ACTA MEDICA SALINIANA

ORIGINAL PAPERS

# METAL COMPLEXES AS POTENTIAL AGENTS FOR NEUTRALIZING FREE RADICALS: A REVIEW OF *IN VITRO* STUDIES

**Merima Imamović[1], Emir Horozić[2], Vedad Salković[3], Armina Pašalić[4], Nermina Hadžić[5]**


ISSN 0350-364X

DOI: 10.5457/618

Merima Imamović, Emir Horozić, Vedad Salković, Armina Pašalić, Nermina Hadžić

ABSTRACT

Metal complexes due to their antibacterial, antifungal, antitumor and antioxidant properties have been extremely studied today. A large number of metal complexes have the ability to neutralize free radicals, which most often occur as a result of metabolic processes in the body, smoking, exposure to radiation and chemical substances. Testing the antioxidant capacity of metal complexes can be tested by various methods *in vitro* and *in vivo*. This paper will present the efficiency of neutralization of free radicals by metal complexes synthesized in the past few years using different ligands, as well as the most commonly used methods for testing the antioxidant capacity of this group of compounds.

## INTRODUCTION

Free radicals can be defined as extremely reactive and unstable molecular species with an unpaired electron. In order to stabilize, radical molecules can donate or accept an electron, which is why they are called oxidants or reductants. [1] Due to their pronounced reactivity, these molecules can damage biologically important molecules such as DNA, proteins, lipids or carbohydrates in the cell nucleus or cell membranes, thus interfering with the homeostasis of the organism. [2] The formation of free radicals in the body is the result of enzymatic and non-enzymatic reactions. Exposure to X-rays, chemicals and other polluting and toxic agents, cigarette consumption, etc. are the most common causes of the formation of free reactive oxygen species (ROS) and reactive nitrogen species (RNS) [3,4] ROS and RNS are responsible for oxidative stress in different pathophysiological conditions. [5] Table 1 shows the list of reactive oxygen species and nitrogen.

**Table 1.** List of ROS and RNS [6,7]

| Reactive oxygen species (ROS) | | Reactive nitrogen species (RNS) | |
|---|---|---|---|
| Symbol | Name | Symbol | Name |
| $O_2$ | Singlet oxygen | $NO^{\cdot}$ | Nitrous oxide |
| $O_2^{\cdot -}$ | Superoxide anion radical | $NOO^-$ | Peroxynitrate |
| $^{\cdot}OH$ | Hydroxyl radical | $NO^-$ | Nitroxyl anion |
| $RO^{\cdot}$ | Alkoxyl radical | $NO_2^{\cdot}$ | Nitrogen dioxide |
| $ROO^{\cdot}$ | Peroxyl radical | $N_2O_3$ | Dinitrogen trioxide |
| $H_2O_2$ | Hydrogen peroxide | $NO_2Cl$ | Nitryl chloride |

Oxidative stress resulting from an imbalance between free radical production and antioxidant defense is associated with damage to a wide range of biomolecules. [8] The cellular constituents of our body change under conditions of oxidative stress, resulting in various diseases. Short-term oxidative stress can occur in tissues injured by trauma, infections, heat injuries, hypertoxia, and toxins. Cancer initiation, promotion, and progression, as well as the side effects of radiation and chemotherapy, are associated with an imbalance between ROS and the antioxidant defense system. ROS has been implicated in the induction and complications of diabetes mellitus, age-related eye diseases, and neurodegenerative diseases such as Parkinson's disease. [9]

**Affiliations:**
[1]Public Health Institution Health Center Lukavac, Kulina bana bb, 75 300 Lukavac, Bosnia and Herzegovina, [2]Faculty of Technology, University of Tuzla, Urfeta Vejzagića 8, 75 000 Tuzla, Bosnia and Herzegovina, [3]Public Health Institution University Clinical Center Tuzla, Prof. dr. Ibre Pašića, 75 000 Tuzla, Bosnia and Herzegovina, [4]Faculty of Medicine, University of Tuzla, Univerzitetska 1, 75 000 Tuzla, Bosnia and Herzegovina, [5]Faculty of Pharmacy, University of Tuzla, Urfeta Vejzagića 8, 75 000 Tuzla, Bosnia and Herzegovina

**Received:**
26.11.2021.

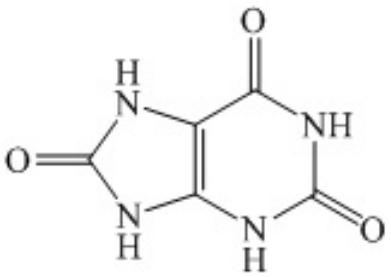
**Accepted:**
15.11.2022.

**Corresponding author:**
Emir Horozić
Email: emir.horozic@untz.ba

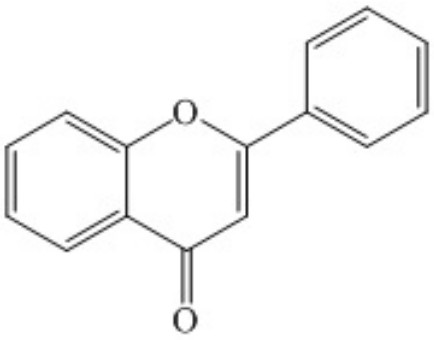
**Funding:** none

**Competing interests:** none

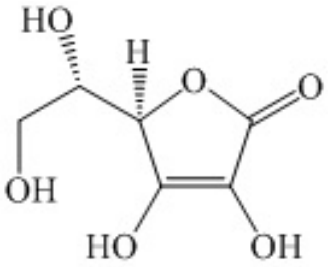
Neutralization of free radicals is done by molecules called antioxidants. Antioxidants have the ability to inhibit and prevent reactions caused by free radicals in the body, thereby delaying or completely inhibiting cell damage. Antioxidants exists both in enzymatic and non-enzymatic forms in the intracellular and extracellular environment. [6] Examples and structures of some known antioxidants are shown in Figure 1. Antioxidant compounds are classified after their type of action into scavengers and "preventive" antioxidants: scavengers (compounds that prevent oxidative stress by capturing free radicals) and "preventive" antioxidants (compounds that act by sequestrating the ions of transition metals, preventing the Fenton reaction). [10]



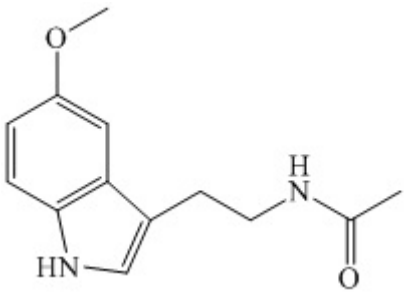
Uric acid



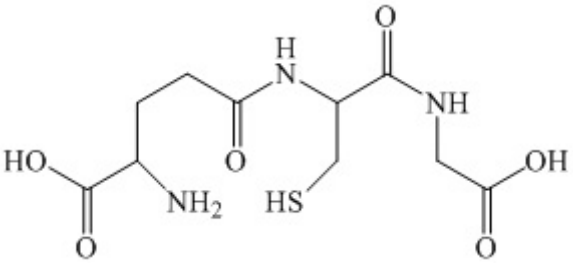
Flavonoid



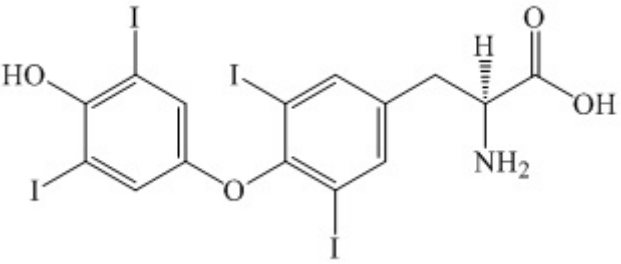
Ascorbic acid



Melatonin



Glutathione



Thyroxine

α-Tocopherol

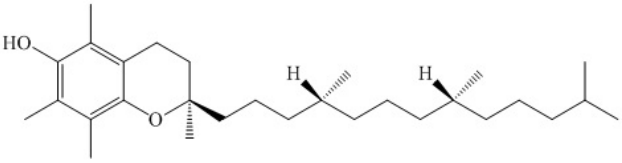


**Fig. 1.** Structures of some antioxidants

Cells are protected against oxidative stress by an interacting network of antioxidant enzymes. [11] Here, the superoxide released by processes such as oxidative phosphorylation is first converted to hydrogen peroxide and then further reduced to give water. This detoxification pathway is the result of multiple enzymes, with superoxide dismutases catalyzing the first step and then catalases and various peroxidases removing hydrogen peroxide. [12]

Today, a significant number of synthetic antioxidants are known. Many of these compounds are of organic origin and contain heteroatoms such as oxygen, sulfur or nitrogen. The structure of these compounds often includes biogenic metals such as iron, copper, zinc, nickel, etc. In the last few years, several analyzes of the antioxidant capacity of metal complexes with different types of ligands have been performed.

## METAL COMPLEXES

Metal complexes are formed by the binding of metals to various ligands, mostly of organic origin. With a wide range of potential ligands and diverse synthetic chemistry, metal complexes represent versatile scaffolds that can be tailored to overcome the specific limitations of a broad spectrum of drugs. [13] A large number of metal complexes synthesized so far with various oxygen, nitrogen, sulfur or combined ligands have shown antitumor, antibacterial, antifungal, antituberculous and

antihypertensive effects. [14-17] In addition, complexes are now being studied as potential synthetic antioxidants and yielding promising results in *in vitro* and *in vivo* studies.

Studies conducted in the past two years on complexes of selected d-block metals and Schiff bases derived from ninhydrin and amino acids have shown significant reduction potential and a high degree of neutralization of DPPH radicals in *in vitro* studies [18-21]. Figure 2 shows the general structure of metal complexes with Schiff bases derived from selected amino acids and ninhydrins, where R is the amino acid residue (phenylalanine, glycine, tryptophan, methionine, histidine, cysteine) and M is the selected metals (copper, cobalt, iron, nickel, silver).

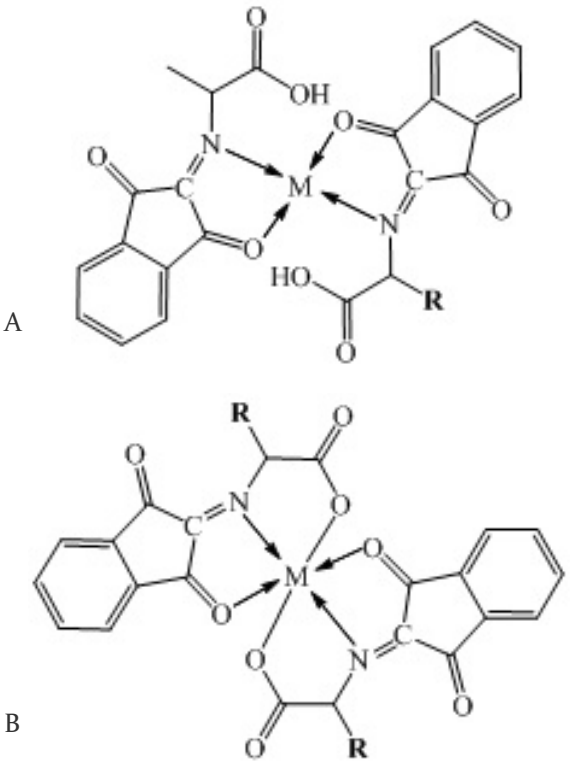



**Fig. 2.** Assumed structures of metal complexes with Schiff bases derived from ninhydrin and selected amino acids: **(A)** structure of complex Co(II) with Schiff bases containing phenylalanine and methionine and Ni(II) complex with Schiff base containing methionine and **(B)** structures of other M(II) complexes with Schiff bases derived from ninhydrin and amino acids

The greatest reduction potential from this series of synthesized complexes was shown by the Co(II) complex containing phenylalanine in the structure, probably due to the different coordination of the ligand to the metal center. Other complexes showed mostly approximate value of DPPH radical neutralization and reduction ability.

Choudhary et al. synthesized a series of metal complexes of Fe(III), Co(II) and Cu(II) containing the bidentate N,O and N,S donor ligand, camphor semicarbazone (1,7,7-trimethylbicyclo [2,2,1]heptanesemicarbazone, TBHSC) and camphor thiosemicarbazone (1,7,7- trimethylbicyclo [2,2,1]heptanethiosemicarbazone, TBHTSC). The results of the free radical scavenging activity of the methanolic solutions of these compounds indicate that the free radical scavenging activity of these compounds was concentration dependent. Among the tested compounds, the $[Cu(C_{11}H_{19}N_3S)_2Cl_2$ complex showed strong interactive ability with concentration-dependent DPPH, and this compound showed an $IC_{50}$ value of 111.0 μg/mL lower than the ascorbic acid value (136.0 μg/mL) and catachin (203.0 μg/mL) used as standards. Maximum free radical scavenging activity (96.09%) was found in $[Cu(C_{11}H_{19}N_3S)_2Cl_2]$ and then (91.10%) in TBHTSC, while the lowest activity (8.60%) was observed in TBHSC. [22]

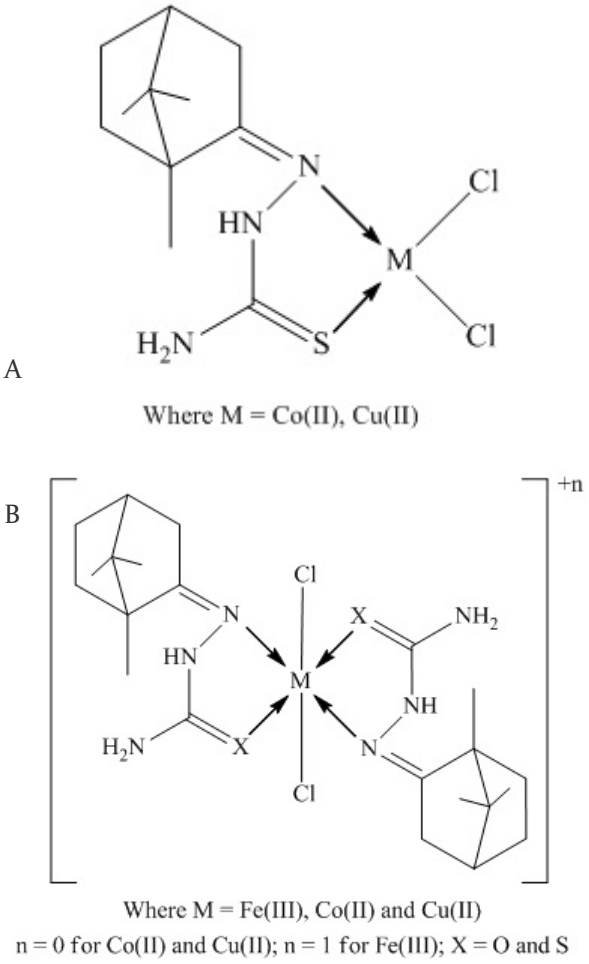
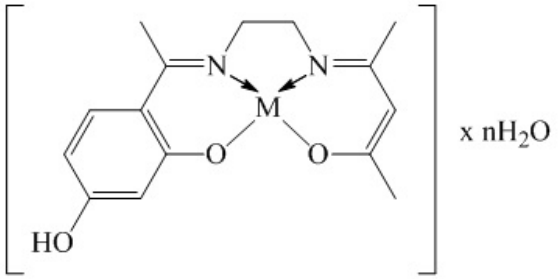



**Fig. 3.** Proposed structural formula for the complexes: **(A)** $[M(LH)Cl_2]$ and **(B)** $[M(LH)_2Cl_2]$ [22]

The nickel(II), iron(III), oxovanadium(IV) complexes of the 3-hydroxysalicylidene-S-methyl-thiosemicarbazone (L) were obtained from the 3-hydroxysalicyldehyde-S-methylthiosemicarbazone with the R1-substituted-salicylaldehyde (R1: H, 3-OH). The value of antioxidant capacity of trolox equivalent (TEAC) of iron (III) complex (TEACCUPRAC = 3.27) was higher than the value of other complexes. Furthermore, the antioxidant activity of free ligand and its complexes was determined by in vitro methods of measuring the purification activity of reactive oxygen species (ROS), including hydroxyl radical (•OH), superoxide anionic radical ($O_2^{\bullet -}$) and hydrogen peroxide ($H_2O_2$), indicat-

ing that complexes V(IV) and Fe(III) in particular had significant ROS removal activity. [23]

Ejidike and Ajibade synthesized complexes of Co (II), Ni (II), Cu (II) and Zn (II) with (4E)-4-[(2-(E)-[1-(2,4-dihydroxyphenyl)ethylidene]aminoethyl)imino] pentan-2-one. The assay was performed using different concentrations of Schiff base and complexes with DPPH and ABTS radicals.




M = Cu(II), Ni(II), Co(II) and Zn(II)
where n = 1 for Zn(II); n = 2 for Cu(II) and Ni(II); n = 3 for Co(II)

**Fig. 4.** Proposed structure of metal complexes with (4E)-4-[(2-(E)-[1-(2,4-dihydroxyphenyl)ethylidene] aminoethyl)imino]pentan-2-one [24]

The activity of removing DPPH radicals by metal complexes is significantly higher than the activity of free ligand, which indicates that the complex neutralizes free radicals much better, but weaker compared to ascorbic acid (vitamin C) and rutin as standard. The Cu(II) and Ni(II) complexes have a higher antioxidant potential ($IC_{50}$) than rutin, but lower than vitamin C (standard drugs). The order can be given as vitamin C > Cu(II) complex > Ni(II) complex > Co(II) complex > Rutin > Zn(II) complex > Ligand. The oxidation potentials of the samples are associated with the presence of compounds that act by breaking the free radical chain by donating hydrogen atoms. Schiff base, Zn(II), Ni(II), Cu(II) and Co(II) metal complexes were moderate and effective scavengers of ABTS radicals. The Co(II) complex showed the highest activity with an $IC_{50}$ of about 1.83±1.08 μM among the synthesized metal complexes. The ability of ABTS to remove radicals for testing can be arranged in the order BHT > Co(II) complex > Ligand > Cu(II) complex > Rutin > Zn(II) complex > Ni(II) complex [24].

Hadjer and coworkers examined the antioxidant activity of Cu (II), Co (II) and Fe (III) complexes with non-natural amino acids containing quaternary ammonium salts moieties used as ligand derived from 2-(diethylamino)ethylmethacylate (DEAEMA). The results showed that metal complexes with copper and iron have the highest antioxidant activity with $IC_{50}$ values of 0.84 and 1.43 mg/mL. The parent ligand and cobalt complexes showed moderate activity with $IC_{50}$ values of 5.13 and 4.95 mg/mL, respectively. [25]

Yapati et al. they synthesized complexes of 1-(benzo[d] thiazol-2-yl)thiourea and examined their ability to remove free radicals (DPPH, $H_2O_2$ and NO) and examined their reducing properties by the FRAP method. The ligand showed moderate antioxidant activity, while the metal complexes showed better antioxidant activity than the ligand. The results of four methods proved that the copper complex is the most powerful antioxidant among all tested compounds. [26] Figure 5 shows the proposed structure of the complexes synthesized by Yapati et al.

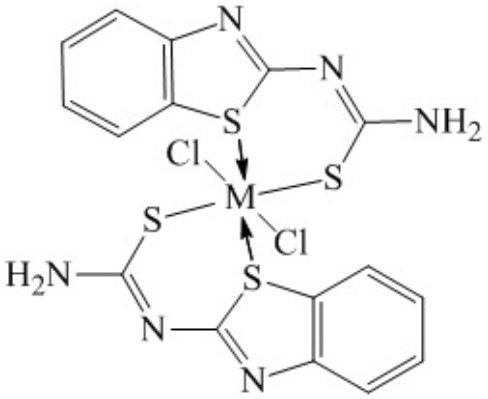



**Fig. 5.** Structure of metal complexes with 1-(benzo[d] thiazol-2-yl)thiourea [26]

Wani et al. have synthesized several metal complexes with para-aminosalicylic acid (PAS). The structure of the synthesized complexes is shown in Figure 6. Compared to the parent ligand, the synthesized metal complexes showed a significantly better effect of neutralizing DPPH radicals. The $IC_{50}$ value of metal complexes ranges from 0.88-1.98 μg/mL. The Ni(II)-PAS complex has the lowest $IC_{50}$ value.

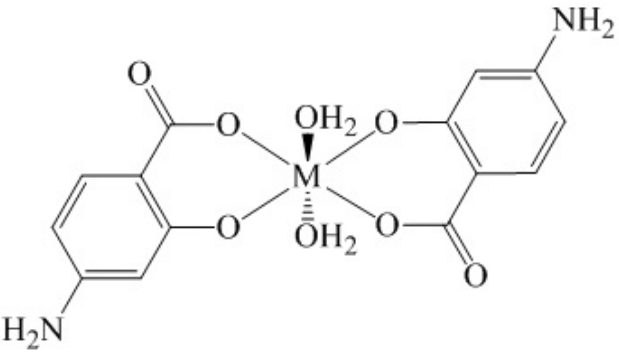



**Fig. 6.** Structure of metal complexes with para-aminosalicylic acid (PAS) [27]

Turan and Buldurun synthesized and tested the antioxidant activity of Fe(II), Mn(II), Zn(II) and Ru(II) complexes with ethyl-2-(2-hydroxy-3-methoxybenzylideneamino)-6-methyl-4,5,6-tetrahydrobenzo[b]thiophene-3-carboxylate. The effect of the ligand and its complexes on the removal of DPPH free radicals was enhanced by their increasing concentration, and the Ru(II) complex showed the greatest ability to remove DPPH radicals. According to the results of this study, the free radical inhibition of standard antioxidants, ligands and its metal complexes was reduced in the following order: ascorbic acid (94.8%) > Ru(II) (94.4%) > BHA (90.8%) > BHT (81.1%) > Mn(II) complex (52.2%)

> Zn(II) complex (49.8%) > Fe(II) complex (31.1%) > Ligand (22.9%) , in the presence of the same concentration (30 μg/mL). [28] The structures of the synthesized metal complexes with ethyl-2-(2-hydroxy-3-methoxybenzylideneamino)-6-methyl-4,5,6-tetrahydrobenzo[b]thiophene-3-carboxylate are shown in Figure 7.

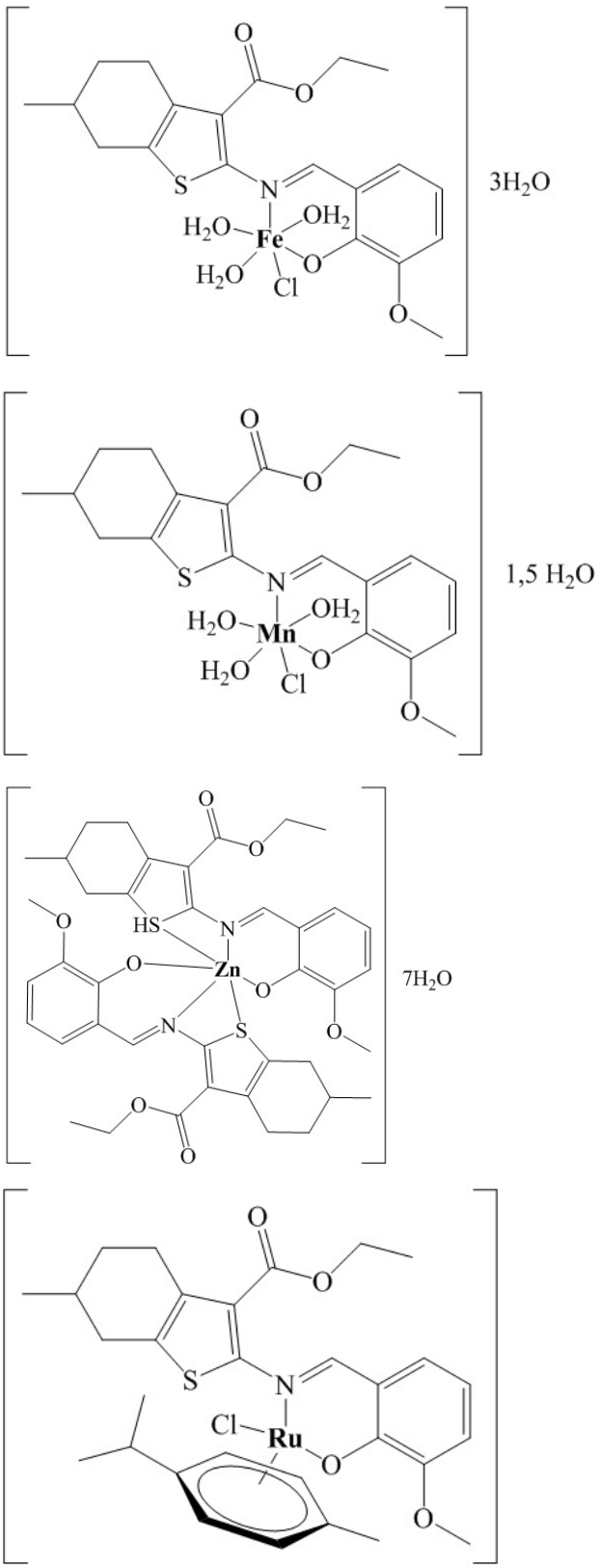



**Fig. 7.** Structure of metal complexes with ethyl-2-(2-hydroxy-3-methoxybenzylideneamino)-6-methyl-4,5,6-tetrahydrobenzo[b]thiophene-3-carboxylate [28]

## CONCLUSION

The presented results clearly show that metal complexes have a great ability to neutralize free radicals, which classifies them as potentially good agents for preventing diseases caused by excessive production of free radicals in the body. In most cases, it has been reported that metal-containing compounds have higher antioxidant activity compared to the ligands used to synthesize the metal complex. It should be emphasized that most of these studies were conducted *in vitro*, which indicates the need for further *in vivo* testing, all with a view to possible application in therapy.

## REFERENCES

1. Cheeseman K.H., Slater T.F. An introduction to free radical biochemistry. British Medical Bulletin 1993; 49(3): 481-493.
2. Young I.S., Woodside J.V. Antioxidants in health and disease. Journal of Clinical Pathology 2001; 54(3): 176-186.
3. Bagchi K., Puri S. Free radicals and antioxidants in health and disease: a review. EMHJ - Eastern Mediterranean Health Journal 1998; 4(‍2) ‍, 350-360.
4. Lobo V., Patil A., Phatak A., Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews 2010; 4(8): 118-126.
5. Young-Woong K., Byzova T.V. Oxidative stress in angiogenesis and vascular disease. Blood 2014; 123(5): 625-631.
6. Nimse S.B., Pal D. Free radicals, natural antioxidants, and their reaction mechanisms. RSC Advances 2015; 5: 27986-28006.
7. Wojtunik-Kulesza K.A., Oniszczuk A., Oniszczuk T., Waksmundzka-Hajnos M. The influence of common free radicals and antioxidants ondevelopment of Alzheimer's Disease. Biomedicine & Pharmacotherapy 2016; 78: 39-49.
8. McCord J.M. The evolution of free radicals and oxidative stress. The American Journal of Medicine 2000; 108(8): 652-659.
9. Rao A.L., Bharani M., Pallavi V. Role of antioxidants and free radicals in health and disease. Advances in Pharmacology and Toxicology 2006; 7: 29-38.
10. Butnariu M. Action and Protection Mechanisms of Free Radicals. Journal of Pharmacogenomics & Pharmacoproteomics 2012; 3(6).
11. Sies H. Oxidative stress: oxidants and antioxidants. Experimental Physiology 1997; 82(2): 291-295.
12. Ho Y.S., Magnenat J.L., Gargano M., Cao J. The nature of antioxidant defense mechanisms: a lesson from transgenic studies. Environmental Health Perspectives 1998; 106 Suppl 5(Suppl 5): 1219-1228.
13. Renfrew A.K. Transition metal complexes with bioactive ligands: mechanisms for selective ligand release and applications for drug delivery. Metallomics 2014; 6: 1324-1335.
14. Horozić E., Cipurković A., Ljubijankić N. Anti-infective and anti-tumor activity of some metal complexes ($M^{II}$-$M^{IV}$) with Schiff bases. Technologica Acta 2017; 10(1): 27-34.
15. Rehman S., Ikram M., Subhan F., Sinnokrot M., Khan W. Antibacterial Activities of Transition Metal complexes

of Mesocyclic Amidine 1,4-diazacycloheptane (DACH). Open Chemistry 2019; 17(1): 936-942.

16. Gundu M., Synthesis, Characterization and Biological Activity of Schiff Base Metal Complexes. Asian Journal of Research in Chemistry 2017; 10(4): 587-592.
17. Sahoo J., Paidesetty SK. Biological investigation of novel metal complexes of 2-amino-4-substituted phenylthiazole Schiff bases. Journal of Taibah University Medical Sciences 2017; 13(2): 142-155.
18. Horozić E., Suljagić J., Suljkanović M. Synthesis, Characterization, Antioxidant and Antimicrobial Activity of Copper(II) Complex with Schiff Base Derived from 2,2-dihydroxyindane-1,3-dione and Tryptophan. American Journal of Organic Chemistry 2019; 9(1): 9-13.
19. Horozić E., Šljivić Husejnović M., Bajrić A., Ibišević M., Karić E., Džambić A., Husejnagić D., Nurković E. BSA binding affinity, free radical scavenging capacity and antibacterial activity of new Silver(I) complexes with Schiff base ligands. International Journal of Basic and Applied Sciences 2020; 9(1): 12-16.
20. Marić S., Hasić N., Horozić E., Cipurković A., Antić A., Mujić Z., Pelemiš K., Brkić K., Bjelošević D. Synthesis, characterization and bioactivity of selected metal complexes with imine ligands. Technologica Acta 2020; 13(2): 39-42.
21. Horozić E., Antić A., Mujić Z., Brkić K., Pelemiš K., Marić S. *In vitro* antibacterial activity and ferric reducing antioxidant power of selected metal complexes with Schiff bases derived from 2,2-dihydroxyindane-1,3-dione and selected amino acids. Acta Medica Saliniana, *accepted*.
22. Choudhary A., Sharma R., Nagar M., Mohsin M., Meena H.S. synthesis, characterization and antioxidant activity of some transition metal complexes with terpenoid derivatives. Journal of the Chilean Chemical Society 2011; 56(4): 911-917.
23. Bal-Demirci T., Sahin M., Kondakçı E., Özyürek M., Ülküseven B., Apak R. Synthesis and antioxidant activities of transition metal complexes based 3-hydroxysalicylaldehyde-S-methylthiosemicarbazone. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 2015; 138: 866-872.
24. Ejidike I.P., Ajibade P.A. Synthesis, Characterization, Antioxidant, and Antibacterial Studies of Some Metal(II) Complexes of Tetradentate Schiff Base Ligand: (4E)-4-[(2-(E)-[1-(2,4-Dihydroxyphenyl)ethylidene]aminoethyl)imino]pentan-2-one. Bioinorganic Chemistry and Applications 2015; 2015. Article ID 890734
25. Hadjer F., Tahar B., Djallal. Eddine A., Sofiane D. Antioxidant and Antimicrobial Activity of Some Transition Metal Complexes with Non-natural Amino Acids Used As Ligand. Journal of Materials and Environmental Sciences 2018; 9(7): 2153-2157.
26. Yapati H., Devineni S.R., Chirumamilla S., Kalluru S. Synthesis, characterization and studies on antioxidant and molecular docking of metal complexes of 1-(benzo[d]thiazol-2-yl)thiourea. Journal of Chemical Sciences 2016; 128(1): 43-51.
27. Wani A.B., Singh J., Upadhyay N. Synthesis and Characterization of Transition Metal Complexes of Para-Aminosalicylic Acid With Evaluation of Their Antioxidant Activities. Oriental Journal of Chemistry 2017; 33(3): 1120-1126.
28. Turan N., Buldurun K. Synthesis, characterization and antioxidant activity of Schiff base and its metal complexes with Fe(II), Mn(II), Zn(II), and Ru(II) ions: Catalytic activity of ruthenium(II) complex. European Journal of Chemistry 2018; 9(1): 22-29.

Scan this QR code with your mobile device for instant access to the current Issue of Acta Medica Saliniana