ICONTECH INTERNATIONAL JOURNAL OF SURVEYS, ENGINEERING, TECHNOLOGY
ISSN 2717-7270
Journal homepage: http://icontechjournal.com/index.php/iij
Volume 5 (2021) Issue 3

IN VITRO ANTIOXIDANT PROPERTIES OF 2-(4-(2-HYDROXYBENZYLIDENEAMINO)BENZYLIDENEAMINO)BENZOIC ACID

Zehra Kubra YILMAZ

MSc, Gazi University, Faculty of Science, Department of Biology, Ankara, Turkey

ORCID ID: 0000-0003-4574-070X

Belma ASLIM

Prof. Dr., Gazi University, Faculty of Science, Department of Biology, Ankara, Turkey

ORCID ID: 0000-0002-0595-7237

Ozlem OZDEMIR

Assoc. Prof. Dr., Gazi University, Faculty of Science, Department of Chemistry, Ankara, Turkey

ORCID ID: 0000-0003-2748-9179

ABSTRACT

In recent years, it has attracted great attention to determine and appraise synthetic antioxidants with high free radical scavenging capacity associated with various disorders. Because synthetic antioxidants are widely used in place of native antioxidants as they are influential and less expensive. In this study, the antioxidant activity of the newly synthesized asymmetric diimine Schiff base was determined by using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, ferrous ion chelation (FIC) activity and total antioxidant activity (Phosphomolybdenum assay) methods. The effective concentration (IC_{50}) values were calculated for the Schiff base and standards. According to the results of DPPH radical scavenging, ferrous ion chelation and total antioxidant methods, Schiff base showed a well antioxidant activity. It was determined that Schiff base is reactive towards DPPH radicals (IC_{50}, 180.0 μM) and especially Fe^{+2} ions (IC_{50}, 76.3 μM). It was also found that it actively reduces the Mo(VI) ion to the Mo(V) form (IC_{50}, 121.5 μM). The our results indicate Schiff base, may be a fine candidate as a source of synthetic antioxidants and possibly strong drug.

Keywords: Free radicals; oxidative stress; DPPH; Schiff base.

1. INTRODUCTION

Free radicals and reactive oxygen species (ROS) like as superoxide and hydrogen peroxide anions and hydroxyl radical are produced in specific organelles of the cell under physiological conditions (Haraguchi, 2001). The balance between antioxidants and free radicals is requisite for appropriate physiological function. Free radicals attack significant macromolecules causing cell damage and homeostatic disruption. All kinds of molecules in the body are targets for free radicals. In addition, free radicals can reason oxidative stress (Aruoma, 1996). Oxidative stress, which occurs as a result of the imbalance between antioxidant defenses and free radical production causes severe damage to a wide variety of molecular species, including lipids, proteins, and DNA, leading to the progression of serious diseases (McCord, 2000; Lobo et al., 2010). For this, it is very important to develop new compounds with higher antioxidant activity to blocking the generation of ROS and free radicals and, accordingly, to treat these radical-related diseases.

In this paper, it was aimed to investigate the antioxidant activity of the asymmetric diimine Schiff base 2-(4-(2-hydroxybenzylideneamino)benzylideneamino)benzoic acid.

2. MATERIAL AND METHODS

2.1. Synthesis of the asymmetric diimine Schiff base

The synthesis of the asymmetric Schiff base was reported firstly in our previous work (Yilmaz et al., 2017; Yilmaz et al., 2018).

Received 14 August 2021; Received in revised form 25 August 2021; Accepted 18 September 2021;
Available online 25 September 2021;
doi: 10.46291/ICONTECHvol5iss3pp7-12

2.2. Determination of antioxidant activities

2.2.1. DPPH radical scavenging assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay described Ogunmoyole et al. (2013) was utilized to quantify the scavenging activity of the asymmetric Schiff base. Various concentrations (125, 250, 375, 500 and 625 µM) of the asymmetric Schiff base were confused with a DPPH solution (4 mg/100 mL), which was followed by placing the mixtures in an upright position in the dark at room temperature. After 30 min of incubation, absorbance reduction was recorded at 517 nm with a microplate reader (Epoch, Biotek, USA). Ascorbic acid in the same concentrations with the asymmetric Schiff base served as a positive control. The % inhibition of both ascorbic acid and asymmetric Schiff base was calculated by using the following formula (Ibrahim et al., 2017):

$$\text{Inhibition (\%)} = {}^{(Ac - As)}/_{Ac} \times 100$$

where A_c = Measured absorbance of the control, A_s = Measured absorbance of the sample (Ibrahim et al., 2017; Ahmad et al., 2017). IC_{50} values correspond to a sample concentration which is essential for scavenging half of DPPH-free radicals.

2.2.2. Ferrous ion chelating assay

The iron chelating propertiy of the asymmetric Schiff base was evaluated by using O-phenantroline color procedure (Sánchez-Moreno et al., 2002). Different concentrations (100, 200, 300 and 400 µM) of the asymmetric Schiff base were added to methanolic solution (3.7 mL) of ferrous chloride (0.1 mL, 2 mM). The reaction was started by the adding of ferrozine (0.2 mL, 5 mM), then the mixture was shaken vigorously. After 10 min of incubation, absorbance reduction was determined at 562 nm with a microplate reader (Epoch, Biotek, USA). EDTA (ethylenediaminetetraacetic acid) in the same concentrations with the asymmetric Schiff base served as a positive control (Şirin and Aslım, 2019). The Fe^{2+} chelating activity was calculated by using the following formula (Ibrahim et al., 2017):

$$\text{Chelating effect (\%)} = {}^{(Ac - As)}/_{Ac} \times 100$$

where A_c = Measured absorbance of the control, A_s = Measured absorbance of the sample (Ibrahim et al., 2017; Ahmad et al., 2017). The IC_{50} values correspond to a sample concentration required to chelate half of the ferrozine-Fe^{2+} complex formation.

2.2.3. Total antioxidant activity (Phosphomolybdenum assay)

Total antioxidant activity of the asymmetric Schiff base was evaluated by using the phosphomolybdenum procedure (Uyoh et al., 2013). At acidic pH, molybdenum Mo(VI) ion is reduced to Mo(V) by removing an electron from the antioxidant. Different concentrations (50, 100, 150, 200, 250 and 500 µM) of the asymmetric Schiff base were mixed with reagent solution (3.0 mL) containg sodium phosphate (28 mM), ammonium molybdate (4 mM) and sulfuric acid (0.6 M). The mixture was incubated at 95 °C for 90 min, then was cooled to room temperature. The rise in absorbance of the mixture was recorded at 695 nm by using a microplate reader (Epoch, Biotek, USA). Ascorbic acid and the sample mixture containing no complexes were employed as the reference standard and the control, respectively (Ibrahim et al., 2017). The following formula was used to calculate the reducing power:

$$\text{Reducing power (\%)} = {}^{(Ac - As)}/_{Ac} \times 100$$

where A_s = Measured absorbance of the control, A_s = Measured absorbance of the sample (Ahmad et al., 2017). The effective concentration (IC_{50}) value was calculated for the asymmetric Schiff base and standard.

2.3. Statistical analysis

Statistical analyzes are given as statistical and mean value ± standard deviation (SD) using IBM SPSS 20.0 program. Significant differences among the means of data were indicated using the One-Way Anova Tukey' HSD test. Statistical significance was determined as $p < 0.05$ at the 95% significance level. All antioxidant tests were performed in at least three replicates.

Received 14 August 2021; Received in revised form 25 August 2021; Accepted 18 September 2021;
Available online 25 September 2021;
doi: 10.46291/ICONTECHvol5iss3pp7-12

3. RESULTS AND DISCUSSION

The new asymmetric diimine Schiff base having free carboxyl group (Figure 1) was synthesized and characterized by different spectroscopic methods and elemental analysis in our previous work (Yilmaz et al., 2017; Yilmaz et al., 2018).

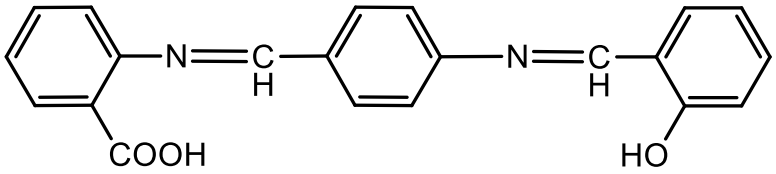

Figure 1. The asymmetric diimine Schiff base

3.1. Antioxidant activity of the asymmetric Schiff base

3.1.1. DPPH radical scavanging activity

DPPH scavenging activity method have been widely used to determine antioxidant properties (Kahriman et al., 2013; Dudonne et al., 2009; Ünver et al., 2016; Naik et al., 2013; Nawaz et al., 2009).

In this paper, the asymmetric Schiff base was researched comparison with standard ascorbic acid. The IC_{50} values found for the asymmetric Schiff base and the natural antioxidant ascorbic acid are shown in Table 1. Measurements showed that the asymmetric Schiff base have significant radical scavenging ability depending on the dose ($*p < 0.05$). The asymmetric Schiff base exhibited a lower scavenging activity than ascorbic acid. Although the asymmetric Schiff base showed lower activity than ascorbic acid, it has higher activity than many complexes in the literature. Ibrahim et al. (2017) reported that Schiff base ligand and their metal complexes present low radical inhibitory effect with high IC_{50} values ranged from 328.05 to 558.31 µM. According to this literature, it can be suggested that the inhibitory effect of the new asymmetric Schiff base is much better than these compounds.

Table 1. Scavenging activity results of the asymmetric Schiff base.

Compounds	% Inhibiton Mean (n=3) ± SD					IC_{50} (µM)
	125 µM	250 µM	375 µM		500 µM	625 µM
Schiff base	48.25±0.02	51.34±0	52.15±0.03	55.78±0.03	58.60±0	180.0±0.03
Ascorbic acid	81.57±0	81.87±0	82.02±0.001	82.18±0	82.48±0	27.0±0.12

Data are given as the mean ± SD. $*p < 0.05$.

3.1.2. Ferrous ion chelating activity (FICA)

Among the transition metals, the most significant lipid prooxidant owing to its elevated reactivity is iron. Fe^{2+} ion (ferrous ion) may cause ROS generation in living systems. Thence ferrous ion chelating ability of compounds may be an important indicator of antioxidant capacity (Halliwell and Gutteridge, 1984; Güder and Korkmaz, 2012; Temel et al., 2015).

In this paper, it was determined that the asymmetric Schiff base affects the formation of ferrous complex with the ferrozine and thus had chelating activity ($*p < 0.05$). The asymmetric Schiff base showed dose dependent a high Fe^{2+} chelating activity close to standard EDTA (Table 2). In addition, the asymmetric Schiff base has Fe^{2+} chelating activity higher activity than many complexes in the literature. Ibrahim et al. (2017) reported that Schiff base and transition metal complexes have high IC_{50} values ranged between 238.26 and 419.56 µM showing their low

Received 14 August 2021; Received in revised form 25 August 2021; Accepted 18 September 2021;
Available online 25 September 2021;
doi: 10.46291/ICONTECHvol5iss3pp7-12

chelating activity. This indicated that the chelating activity of the asymmetric Schiff base is much better than these compounds.

Table 2. Ferrous ion chelating activity results of the asymmetric Schiff base.

Compounds	% Chelation Mean (n=3) ± SD				IC_{50} (µM)
	100 µM	200 µM	300 µM	400 µM	
Schiff base	89.26±0.02	91.65±0.01	92.78±0.001	93.47±0	76.25±0.02
EDTA	96.01±0	96.05±0	97.85±0.03	97.85±0.02	55.0±0.69

Data are presented as the mean ± SD. *$p < 0.05$.

3.1.3. Total antioxidant capacity

The asymmetric Schiff base studied using phosphomolybdate method showed substantial antioxidant activity, with the reducing power having increased as the concentration of the compound was elevated (*$p < 0.05$). Thus, it was seen that the asymmetric Schiff base have higher Mo(VI) reducing activity at higher concentrations. The asymmetric Schiff base showed less molybdenum ion reducing activity than ascorbic acid (Table 3), however were much better than IC_{50} results in the range of 327.48-524.69 µM given in literature for a Schiff base and their transition metal complexes (Ibrahim et al., 2017).

Table 3. Molybdenum ion reducing activity results of the asymmetric Schiff base.

Compounds	% Reduction Mean (n=3) ± SD						IC_{50} (µM)
	50 µM	100 µM	150 µM	200 µM	250 µM	500 µM	
Schiff base	24.19±0.01	41.98±0	54.81±0.03	61.79±0	66.67±0.1	80.89±0	121.47±0.1
Ascorbic acid	31.88±0	54.15±0.01	60.34±0.001	67.13±0.001	71.52±0.001	82.26±0.003	89.47±0.02

Data are presented as the mean ± SD. *$p < 0.05$.

4. CONCLUSION

In this study, to reveal the antioxidant potential of the asymmetric diimine Schiff base, its DPPH radical scavenging, ferrous ion chelation and total antioxidant activities were investigated. The results showed that the asymmetric Schiff base is reactive versus DPPH radicals and Fe^{+2} ions. In addition, it was determined that the asymmetric Schiff base have Mo(VI) reducing activity. The asymmetric Schiff base indicated the most active chelating activity among other antioxidant activity studies.

As a result, it was determined that the Schiff base exhibited a significant antioxidant activity. Therefore, the asymmetric Schiff base may be suggested as potent a synthetic antioxidant for the treatment of oxidative damage related dysfunction and diseases.

Received 14 August 2021; Received in revised form 25 August 2021; Accepted 18 September 2021;
Available online 25 September 2021;
doi: 10.46291/ICONTECHvol5iss3pp7-12

REFERENCES

Ahmad, J.A.M.I.L. Rehman, W. Said, M. Iqbal, Z.A.F.A.R. Gul, S.N. Ahmad, S. Khan, N.Bibi, I. and Qureshi, M.T. 2017. Antioxidants and Antifungal Activities of Substituted Guanidines and their Copper Complexes. Acta poloniae pharmaceutica, 74 (2): 425-434, https://www.ptfarm.pl/pub/File/Acta_Poloniae/2017/2/425.pdf.

Aruoma, O.I. 1996. Assessment of potential prooxidant and antioxidant actions. Journal of the American Oil Chemists' Society, 73 (12): 1617-1625, https://link.springer.com/article/10.1007/BF02517962.

Dudonne, S. Vitrac, X. Coutiere, P. Woillez, M. and Mérillon, J.M. 2009. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. Journal of Agricultural and Food Chemistry, 57: 1768–1774, https://pubs.acs.org/doi/10.1021/jf803011r.

Güder, A. and Korkmaz, H. 2012. Investigation of Antioxidant Activity and Total Anthocyanins from Blackberry (Rubus hirtus Waldst. and Kit) and Cherry Laurel (Laurocerasus officinalis Roem). Asian Journal of Chemistry, 24 (10), https://www.researchgate.net/publication/264388535_Investigation_of_Antioxidant_Activity_and_Total_Anthocyanins_from_Blackberry_Rubus_hirtus_Waldst_and_Kit_and_Cherry_Laurel_Laurocerasus_officinalis_Roem.

Haraguchi, H. 2001. Antioxidative Plant Constituents. In: Bioactive Compounds from Natural Sources. (Ed: C. Tingali.), New York: Taylor and Francis, pp 338 – 377.

Halliwell, B. and Gutteridge, J. 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochemical journal, 219 (1): 1, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1153442/pdf/biochemj00330-0011.pdf.

Ibrahim, M. Khan, A. Ikram, M. Rehman, S. Shah, M. Nabi, H.H. and Achuchaogu, A.A. 2017. In Vitro Antioxidant Properties of Novel Schiff Base Complexes. Asian Journal of Chemical Sciences, 2: 1-12, https://www.journalajocs.com/index.php/AJOCS/article/view/6176/10970.

Kahriman, N. Yayli, B. Aktas, A. İskefiyeli, Z. Beriş, F.Ş. and Yaylı, N. 2013. Synthesis, antibacterial and antioxidant activities of new 1-alkyl-4-(1-alkyl-4-oxo-1,4-dihydroquinolin-2-yl)pyridinium bromides. European Journal of Medicinal Chemistry, 69: 348–55, https://www.sciencedirect.com/science/article/abs/pii/S0223523413005680.

Lobo, V. Patil, A. Phatak, A. and Chandra, N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy reviews, 4 (8): 118, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3249911/.

McCord, J.M. 2000. The evolution of free radicals and oxidative stress. The American journal of medicine, 108 (8): 652-659, https://www.amjmed.com/action/showPdf?pii=S0002-9343%2800%2900412-5.

Sánchez-Moreno, C. 2002. Methods used to evaluate the free radical scavenging activity in foods and biological systems. Food science and technology international, 8 (3): 121-137, https://journals.sagepub.com/doi/pdf/10.1106/108201302026770.

Naik, G.H. Priyadarsini, K.I. Satav, J.G. Banavalikar, M.M. Sohoni, D.P. Biyani, M.K. and Mohan, H. 2003. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. Phytochemistry, 63 (1): 97-104, https://www.sciencedirect.com/science/article/abs/pii/S0031942202007549?via%3Dihub.

Nawaz, H. Akhter, Z. Yameen, S. Siddiqi, H.M. Mirza, B. and Rifat, A. 2009. Synthesis and biological evaluations of some Schiff-base esters of ferrocenyl aniline and simple aniline. Journal of Organometallic Chemistry, 694 (14): 2198-2203, https://www.sciencedirect.com/science/article/abs/pii/S0022328X09001557.

Ogunmoyole, T. Inaboya, S. Makun, J.O. and Kade, I.J. 2013. Differential antioxidant properties of ethanol and water soluble phytochemicals of false nutmeg (Monodoramyristica) seeds. International Journal of Biology and Biotechnology, 2: 253-262, https://www.internationalscholarsjournals.com/articles/differential-antioxidant-properties-of-ethanol-and-water-soluble-phytochemicals-of-false-nutmeg-monodora-myristica-seeds.pdf.

Received 14 August 2021; Received in revised form 25 August 2021; Accepted 18 September 2021;
Available online 25 September 2021;
doi: 10.46291/ICONTECHvol5iss3pp7-12

Şirin, S. and Aslım, B. 2019. Determination of antioxidant capacity, phenolic acid composition and antiproliferative effect associated with phenylalanine ammonia lyase (PAL) activity in some plants naturally growing under salt stress. Medicinal Chemistry Research, 28 (3): 229-238, https://link.springer.com/article/10.1007/s00044-018-2278-6.

Temel, E. Alaşalvar, C. Gökçe, H. Güder, A. Albayrak, Ç. Alpaslan, Y.B. Alpaslan, G. and Dilek, N. 2015. DFT calculations, spectroscopy and antioxidant activity studies on (E)-2-nitro-4-[(phenylimino) methyl] phenol. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 136: 534-546, https://www.sciencedirect.com/science/article/abs/pii/S1386142514014255?via%3Dihub.

Uyoh, E.A. Chukwurah, P.N. Urua, I.S. and Umoffia, H.M. 2013. Upgrading the medicinal value chain of neglected and underutilized eremomastax (Lindau) species through antioxidant health benefitts. International Journal of Medical Aroma Plants, 3: 334-342, https://www.scinapse.io/papers/2169719732.

Ünver, Y. Deniz, S. Çelik, F. Akar, Z. Küçük, M. and Sancak, K. 2016. Synthesis of new 1, 2, 4-triazole compounds containing Schiff and Mannich bases (morpholine) with antioxidant and antimicrobial activities. Journal of enzyme inhibition and medicinal chemistry, 31 (3): 89-95, https://www.tandfonline.com/doi/full/10.1080/14756366.2016.1206088.

Yilmaz, K.Z., Aslim, B., Ozdemir, O. 2017. New synthesis of asymmetric diimine Schiff base for the development of DNA targeted anticancer drug. 5th International BAU-Drug Design Congress, October 19-21, ISTANBUL, 262-263.

Yilmaz, K.Z., Aslim, B., Ozdemir, O. 2018. Asymmetric diimine Schiff base as potential chemotherapeutic drug: antiproliferative activity and micronucleus formation in human cancer cell lines. International Eurasian Conference on Biological and Chemical Sciences, April 26-27, ANKARA, 965.

Yilmaz, K.Z., Aslim, B., Ozdemir, O. 2018. Effect of the Schiff base artificial nucleases on the DNA cleavage activity in presence of activators and reactive oxygen species (ROS) for molecular target DNA. International Eurasian Conference on Biological and Chemical Sciences, April 26-27, ANKARA, 1408.

Received 14 August 2021; Received in revised form 25 August 2021; Accepted 18 September 2021;
Available online 25 September 2021;
doi: 10.46291/ICONTECHvol5iss3pp7-12