoo.fr;
Phone: +213661455427

Abstract

In this study, our main objective was to evaluate the antioxidant and antimicrobial activity of three new metal complexes of Cu(II), Fe(III), Co(II), with non-natural amino acids containing quaternary ammonium salts moieties used as ligand derived from 2-(diethylamino)ethylmethacylate (DEAEMA). All the compounds tested in this work were evaluated for their in vitro antimicrobial activities. And for their possible in-vitro antioxidant activity through free radical scavenging activity using DPPH (1,1-diphenyl-2-picryl-hydrazyl) method. All the compounds showed DPPH radical scavenging activity, where compound CuL_2 and FeL_3 was the best radical scavenger by IC_{50} of 0.84 and 1.43 mg/ml, respectively. The results of antimicrobial activity showed that the tested compounds exhibited good to moderate activity against the tested bacterial and fungal strains.

1. Introduction

The search for new antimicrobial agents is an important line of research because of the resistance acquired by several pathogenic microorganisms [1], which cause damage to human health [2], exhibit drug resistance due to inadequate use of antibiotics [3]. Thus, there is a need for the discovery of new substances. The field of bioactive *coordination* chemistry, which deals with the study of role of metal complexes in biological systems during the past decades, much attention has been given to the synthesis of new metal complexes and the evaluation of these agents for antibacterial activity [4, 5]. Metal ions are required for many critical functions in the Biological periodic system. [6] Metal complexes or coordination complexes; have been widely used in medicine [7] and pharmaceutical fields [8] because of their broad bioactivities of antibacterial, antifungal [9, 10]. They have been evaluated against several pathogenic fungi and bacteria with promising results. Some of the recent studies have shown that metal complexes are reported to possess antitumor [11], antiviral [12], they are also used in the treatment for diabetes [13] and anti-HIV [14]. Some metals complexes have been used as drugs [15] and diagnostic agents [16] to treat a variety of diseases and conditions [17]. The importance of metal ions in biological systems is well known. One of the most interesting features of metal coordinated systems is the concerted spatial arrangement of the ligands around the metal ion [18].

In the recent years quaternary ammonium compounds (QAC) have received much attention because of many uses for a variety of clinical purposes [19]. They have been extensively employed as antimicrobial [20], antifungal [21], antimalarial [22], disinfectants [23], biocides, insecticidal [24], herbicidal [25], muscle relaxants [26] anti-inflammatory [27], cytotoxicity [28]. Metal complexes with ligands containing quaternary ammonium moieties have interesting properties due to their diverse multifunctional groups. Thus, the development of this compound is now attracting the attention of medicinal chemists. In the literature, there are few reports concerning biological properties of metal complexes containing QAC. In view of the previous rationale and as a

part of continuation of our extensive research program aimed at the discovery of novel bioactive compounds, the aim of this study was to determine the antioxidant and antimicrobial properties of new ligand and its metal complexes of Cu (II), Fe (III), and Co (II) and we summarize our results.

2. Material and Methods

2.1.1 Tested compounds

The tested compounds were synthesized and confirmed by spectral data by our group [29]. Chemical structures of free ligand and its metal complexes are shown in (Figure 1).

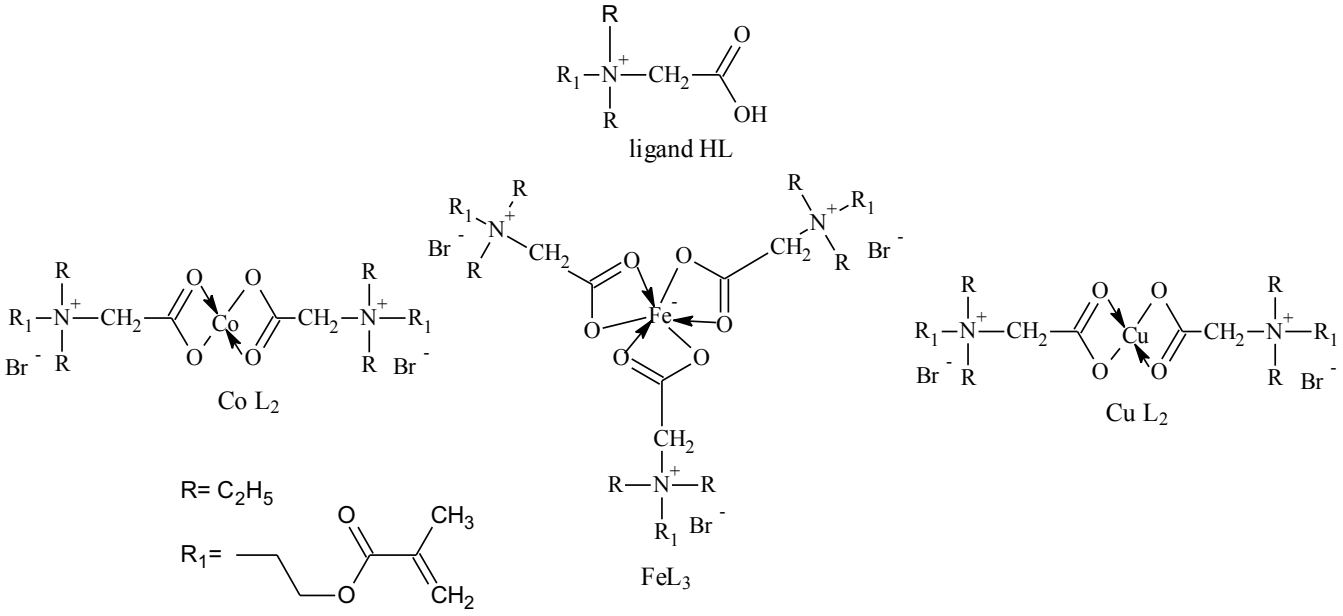

Figure 1: Chemical structures of the ligand and its metal complexes.

2.1.2 Antioxidant activity

The free radical scavenging activity of the tested compounds was studied *in vitro* by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay method [30], methanolic solution of each tested compounds having different concentrations (0.12, 0.24, 0.48, 0.79, 1.49, 3.89 and 7.79 mg/ ml) was mixed with DPPH methanol solution. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. then; absorbance was measured at 517 nm. by using spectrophotometer. L-Ascorbic acid were used as positive controls. The radical scavenging activities were expressed as the inhibition percentage and were calculated using the formula:

$$\text{Radical scavenging activity (\%)} = [(Ac\text{-}As)/Ac) \times 100]$$

Where Ac is absorbance of the control (without compound) and As is absorbance of the tested compounds. The IC50 parameter, which represents the concentration of the material in question necessary to inhibit 50% of free radicals. Thus, a lower IC50 value indicates a greater ability to neutralize free radicals. The radical scavenging activities of the tested compounds are summarized in Table 1.

Table 1: Antioxidant activity of the tested compounds

Compounds	**DPPH scavenging activity Concentration (mg/ ml) and % inhibition**							**IC50 Value (mg/ ml)**
[C] (mg/ml)	**0.12**	**0.24**	**0.48**	**0.79**	**1.49**	**3.89**	**7.79**	
HL	4.13	25.45	34.38	39.00	46.77	50.90	55.37	5.13
CuL_2	13.11	50	56.66	62.11	64.22	68.88	77.66	0.84
CoL_2	12.06	27.1	36.03	42.31	50.08	53.38	54.04	4.95
FeL_3	3.11	47.55	54.88	61.88	63.66	66.88	76.66	1.43

As indicated in table 1, the percentage inhibition of the free radical appears to increase with increasing concentration. Metal complexes CuL_2 and FeL_3 exhibited the highest antioxidant activity of 77.66 at 76.66 %,

with the *IC*50 value of 0.84 and 1.43 mg/ml. Other moderately active compounds of the ligand HL and metal complexes CoL_2 showed the *IC*50 values of 5.13 and 4.95 mg/ml, respectively.

2.1.3 Microorganisms used

Antimicrobial activities of free ligand and its metal complexes were tested against twelve microorganisms, including six strains of bacteria: *Enterococcus faecalis* ATCC 49452, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25933, *Campylobactr fetus* ATCC 27374 and *Enterobacter cloacae* ATCC 13047, the anti-yeast activities were tested against two *registered* yeast species, *Candida albicans* ATCC 10231 and *Candida albicans* IPP444. For antifungal activity four species were selected, *Aspergillus niger*, *Alternaria alternata*, *Rhizopus stolinifer* and *Aspergillus flavus.* All the microorganisms were obtained from the culture collection of the Laboratory of Biotoxicology, Pharmacognosy and Biological recovery of plants, Department of Biology, Faculty of Sciences, University of Saida Dr Moulay Taher, Algeria.

2.1.4 Antibacterial activity

The antibacterial activities of the tested compounds were screened for in *vitro* antimicrobial activity by measuring the Minimum Inhibitory concentrations (MIC) and the minimum bactericidal concentration (MBC) which give the lowest concentrations of compound inhibiting visible growth, according to the broth macrodilution method [31]. Three Gram-positive and three Gram-negative bacteria. A solution of the activated bacterial strain was made in sterile normal saline, the turbidity was adjusted using 0.5 McFarland standards. The tested compounds were dissolved in dimethyl sulfoxide (DMSO) to get various concentrations. A series of dilutions of each tested compound in the range concentration of 10, 5, 2.5, 1.5, 0.625, 0.3125, 0.156, 0.078, and 0.039 mg/ml were prepared. Fresh cultures of bacteria obtained by inoculating bacteria in Muller-Hinton broth. The plates were incubated at 37°C for 24 hours. Test was repeated twice and the average values of MIC and MBC results are presented in table 2.

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the tested compounds

Compounds	values of MIC/MBC (mg/ml)											
	Gram positive bacteria						**Gram negative bacteria**					
	Enterococcus faecalis ATCC 49452		*Bacillus subtilis* ATCC 6633		*Bacillus cereus* ATCC 11778		*Escherichia coli* ATCC 25933		*Campylobactr fetus* ATCC 27374		*Enterobacter cloacae* ATCC 13047	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
HL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
CuL_2	0.625	1.5	10	ND	5	10	ND	ND	ND	ND	ND	ND
CoL_2	0.625	2.5	0.312	5	1.5	5	2.5	5	2.5	5	10	ND
FeL_3	10	ND	10	ND	ND	ND	ND	ND	ND	ND	ND	ND

Samples with CMI>10 mg /ml were considered not determined (ND)

The tested compounds exhibited variable antibacterial activity against the above tested bacterial strains. The results of the minimum inhibitory concentration and minimum bactericidal concentration of tested compounds showed that varying antibacterial activity against tested bacterial. Compounds $\mathbf{CoL_2}$ exhibited very good antibacterial activity against all tested bacteria, metal complexes of $\mathbf{CuL_2}$ exhibited promising antibacterial activities against Gram positive bacteria, $\mathbf{FeL_3}$ showed moderate antibacterial activity against *Enterococcus faecalis* and *Bacillus subtilis.*

2.1.5 Antifungal activity

The antifungal activities were tested by the reported method[32], all metal complexes and free ligand were studied against four fungal cultures, the compounds were dissolved in dimethyl sulfoxide and tested at a concentration of 0.25, 0.5, 1.25 and 2.5 mg/ml, and mixed with 20 ml of sterile potato dextrose agar medium (P.D.A), the mixture was transferred to sterile Petri dishes of 9 cm diameter and allowed to solidify, then 0,6 cm disc of 3-7 day-old culture of the test fungi were placed at the center of the petriplates and incubated at 28 ± 4°C for 7 days, three replicates were conducted; the antifungal index was calculated as follows:

Antifungal index (%) = (1-Da/Db) × 100

Da: Area of mycelial colony grown with oil sample.
Db: Area of mycelial colony grown in control.

The values of Antifungal index results are presented in table 3.

Table 3: Antifungal index of the tested compounds

	Antifungal index (%) of the tested compounds															
compounds	***A.niger***				***A.alternata***				***R.stolinifer***				***A.flavus***			
Concentration (mg/ml)	**0.25**	**0.5**	**1.25**	**2.5**	**0.25**	**0.5**	**1.25**	**2.5**	**0.25**	**0.5**	**1.25**	**2.5**	**0.25**	**0.5**	**1.25**	**2.5**
HL	8.3	56.6	73.3	78.3	27.2	54.5	63.6	69	10	16.2	30.6	51.6	22	40	50	60
Cu L_2	55	62	65	81.6	45	58.1	60	62	6.6	10	11.1	16.6	9	11	18	32
Co L_2	8.3	75	81.6	88.3	40	45.4	54.5	53	22.5	28.7	43.1	77.5	42	60	70	84
Fe L_3	70	83	85	88.3	64	69.0	73	84	25	38	59	66	55	67	79	87

The results of the in vitro antifungal activity of compounds showed that metal complex compound **FeL_3** and **CoL_2** displayed good activity against all tested fungi at high concentration, while compound Cu L_2 showed moderate activity against *R.stolinifer* and *A.flavus,* the ligand **HL** displayed good activity against *A.niger*, *A.alternata* and moderate against *R.stolinifer*, *A.flavus.*

2.1.6 Anti-yeast activities

The yeast cultures used in these studies are *Candida albicans* ATCC 10231, *Candida albicans* IP 444, They are maintained into sterile Sabouraud dextrose liquid medium. The inoculum was prepared in saline solution. Its turbidity was adjusted in accordance with McFarland scale (0.5), which was equivalent to the absorbance of 0.08- 0.10 at 625 nm corresponding to 5 x 10^6 CFU/ml. Tests were performed in triplicate and plates were incubated at room temperature for 2-7days. A Reference method for broth dilution antifungal susceptibility testing of yeasts was used to determine minimal inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). Test was performed twice and average values of MIC and MFC are shown in Table 4.

Table 4: Minimum inhibitory and fungicidal concentrations of

Compounds	**Species of yeast**			
	***Candida albicans* ATCC 10231**		***Candida albicans* IP 444**	
	MIC mg/ml	**MFC mg/ ml**	**MIC mg/ml**	**MFC mg/ml**
HL	2.5	2.5	5	5
CuL_2	10	10	10	10
CoL_2	5	5	1.5	5
FeL_3	2.5	5	5	10

From Table 4 it is found that the ligand and all the metal complexes exhibited very significant Anti-yeast activity, the most active compound **CoL_2** with lowest MIC against *Candida albicans* IP 444. The copper complex was moderately active for species of yeast, the ligand exhibited good activity against *Candida albicans.*

Conclusion

We have reported the biological activity of new non-natural amino acid used as ligand and its metal complex of Cu(II), Co(II), Fe(III), The result of *in vitro* antimicrobial activity study indicated that all the compounds showed significant activity against several Gram positive, Gram negative bacteria and fungi strains. The results of antioxidant activity indicate higher free radical scavenging activity of metal complexes. These results make metal complex with non natural amino acids bearing quaternary amino groups interesting lead molecules for further synthetic and biological evaluation.

References

1. D. Julian, D. Dorothy, *Microbiol Mol Biol Rev*. 74-3 (2010) 417-433.
2. M. Fernández, J. A. Hudson, R. Korpela, C. G. de los Reyes-Gavilán , *Biomed Res Int*, 2015, (2015), 412714.
3. O. Uri, D.G. Eynat, Y.S. Gideon, H. Lilach, *Infect Genet Evol*. 40 (2016) 155-161.
4. S. Sangilipandi, S. Dipankar, B. Kaushik, K. Werner, S.R. Joshi, K.C. Asit, K.R. Mohan, *Inorg. Chim. Acta.* 441 (2016) 95-108.
5. I. Halil, Y. Cengiz, G. Aysel, S. Musa, *J Mol Struct.* 1120 (2016) 25-33.
6. M. Wolfgang, *Int. J. Mol. Sci*. 17- 66 (2016) 1-8.
7. B. Atreyee, *Sci. Revs. Chem. Commun*. 5-2 (2015) 77-87.
8. F.M.E. Reda, A.R. Ayaat, A.E. Emtithal, *Carbohydr Polym*.146 (2016) 376-387.
9. L. Zorica, V. Danijela, K. Milica, L. Nedeljko, Đ. Marijana, V. Aleksandar, *Polyhedron.* 80 (2014) 233-242.
10. S. Urvashi, M.M. Ali, A.B. Imtiyaz, A.A. Ajaz, N.B. Mohd, B. Sneha, A. Syed, A.H. Athar, *Microb Pathog*. 93 (2016) 172-179.
11. J. Lei, X. Jun, Z. Xiaolei, S. Shanshan, Z. Tao, X. Zhouqing, Z. Taofeng, C. Ruhua, M. Tieliang, X. Jing, D. Kun, H. Jiancui, *J. Inorg. Biochem*. 159 (2016) 107-119.
12. D. Rogolino, M. Carcelli, A. Bacchi, C. Compari, C. Laura, E. Fisicaro, A. Gatti, M. Sechi, A. Stevaert, L. Naesens, *J. Inorg. Biochem.* 150 (2015) 9-17.
13. A.P. Mohini, J.P. Manohar, A. Konde, *Saudi J Biol Sci.* 23(2016) 420-425.
14. S. Oriel, G. Sorenlis, R.H.P. Ángel, L. Yokoy, C. David, F. Mercedes, T. Peter, U. Izaskun, R.R. Héctor, T.O. Joseph, C. William, C.G. María, *Polyhedron.* 110 (2016) 14-23.
15. A.A. Warra, *J. Chem. Pharm. Res*. 3-4 (2011) 951-958.
16. W.Y.C. Kannie, W. Wing-Tak, *Coord. Chem. Rev.* 251 (2007) 2428-2451.
17. S.R. Pattan, S.B. Pawar, S.S. Vetal, U.D. Gharate, S.B. Bhawar, *Indian Drugs*. 49 (11) 2012.
18. P. G. Jenny, K. K. Amy, W. B. Charles, *The Rigaku J*. 16-2 (1999).
19. W.M.Yuen John, Y.K. Yung Jolene, *Mod Chem appl*. (2013) 1-3.
20. Z.S. Xu, W. Ning, C. Di, Y.H. Zi, M. Pu, B.Z. Hai, H.Z. Xiao, *Chin. Chem. Lett*. 22-8 (2011) 887-890.
21. O. Ewa, P. Agata, K. Anna, Ł. Jacek, *Microbiol. Res.* 168 (2013) 630-638.
22. B. Nicoletta, M. Mara, P.I. Denise, T. Donatella, S. Riccardo, P. Elena, *Bioorg. Med. Chem*. 23 (2015) 4681-4687.
23. T. Ulas, G.P. Spyros, *Curr Opin Biotechnol*. 33 (2015) 296-304.
24. A. Liu, X. Ou, M. Huang, X. Wang, X. Liu, Y. Wang, C. Chen, J. Yao, *Pest Manag Sci*. 61-2 (2005)166-70.
25. K.M. Roksana, R. Hubert, M. Bartosz, W. Filip, S. Agata, M. Katarzyna, P. Tadeusz, P. Juliusz, *Tetrahedron*. 70 (2014) 4784-4789.
26. B. Ranju, G. Sheetal, C.Y. Louise, L.H. Alan, *Steroids*. 76 (2011) 254-260.
27. M.C. Filion, N.C. Phillips, *Br J Pharmacol.* 122-3 (1997) 551-557.
28. S.S. Anastasiya, I.Y. Olga, V.S. Andrey, A.P. Michail, G.P. Andrey, A.L. Valentina, V.Z. Vladimir, S.T. Tatiana, M.A. Pavel, I.K. Oleg, B.B. Anatoly, F.S. Nariman, *Bioorg. Med. Chem.* 21 (2013) 6690-6698.
29. O.K. Zahra, B. Tahar, D. Sofiane, M. Mohamed, *Chem. Sci. Trans*. 4-2 (2015) 588-594.
30. C. Alasalvar, M. Karamac, R. Amarowicz, F. Shahidi, *J Agric Food Chem*. 54 (2006) 4826-4832.
31. S.H Siddiqi , L.B Heifets , M.H Cynamon , N.M Hooper , A. Laszlo , J.P. Libonati , P.J. Lindholm-Levy , N. Pearson, *J Clin Microbiol*. 31-9 (1993) 2332-8.
32. P. Fandohan, B. Gnonlonfin, K. Hell, W.F.O. Marasas, M.J.Wingfield, *Int J Food Microbiol*. 99 (2005) 173-183.

(2018) ; http://www.jmaterenvironsci.com