Bitlis Eren Üniversitesi Fen Bilimleri Dergisi
BİTLİS EREN UNIVERSITY JOURNAL OF SCIENCE
ISSN: 2147-3129/e-ISSN: 2147-3188
VOLUME: 12 NO: 2 PAGE: 329-336 YEAR: 2023
DOI:10.17798/bitlisfen.1185118

Antioxidant and Antibacterial Activities of Salen-type Schiff Base and Metal Complexes

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Keywords: Schiff base, Metal complexes, FRAP, DPPH, CUPRAC, Antibacterial activity.

Abstract
2-Aminobenzylamine derived Schiff base and its Cu(II), Ni(II),Co(II) and Fe(III) metal complexes were researched in terms of their antioxidant and antibacterial activities in this study. 1,1-Diphenyl-2-picrylhydrazyl radical scavenging (DPPH) method, ferric reducing antioxidant power (FRAP) method and cupric reducing antioxidant capacity (CUPRAC) method were carried out for determination of antioxidant effects of compounds. The antioxidant activity of the compounds was compared with the standard antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Fe, Ni and Cu complexes exhibited more scavenging activity than BHT. All compounds exhibit lower ferric reducing activity than BHA and BHT. Fe complex, Ni complex and the ligand have more antioxidant capacity than corresponding to BHT according to CUPRAC method. These assays showed that all compounds researched can use as synthetic antioxidants. Antibacterial effects of Schiff base and its metal complexes were investigated by agar disc diffusion method. Antibacterial studies of the compounds were achieved against some gram-positive and gram-negative bacteria. *Enterococcus faecium*, *Pseudomonas aeroginosa*, *Klebsiella pneumoniae* and *Escherichia coli* were used as bacterial strains. The beneficial datas were acquired.

1. Introduction

Shiff bases are obtained from the reaction of aldehydes and ketones with primary amines. Then, these synthesized Schiff bases form complex compounds with transition metal ions [1]. In addition, Schiff bases are useful ligands and employed in biochemistry field such as effects of antioxidants to oxidative stress damage of cells [2]. Therefore, they have been researched for their antioxidant, antibacterial and antifungal effects [3-5]. Similarly, Schiff bases and their metal complexes show important biological activity and they have been considered because of these properties recently [6-11].

Various properties of metal complexes are effected by the structure of the metal ion and the ligand. Metal ions increase the activity of the ligand due to the synergistic effect. Accordingly, metal complexes of salen-type Schiff bases are found to have antioxidant effects [12]. Moreover salen-type Schiff bases show antimicrobial activity as they have donor O and N atoms [13].

After the COVID-19 epidemic, which effected the whole world, studies on new antioxidant and antibacterial agents has increased. Because, antioxidant substances make the body more resistant to diseases, while antibacterial substances are seen as an important factor in preventing the further spread of this epidemic. As a result, Schiff bases and metal complexes appear as alternative substances that can be investigated with their antioxidant and antibacterial properties. For that reason, 2-Aminobenzylamine derived Schiff base and its Cu(II), Ni(II), Co(II) and Fe(III) metal complexes were investigated in terms of their antioxidant and antibacterial activities in this study. Different methods were used for antioxidant analysis of the compounds. Antibacterial effects of Schiff base and

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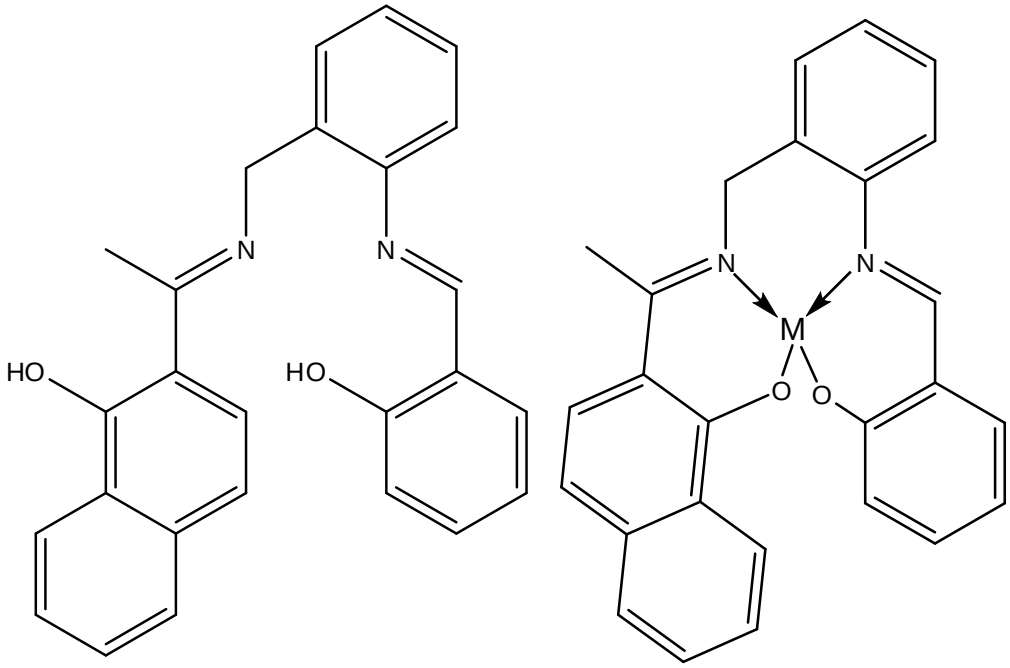
Received: 06.10.2022, Accepted: 08.06.2023

its metal complexes were investigated by agar disc diffusion method.

2. Material and Method

2.1. Material

Schiff base and its metal complexes were prepared according to reported method [14]. Structures of the compounds are shown in Figure 1. 1 represents Schiff base structure. 2 represents general formulas of the metal complexes of Schiff base which M indicates Cu(II), Ni(II), Co(II) and Fe(III) seperately. Other chemicals were bought from Sigma Aldrich. Bacterial strains were obtained from Hatay Mustafa Kemal University Research Hospital Microbiology Laboratory. Antioxidant effects of samples were examined in spectrophotometer. Antibacterial studies were carried out with incubator. Autoclave was used for sterilization of tools. The assays were achieved in triplicate.



H_2L M: Cu(II), Ni(II), Co(II) and Fe(III)

1 **2**

Figure 1. Structures of the compounds

1: FT-IR (U-ATR, cm^{-1}): 3062 cm^{-1} (Ar-H), 2978–2850 cm^{-1} (CH_2), 1611 cm^{-1} (CH=N).

^{1}H NMR ($CDCl_3$ as solvent, δ in ppm): 16.0 (s, OH), 12.9 (s, OH), 8.6 (s, CH=N), 8.5–6.7 (m, Ar-H), 4.9 (s, CH_2), and 2.5 ppm (s, -CH_3).

^{13}C NMR ($CDCl_3$ as solvent, δ in ppm): 175 (C=N), 172 (CH=N), 163 (C-OH), 144–109 (Ar), 45 (-CH_2-), and 15 ppm (-CH_3).

[CuL]; FT-IR (U-ATR, cm^{-1}): 3442 (O-H), 3047 (Ar,C-H), 2926 (C-H), 1608 (C=N), 1258 (C-O), 549 (Cu-O), 459 (Cu-N).

[NiL]; FT-IR (U-ATR, cm^{-1}): 3045 (Ar,C-H), 2997 (C-H), 1608 (C=N), 1255 (C-O), 556 (Ni-O), 465 (Ni-N).

[CoL]; FT-IR (U-ATR, cm^{-1}): 3300 (O-H), 3049 (Ar, C-H), 2950 (C-H), 1594 (C=N), 1259 (C-O), 582 (Co-O), 462 (Co-N).

[FeL]; FT-IR (U-ATR, cm^{-1}): 3048 (Ar, C-H), 2920 (C-H), 1609 (C=N), 1251 (C-O), 574 (Fe-O), 477 (Fe-N).

2.2. Methods

2.2.1. Antioxidant Activity

2.2.1.1. Free Radical Scavenging (DPPH) Assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to determinate radical scavenging activities of samples. The method was modified by Brand-Williams [15] and used by Blois firstly [16]. 3.75 mL of 0.06 mM DPPH in methanol was added into various concentrations (1.25 mL) of DMSO solutions of compounds. Absorbances of solutions were observed at 517 nm by spectrophotometrically. BHA and BHT were used as standards. Percentage inhibition was calculated by using the following formula:

$$\% \text{ Inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (1)$$

$A_{control}$: DPPH absorbance only, A_{sample}: DPPH absorbance with an antioxidant.

Furthermore, IC_{50} values mean that concentrations of antioxidants cause 50 % scavenging of DPPH radical were calculated.

2.2.1.2. Ferric Reducing Antioxidant Power (FRAP) Assay

Reducing activities of compounds were investigated according to the FRAP method [17]. DMSO solutions of samples in various concentrations added to phosphate buffer. Then, the mixtures were incubated at 50 °C for 20 min. After addition of potassium ferricyanide (1.0 mL, 1.0%). Trichloroacetic acid (10%) was added, and the mixtures were utilized for 10 min. centrifugation. 1.0 mL of the supernatants were mixed with $FeCl_3$ and the absorbances were measured at 700 nm. FRAP values were calculated for the compounds as the ratio of compounds' molar absorptivities to ascorbic acid's molar absorptivity.

2.2.1.3. CUPRAC Antioxidant Capacity Assay

The antioxidant effects of the compounds were investigated by CUPRAC method [18]. $CuCl_2.2H_2O$, Nc, ammonium acetate buffer solution and sample solution were mixed. Mixtures were incubated at 25°C for 30 min., and the absorbances were measured at 450 nm. The ratio of each compound's molar absorptivity to molar absorptivity of trolox were calculated as TEAC values (trolox equivalent antioxidant capacity).

2.2.2. Antibacterial Activity

Antibacterial effects of Schiff base and its metal complexes were investigated by agar disc diffusion method [19]. *Enterococcus faecium*, *Pseudomonas aeroginosa*, *Klebsiella pneumoniae* and *Escherichia coli* were used as bacterial strains. Besides, chloramphenichol was used as standard antibacterial agent. The bacteria were cultured at 37 °C. The bacterial suspensions were transferred to sterile Petri plates covered by Muller Hinton agar medium. Filter paper disks were placed on to sterile Petri plates. Solutions of the compounds were dissolved in DMSO. The disks were saturated with 30 μL of the samples. Incubation was applied to plates at 37 °C. Diameter of the inhibition zones were measured for determination of antibacterial activity of compounds [20].

3. Results and Discussion

3.1. Synthesis

The ^{1}H NMR and ^{13}C NMR spectra of Schiff base are given in Figure 2 and Figure 3, respectively. The observation of peaks in the ^{1}H NMR spectrum at 8.6 ppm and 12.9 ppm attributed to the formation of CH=N and OH, respectively, are the most important evidences of the formation of 1. In addition, the multiplets in the 6.5–8.5 ppm range can be attributed to the protons of benzene rings of H_2L. Also, the -CH_2- protons of these compounds were observed in the 4.9–5.2 ppm region. In the ^{13}C NMR spectrum of H_2L, a band observed at 170 ppm due to CH=N carbon atom. In addition, in the ^{13}C NMR spectrum of this compound, the peaks of the carbon atoms of -CH_3 and -CH_2- are observed at 15 ppm and 45 ppm, respectively.

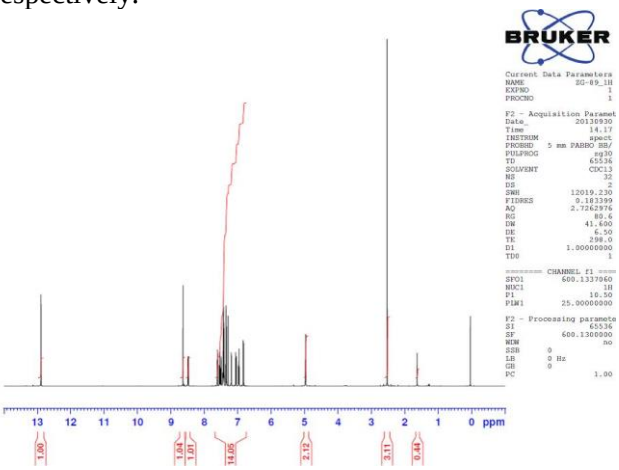


Figure 2. The ^{1}H-NMR spectrum of **1.**

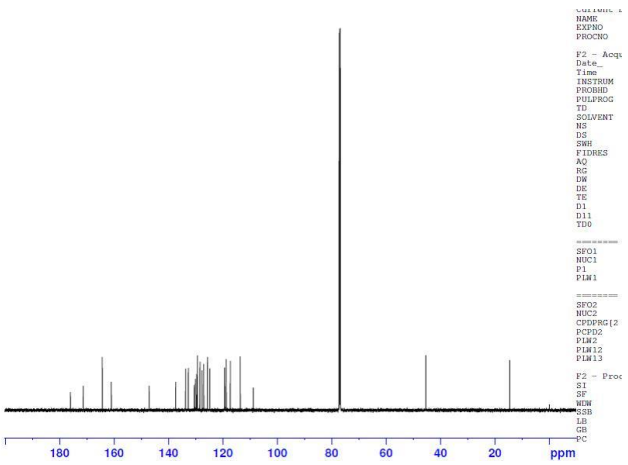


Figure 3. The ^{13}C-NMR spectrum of **1.**

The FT-IR spectra of the ligand and its metal complexes are given in Figure 4. The FT-IR spectrum Schiff base ligand, H_2L, exhibit strong band 1611 cm^{-1}, attributed to the characteristic peak of CH=N stretching vibration frequency. The spectrum also shows several weak bands for the aliphatic and aromatic C-H stretching vibration frequencies at the region of 2845-3070 cm^{-1} and strong bands. The band at 1205 cm^{-1} for H_2L is ascribed to the phenolic C-O stretching vibration.

The FT-IR spectra of the metal complexes also showed new bands in the 582–549 and 477–459 cm^{-1} regions, which are probably due to the formation of M-O and M-N bonds, respectively. The peak observed in the FTIR spectra of metal complexes at around 1600 cm^{-1}, partially differ from the ligand. This can be explained by the formation of a coordination bond between the C=N group of the ligand and the metal ion.

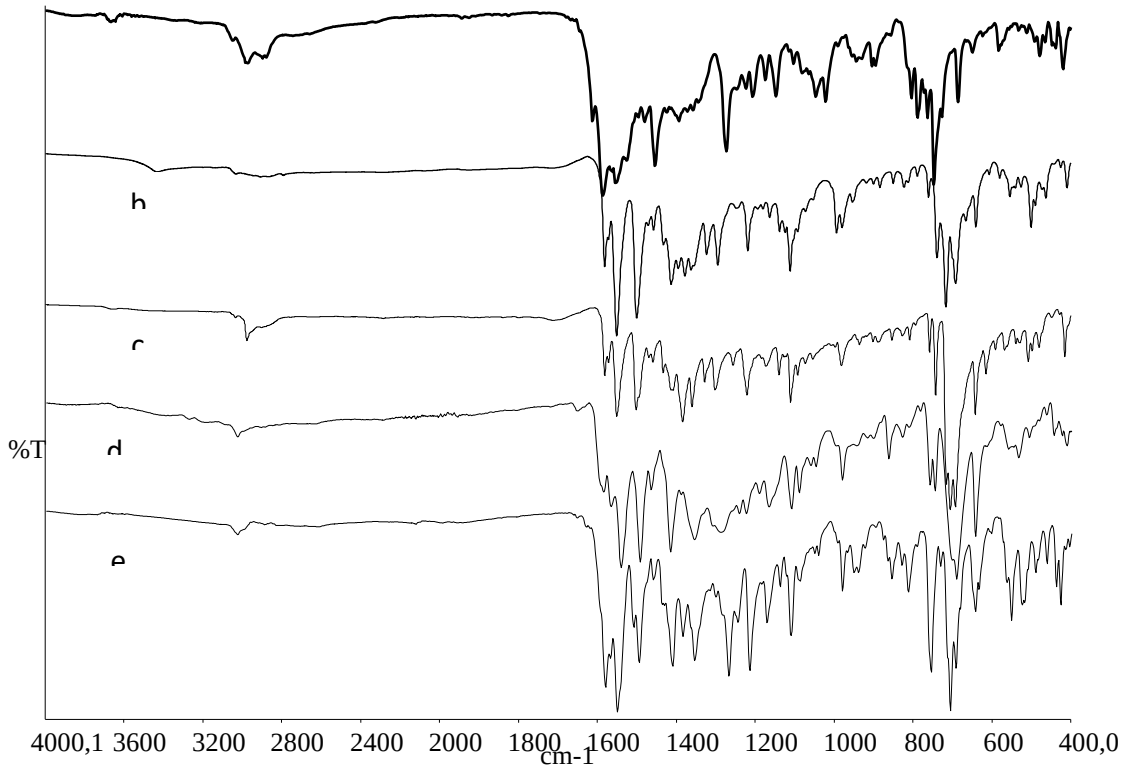


Figure 4. The FT-IR spectra of H_2L(a), [CuL] (b), [NiL](c), [CoL](d) and [FeL](e).

3.2. Antioxidant Activity

3.2.1. Free Radical Scavenging (DPPH) Assay

Radical scavenging activity of the samples and standards were determined by DPPH assay. IC_{50} values (amount of antioxidant that scavenged 50% of DPPH radical) were given in Table 1. Low IC_{50} value represents more scavenging activity. Therefore, BHA is the best antioxidant in Table 1. According to free radical scavenging (DPPH) assay results the metal complexes showed more scavenging activity than the ligand because of chelation theory [21-24]. Ni complex with the lowest IC_{50} value has the highest scavenging activity among the all metal complexes and the ligand has the lowest scavenging activity because of the highest IC_{50} value. Furthermore, Fe, Ni and Cu complexes exhibited more scavenging activity than BHT.

Table 1. IC_{50} values of compounds and standards

Compounds	IC_{50}
Fe complex	0.265 ± 0.056 (mg/mL)
Co complex	0.430 ± 0.018 (mg/mL)
Ni complex	0.050 ± 0.006 (mg/mL)
Cu complex	0.067 ± 0.008 (mg/mL)
Ligand	0.477 ± 0.079 (mg/mL)
BHA	7.335 ± 0.870 (µg/mL)
BHT	0.359 ± 0.013 (mg/mL)

3.2.2. Ferric Reducing Antioxidant Power (FRAP) Assay

Reducing power of the samples and standards were determined by FRAP assay. FRAP values for FRAP assay were given in Table 2. High FRAP value means high activity whereas low FRAP value means low activity. For that reason, the metal complexes showed more reducing activity than the ligand as FRAP assay results [25]. Even if all of the metal complexes have similar reducing activity, it is observed that Cu complex has the most reducing activity. Fe complex comes after Cu complex. Besides, ligand has the lowest reducing activity. Both metal complexes and ligand exhibit lower reducing activity than BHA and BHT.

Table 2. FRAP values of compounds and standards

Compounds	FRAP values
Fe complex	0.153 ± 0.07
Co complex	0.110 ± 0.001
Ni complex	0.103 ± 0.006
Cu complex	0.158 ± 0.002
Ligand	0.015 ± 0.005
BHA	2.695 ± 0.311
BHT	1.479 ± 0.234

3.2.3. CUPRAC Antioxidant Capacity Assay

Cupric reducing antioxidant activity of the Schiff base ligand, its metal complexes and standards were determined by CUPRAC assay. TEAC values for CUPRAC assay were given in Table 3. High TEAC value shows more antioxidant capacity. In accordance with CUPRAC assay results, the metal complexes exhibited less antioxidant activity than the ligand due to donor phenolic hydroxyl groups [26, 27]. Ligand has the most antioxidant capacity. Among the metal complexes, Co complex has the lowest antioxidant capacity while Ni complex has the highest antioxidant capacity. Additionally, Fe complex, Ni complex and the ligand have more antioxidant capacity than corresponding to BHT.

Table 3. TEAC values of compounds and standards

Compounds	TEAC values
Fe complex	1.573 ± 0.119
Co complex	0.997 ± 0.036
Ni complex	2.210 ± 0.031
Cu complex	1.235 ± 0.064
Ligand	2.437 ± 0.026
BHA	3.928 ± 0.181
BHT	1.412 ± 0.248

3.3. Antibacterial Activity

In vitro antibacterial activity of compounds was investigated against *Enterococcus faecium*, *Pseudomonas aeroginosa*, *Klebsiella pneumoniae* and *Escherichia coli* strains by agar disc diffusion

method. Zone of inhibition data was given in Table 4. Accordingly, chloramphenicol performed more antibacterial activity on each bacterium than the other compounds. Fe complex showed antibacterial effect to all bacteria except *Klebsiella pneumoniae*. Co and Cu complexes inhibited the growth of *Pseudomonas aeroginosa* and *Escherichia coli* only. Ni complex exhibited antibacterial effect against to all bacteria except to *Enterococcus faecium.* Finally, ligand showed antibacterial effect to all bacteria.

Compounds	Zone of inhibition (mm)			
	E. faecium	*P. aeroginosa*	*K. pneumoniae*	
Fe complex	13	9	-	
Co complex	-	12	-	
Ni complex	-	10	12	
Cu complex	-	13	-	
Ligand	15	10	11	
Chloramphenichol	35	25	30	

Table 4. Antibacterial activity values of compounds

4. Conclusion and Suggestions

In this study, 2-Aminobenzylamine derived Schiff base and its Cu(II), Ni(II), Co(II) and Fe(III) metal complexes were investigated in terms of their antioxidant and antibacterial activities. Different methods were used for antioxidant analysis of the compounds. According to the results, all of the compounds can be utilized as antioxidant. Likewise, all of the compounds exhibited important antibacterial activities.

Acknowledgment

The authors gratefully acknowledge the support of Prof. Dr. Cahit Demetgül , Department of Chemistry, Faculty of Arts and Sciences, Hatay Mustafa Kemal University, Hatay, Turkey. This research was financially supported by Hatay Mustafa Kemal University Research foundation (Project No. 18. YL. 032).

Contributions of the authors

The contribution of the authors is equal.

Conflict of Interest Statement

There is no conflict of interest between the author.

Statement of Research and Publication Ethics

The study is complied with research and publication ethics

References

[1] I. Gonul, M. Kose, G. Ceyhan, and S. Serin, “Methoxy group containing bidentate Schiff base ligands and their transition metal complexes: Synthesis, structural characterisation, photoluminescence,

antioxidant capacity and superoxide dismutase activity studies", *Inorganica Chimica Acta,* vol. 453, pp. 522–530, 2016.

[2] A.A. Al-Amiery, "Synthesis and antioxidant, antimicrobial evaluation, DFT studies of novel metal complexes derivate from Schiff base", *Res. Chem. Intermed.,* vol.,38, pp. 745–759, 2012.

[3] C. Demetgül, M. Karakaplan, S. Serin, and M. Diğrak, "Synthesis, characterization, and biological properties of Ni(II), Co(II), and Cu(II) complexes of Schiff bases derived from 4-aminobenzylamine", *J. Coord. Chem.,* vol.62, pp. 3544–3551, 2009.

[4] A.A. Al-Amiery, A. A. H. Kadhum, M. Shamel, M. Satar, Y. Khalid, and A. B. Mohamad, "Antioxidant and antimicrobial activities of novel quinazolinones", *Med. Chem. Res.,* vol. 23, pp. 236–242, 2014.

[5] S. Saha, A. Das, K. Acharjee, and B. Sinha, "Synthesis, characterization and antibacterial studies of Mn(II) and Co(II) complexes of an ionic liquid tagged Schiff base", *J. Serb. Chem. Soc.,* vol. 81, pp. 1151–1159, 2016.

[6] M. S. Refat, "Complexes of uranyl(II), vanadyl(II) and zirconyl(II) with orotic acid "vitamin B13": Synthesis, spectroscopic, thermal studies and antibacterial activity", *J. Mol. Struct., vol.* 842, pp. 24–37, 2007.

[7] M. A Neelakantan, F. Rusalraj, J. Dharmaraja, S. Johnsonraja, T. Jeyakumar, and M. S. Pillai, "Spectral characterization, cyclic voltammetry, morphology, biological activities and DNA cleaving studies of amino acid Schiff base metal(II) complexes", *Spectrochim. Acta A,* vol. 71, pp. 1599–1609, 2008.

[8] H. F. Abd El-Halim, M. M. Omar, and G. G. Mohamed, "Synthesis, structural, thermal studies and biological activity of a tridentate Schiff base ligand and their transition metal complexes", *Spectrochim. Acta A,* vol. 78, pp. 36–44, 2011.

[9] M. B. Halli, and R. B. Sumathi, "Synthesis, spectroscopic, antimicrobial and DNA cleavage studies of new Co(II), Ni(II), Cu(II), Cd(II), Zn(II) and Hg(II) complexes with naphthofuran-2-carbohydrazide Schiff base", *J. Mol. Struct.,* vol. 1022, pp. 130–138, 2012.

[10] M. Şahin, N. Koçak, D. Erdenay, and U. Arslan, "Zn(II), Ni(II), Cu(II) and Pb(II) complexes of tridentate asymmetrical Schiff base ligands: Synthesis, characterization, properties and biological activity", *Spectrochim. Acta A,* vol. 103, pp. 400–408, 2013.

[11] O. A. M. Ali, "Synthesis, spectroscopic, fluorescence properties and biological evaluation of novel Pd(II) and Cd(II) complexes of NOON tetradentate Schiff bases", *Spectrochim. Acta A,* vol. 121, pp. 188–195, 2014.

[12] Q. Hasi, Y. Fan, X. Yao, D. Hu, and J. Liu, "Synthesis, characterization, antioxidant and antimicrobial activities of a bidentate Schiff base ligand and its metal complexes", *Polyhedron,* vol. 109, pp. 75–80, 2016.

[13] M. I. Sundararajan, T. Jeyakumar, J. Anandakumaran, and B. K. Selvan, "Synthesis of metal complexes involving Schiff base ligand with methylenedioxy moiety: Spectral, thermal, XRD and antimicrobial

studies", *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* vol. 131, pp. 82-93, 2014.

[14] D. Çakmak, S.,Çakran, S. Yalçinkaya, and C. Demetgül, "Synthesis of salen-type Schiff base metal complexes, electropolymerization on graphite electrode surface and investigation of electrocatalytic effects", *Journal of Electroanalytical Chemistry,* vol. 808, pp. 65-74, 2018.

[15] U. K. Sharma, S. Sood, N. Sharma, P. Rahi, R. Kumar, A. K. Sinha, and A. Gulati, "Synthesis and SAR investigation of natural phenylpropenederived methoxylated cinnamaldehydes and their novel Schiff bases as potent antimicrobial and antioxidant agents", *Med. Chem. Res.,* vol. 22, pp. 5129–5140, 2013.

[16] B. B. Sokmen, N. Gumrukcuoglu, S. Ugras, H. Sahin, Y. Sagkal, and H. I. Ugras, "Synthesis, antibacterial, antiurease, and antioxidant activities of some new 1,2,4-triazole Schiff base and amine derivatives", *Appl. Biochem. Biotechnol.,* vol. 175, pp. 705–714, 2015.

[17] M. Silinsin, and E. Bursal, "UHPLC-MS/MS phenolic profiling and in vitro antioxidant activities of Inula graveolens (L.) Desf.", *Nat. Prod. Res.,* vol. 32, no. 12, pp. 1467-1471, 2018.

[18] R. Apak, K. Güçlü, M. Özyürek, and S. E. Karademir, "Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method", *J. Agric. Food Chem.,* vol. 52, no. 26, pp.7970-7981, 2004.

[19] A. Rehman, M. I. Choudhary, and W. J. Thomsen, *Bioassay Techniques for Drug Development,* Amsterdam, The Netherlands, Harwood Academic Publishers, pp. 9, 2001.

[20] R. S. Varma, Z. K. Khan, and A. P. Singh, "Antifungal agents: past, present and future prospects", *National Academy of Chemistry and Biology,* India, Lucknow, pp. 55, 1998.

[21] S. B. Bukhari, S. Memon, M. Mahroof-Tahir, and M. I. Bhanger, "Synthesis, characterization and antioxidant activity copper-quercetin complex", *Spectrochim, Acta A,* vol.71, no. 5, pp., 2009.

[22] M. R. Karekal, V. Biradar, and M. B. H. Mathada, "Synthesis, characterization, antimicrobial, DNA cleavage, and antioxidant studies of some metal complexes derived from Schiff base containing indole and quinoline moieties", *Bioinorg. Chem. Appl. Res.,* doi: 10.1155/2013/315972, 2013.

[23] H. A. R. Pramanik, P. C. Paul, P. Mondal, C. R. Bhattacharjee, "Mixed ligand complexes of cobalt(III) and iron (III) containing N_2O_2-chelating Schiff Base: Synthesis, characterization, antimicrobial activity, antioxidant and DFT study", *J. Mol. Struct.,* vol. 1100, pp. 496-505, 2015.

[24] R. A. Ammar, A. M. A. Alaghaz, M. E. Zayed, and L. A. Al-Bedair, "Synthesis, spectroscopic, molecular structure, antioxidant, antimicrobial and antitumor behavior of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) complexes of O_2N type tridentate chromone-2-carboxaldehyde Schiff's base ligand", *J. Mol. Struct.,* vol. 1141, pp. 368-381, 2017.

[25] M. Gaber, H. A. El-Ghamry, S. K. Fathalla, and M. A. Mansour, "Synthesis, spectroscopic, thermal and molecular modeling studies of Zn^{2+}, Cd^{2+} and UO_2^{2+} complexes of Schiff bases containing triazole moiety. Antimicrobial, anticancer, antioxidant and DNA binding studies", *Mater. Sci. Eng.,* vol. 83, pp. 78-89, 2018.

[26] T. Bal-Demirci, M. Şahin, E. Kondakçı, M. Özyürek, B. Ülküseven, and R. Apak, "Synthesis and antioxidant activites of transition metal complexes based 3-hydroxysalicylaldehyde-S-methylthiosemicarbazone", *Spectrochim., Acta A,* vol. 138, pp. 866-872, 2015.

[27] W. Al Zoubi, A. A. S. Al-Hamdani, and M. Kaseem, "Synthesis and antioxidant activites of Schiff bases and their complexes: a review", *Appl. Organometal Chem.,* vol. 30, pp. 810-817, 2016.