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RESEARCH ARTICLE

Screening and evaluation of antioxidant activity of some amido-carbonyl oxime derivatives and their radical scavenging activities

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Abstract

The antioxidant activity of some amido-carbonyl oximes containing a C=O and –NH–R adjacent to the oxime group, [Phenyl-C(=O)-C(=N-OH)-N(-H)-Phenyl(-R)] where R= H, 4-chloro, 4-methyl, 4-methoxy, 3,4-dichloro, 3,4-dimethyl, 3-chloro-4-dimethyl, 3-chloro-4-methoxy, naphthyl and an amido-carbonyl dioxime were investigated *in vitro* by ferric thiocyanate, total reducing power by potassium ferricyanide reduction, 1,1-diphenyl-2-picryl-hydrazyl ($DPPH^{\cdot}$) free radical scavenging, ferrous ions chelating, superoxide anion radical scavenging and hydrogen peroxide scavenging activity assays. The results indicated that the amido-carbonyl oximes have powerful antioxidant capacity.

Keywords: *oxime; amido-carbonyl oxime; antioxidant activity; radical scavenging activity*

Introduction

Organic compounds containing the –C=N–OH group have been named as an oxime compounds (Figure 1) [1]. Oxime derivatives are very important compounds because of their biological activity, such as insecticidal, miticidal, nematocidal, and antidote activities towards organophosphorous poisons. Some oxime complexes have anti cancerogenic activities [2–4].

Also, they have been used as anti-skinning agents in paint, blocking agents in the polymer industry and chelators in variable industrial applications. Some oxime derivatives clinical used as anti-inflammatory and anti-allergic agents [5–7].

Recent studies showed the oximes have powerful antioxidants [8–11] antiepileptic drug [12], anti-inflammatory [13–15], antimicrobial [16,17], oxidative effect [18], antihyperglycemic agent [19] and hepatoprotective [20] activities.

In addition to these, the oximes and their derivatives have been used in analytical applications, such as determination and extraction of the metals [21–26].

Oximes have been named as carbonyl oximes or amidoximes relating to position of the groups. While carbonyl oximes have a carbonyl (C=O) group in the α- position to the oxime group (Figure 1), amide-oximes have a –NH–R group in the α- position to the oxime group (Figure 1). [1,2,4] Carbonyl oximes and amide-oximes have been studied extensively, but work on amido-carbonyl oximes (Figure 1), which have –C=O and –NH–R′ groups in the same molecule in the α-position to the oxime group is scantily described [27].

The reactive oxygen species (ROS) are produced as a normal consequence of biochemical processes in the body and due to increased exposure to environmental and/or dietary xenobiotics. It is an imbalance in these

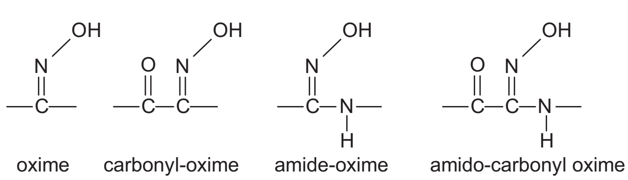

Figure 1. The structure of the oximes and its derivatives.

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oxidants versus antioxidant processes (oxidative stress) that is thought to cause the subsequent cellular damage that leads to the some biological disease processes [28]. Neurodegenerative diseases, such as Alzheimers and Parkinson's disease, are also linked to damage from ROS as a result of an imbalance between the rates of radical generation and scavenging [29].

Antioxidants interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers [30, 31]. Antioxidant supplements or foods containing synthetic antioxidants such as BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), trolox, use to help the human body reduce oxidative damage [32–34]. However, they may cause for liver damage and carcinogenesis in laboratory animals [35]. Therefore, the preparing of more effective new antioxidants is very important research area.

So, the aim of this work were to systemically investigate the total antioxidant activities, reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, and metal chelating activities of the some amido-carbonyl oximes (Subsequently referred to as oxime) and to determine the substitutent effect on this activities (Figure 2). An important goal of this research was to determinate *in vitro* antioxidant effects of oximes against to commercial and standard antioxidants such as trolox, BHA and BHT, commonly used by the pharmaceutical industry.

Materials and methods

Chemicals

Ammonium thiocyanate, ferrous chloride and 1,1-diphenyl-2-picryl-hydrazl ($DPPH^{\cdot}$), nicotine adenine dinucleotide (NADH), BHA, BHT, trolox, trichloracetic acid (TCA), polyoxycthylenesorbitan monolaurate (Tween-20), nitroblue tetrazolium (NBT), nicotinamide adenine dinucleotide (NADH), phenazine methosulphate (PMS), potassium ferricyanide and linoleic acid were commercially available and were reagent grade.

Synthesis of the oximes

The syntheses of the oxime derivatives **1-5, 7-10** used in this work, have been described previously by Taş and et al. [27, 36–42]. Compound **6** was newly prepared for this work from the reaction of ω-chloroisonitrosoacetophenone with 3,4 dimethylaniline using Taş' method [27].

Assay of total antioxidant activity

The ferric thiocyanate method (FTC) was adapted from the model of Mitsuda [43]. The solutions which contains the same concentration oximes and standard antioxidants (5 and 50 μM) in 2.5 mL of potassium phosphate buffer (0.04 M, pH 7.0) was added to linoleic acid emulsion in potassium phosphate buffer (2.5 mL, 0.04 mM, pH 7.0). In addition to these solutions, 5 mL control solution was prepared with linoleic acid emulsion (2.5 mL) and potassium phosphate buffer (2.5 mL, 0.04 M, pH 7.0). The all solutions were shaked and periodical reaction mixture was incubated at 37 °C in dark. The peroxide values were determined by reading the absorbance at 500 nm after reaction with $FeCl_2$ and thiocyanate (SCN^-) at intervals during incubation. During the linoleic acid oxidation, peroxides forms and these compounds oxidize Fe^{2+} to Fe^{+3} and give complex compound with SCN^- ligands which has an absorbance maximum at 500 nm. This step was repeated every 5 h until the control reached its maximum absorbance value. Higher absorbance values indicate higher linoleic acid oxidation. The solutions without oximes or standards were used as a blank sample. The inhibition of lipid peroxidation in percent was calculated by the following equation:

$$\text{Inhibition of lipid peroxidation (\%)} = [(A_0\text{-}A_1)/A_0 \times 100]$$

where A_0 was the absorbance of control incubated with linoleic acid but without the samples (control) and A_1 was absorbance of oximes or the standards which are BHA, BHT and trolox.

Assay of reducing power

The reducing power capacity of oximes was determined according to the method of Oyaizu [44]. The oximes and standards 5 or 50 μM in 1 mL of the corresponding solvent mixed with buffer (2.5 mL, 0.2 M, pH 6.6) and $K_3Fe(CN)_6$ (2.5 mL, 1%), then the mixture was incubated at 50 °C for 20 min. Afterwards, TCA (2.5 mL, 10%) was added to the mixture, which was then centrifuged at 3000 rpm for 15 min. The upper layer of solution (0.5 mL) was mixed 1 mL of distilled water and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm Higher absorbance of the reaction mixture indicated increased reducing power.

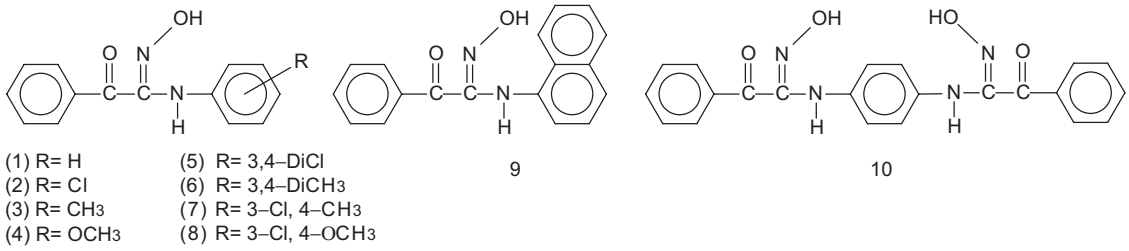

Figure 2. Structure of the amido-carbonyl oximes used in this work.

Assay of antiradical activity

The effect of oximes on $DPPH^{\cdot}$ radical was estimated according to the method of Blois [45] wherein the bleaching rate of a stable free radical, $DPPH^{\cdot}$, is monitored at a characteristic wavelength (517 nm) in the presence of samples. An amount of 0.5 mL of 0.1 mM ethanolic solution of $DPPH^{\cdot}$ was added to 3.0 mL of oximes or standard antioxidants. The mixture was shaken vigorously and waited at room temperature for 30 min. Then the absorbance was measured at 517 nm. The decrease in the absorbance of the $DPPH^{\cdot}$ solution indicates an increasing of $DPPH^{\cdot}$ radical-scavenging activity. The $DPPH^{\cdot}$ concentration (mM) in the reaction medium was calculated from the calibration curve determined by linear regression (R^2:0.9998):

Absorbance = 6.5781 x [$DPPH^{\cdot}$] + 0.058

This activity was calculated by;

$DPPH^{\cdot}$ Scavenging Effect (%) = $[(A_0\text{-}A_1)/A_0 \times 100]$,

where A_0 was the absorbance of control and A_1 was absorbance of oximes or standards. The radical scavenging activity was expressed as IC_{50} which was determined from a calibration curve for each compound.

Assay of metal chelating activity

The chelating of ferrous ions by oximes was determined by the method of Dinis [46]. Briefly, the samples (oximes or standard antioxidants; 5 or 50 μM) were added to a solution of 2 mM $FeCl_2$ (0.05 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for 10 min. The absorbance of the resulting solution was then measured at 562 nm. The metal chelating activities were calculated by the given formula:

Metal chelating effect (%) = $[(A_0\text{-}A_1)/A_0 \times 100]$,

where A_0 was the absorbance of control and A_1 was absorbance of oximes or standards. The control contains $FeCl_2$ and ferrozine.

Assay of superoxide anion scavenging activity

The determination of superoxide anion scavenging activity of oximes was measured according to slightly modified Nishimiki's method [47]. Superoxide radicals are generated in phenazine methosulphate (PMS)-nicotinamide adenine dinucleotide (NADH) systems by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT) [48]. One milliter of oximes solution and standard antioxidants (5 or 50 μM), 1.0 mL NBT solution (156 μM NBT in 100 mM phosphate buffer, pH 7.4) and 1.0 mL NADH solution (468 μM in 100 mM phosphate buffer, pH 7.4) were mixed. The reaction was started by adding 100 μl of PMS solution (60 μM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The mixture was incubated at 25 °C temperature for 5 min, and its absorbance was measured at 560 nm wavelength against blank samples. L-ascorbic acid was used as a control. The decreasing of the absorbance for the mixtures indicates an increasing superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

Inhibition of superoxide anion generation (%) = $[(A_0\text{-}A_1)/A_0 \times 100]$,

where A_0 was the absorbance of control, and A_1 was absorbance of oximes or standards.

Assay of hydrogen peroxide scavenging activity

The ability of oximes to scavenge hydrogen peroxide was determined according to the method of Ruch [49]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (100 mM, pH 7.4). The concentration H_2O_2 was determined from absorption at 230 nm. Samples (oximes or standard antioxidants; 5 or 50 μM, 3.4 mL) were added to the H_2O_2 solution of 0.6 mL. The absorbance of H_2O_2 at 230 nm was determined after 10 minute against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of scavenging H_2O_2 of oximes and standard antioxidants was calculated using the following equation:

Percent Scavenged (H_2O_2) = $[(A_0\text{-}A_1)/A_0 \times 100]$,

where A_0 was the absorbance of control and A_1 was absorbance of oximes or standards.

Statistical analysis

The assays were performed in triplicate. The data were recorded as mean ± standard deviation. They were analysed by SPSS. One-way analysis of variance was performed by ANOVA and Duncan's Multiple Range tests. All results was regarded as $p < 0.05$ (significant) and $p < 0.01$ (very significant).

Results and discussion

Characterization of compound 6.

In the ^{1}H NMR spectrum of **6** (R= 3,4-dimethyl, Figure 2) a singlet peak for the OH proton of oxime group was observed at 11.02 ppm. The N–H protons adjacent to the oxime groups in the ligands resonate at 8.36 ppm. The aromatic C–H protons resonate at 7.90–6.50 ppm while aliphatic CH_3 protons at 2.12–2.06 ppm. The O–H and N–H peaks of the ligands disappeared with the addition of deuterium oxide to the solutions. In the IR spectra of **6**, bands at 3359, 3263, 1687, 1646, and 916 cm^{-1} belong to N–H, O–H, C=O, C=N, and N–O vibrations, respectively. These results are in good agreement with those of related oximes and indicate that the compound **6** have similar structure with previously reported ones (Figure 2).

Total antioxidant activity

The ferric thiocyanate method (FTC) measures the amount of peroxide produced during the initial stages of oxidation, which is the primary product of oxidation. The mechanism of bleaching of the Fe^{3+}-SCN^- complex is a free radical-mediated phenomenon, resulting from the hydro-peroxides formed from linoleic acid. Fe^{3+}-SCN^- complex, in this model system, undergoes rapid discoloration in the absence of an antioxidant. As Fe^{3+}-SCN^- complex loses their electron by oxidation, the compound loses its electron and characteristic

red color, which is monitored spectrophotometrically at 500 nm [48].

The antioxidant activities of oximes, trolox, BHA and BHT were evaluated at 5 and 50 μM using the linoleic acid emulsion model system. Antioxidant activity of the concentration of oximes, trolox, BHA and BHT is shown in Table 1.

The results clearly showed the substitutent and concentration effects on the antioxidant activities of the oximes. The increasing of the oxime functional group numbers indicated the best antioxidant activity. The ligands inductively pulling electron from the benzene ring showed increasing on antioxidant activity. The increasing of the concentration gave whole different lining up.

Increasing the concentration of oximes, trolox, BHA and BHT up to 50 μM resulted in a significant (p<0.05) increase in antioxidant activity. The standard antioxidant (Trolox) showed a maximum activity as ~80% whereas oxime (**10**) showed more activity (~85%) at the concentration of 50 μM. Oxime (**10**) showed ~79% inhibition at 5 μM concentration while standard antioxidant (BHA) showed ~72% inhibition.

Reducing power

The reducing capacity of a compound may help to decide as a significant indicator of its potential antioxidant activity. The presence of reductants such as antioxidant substances in the antioxidant samples causes the reduction of the Fe^{3+}/ferricyanide complex to the Fe^{2+} monitored by measuring the formation of blue color at 700 nm [50]. The antioxidant activity of oximes and standard antioxidants as reflected in their reducing power is presented in Table 2 (as indicated by absorbance at 700 nm). The reducing power of oximes and standard antioxidants increased with increasing concentration of samples. At different concentrations, oximes showed an effective reducing power and these differences were statistically significant (p< 0.05). The reducing power of oximes was in a concentration-dependent manner and substitutent. At the high concentration, reductive capabilities of oximes were lower than the standard BHT except oxime **6**. At the lower concentration, reductive capabilities of oximes were lower than the standard BHT except oximes **1, 2, 5, 6** and **1**. However, the data clearly indicated that the oximes have significant reducing power activity. There was no correlation found between the reducing capabilities and substitutents.

Antiradical activity

Radical scavenging properties are very important because of the decomposition role of free radicals in foods and in biological system. Free radicals induce the oxidation of lipids in foods and decreasing food quality [51]. The effect of antioxidants on $DPPH^{\cdot}$ radical scavenging is thought to be due to their hydrogen donating ability. $DPPH^{\cdot}$ is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [48]. Compounds react with $DPPH^{\cdot}$ which is a nitrogen centered radical with a characteristic absorption at 517 nm, and convert it to stable diamagnetic molecule 1,1-diphenyl-picryl hydrazine, due to its hydrogen donating ability at a very rapid rate. It is visually noticeable as a discoloration from purple to yellow colored 1,1-diphenyl-2-picryl-hydrazine. $DPPH^{\cdot}$ is usually used as a substrate to evaluate antioxidative and antiradical activities of antioxidants. The entire synthesized compounds scavenged $DPPH^{\cdot}$ radical significantly in a concentration-dependent manner (p< 0.01). Their comparable scavenging activities were expressed in IC_{50} (concentration required for 50% inhibition of 0.1 mM $DPPH^{\cdot}$ concentration) value. Trolox, BHT and BHA were used as the positive standard. The radical scavenging activities of the synthesized compounds are summarized in Table 3. Compounds **4, 5, 6, 9** and **10** showed appreciable radical scavenging activity higher than BHT and Trolox. These results indicated that oximes have a noticeable effect on scavenging free radicals. These data clearly indicate that 10 is a powerful free radical inhibitor or scavenger and the radical scavenging activity increased by the electron donor substitiuents.

Table 1. Total antioxidant activities (%) of the oximes, BHA, BHT and trolox at different concentration (5 and 50 μM) in the linoleic acid emulsion system by the FTC method.

		Total antioxidant activity,%	
Compounds	R	5 μM	50 μM
1	H	61.13±1.11	55.55±2.42
2	Cl	72.69±2.70	45.17±1.21
3	CH_3	64.87±1.15	66.77±3.11
4	OCH_3	53.82±4.62	69.29±1.80
5	3,4-diCl	74.42±3.17	78.61±2.73
6	3,4- diCH_3	71.07±4.11	72.64±3.75
7	3-Cl-4-CH_3	50.75±1.20	68.62±1.94
8	3Cl-4-OCH_3	56.56±1.16	82.06±1.43
9	Naphthyl	76.71±3.03	75.71±1.94
10	Dioxim	79.09±4.72	84.73±0.82
11	Trolox	67.25±3.74	79.50±2.41
12	BHA	59.47±1.41	72.75±1.66
13	BHA	71.62±1.31	77.72±2.82

Table 2. Reducing power of different concentrations (5 and 50 μM) of oximes, BHA, BHT and trolox.

		Reducing power,Abs	
Compounds	R	5 μM	50 μM
1	H	0.16±0.007	0.17±0.013
2	Cl	0.153±0.011	0.185±0.008
3	CH_3	0.132±0.018	0.187±0.004
4	OCH_3	0.132±0.009	0.205±0.007
5	3,4-diCl	0.157±0.007	0.169±0.004
6	3,4- diCH_3	0.158±0.008	0.233±0.015
7	3-Cl-4-CH_3	0.146±0.017	0.176±0.017
8	3Cl-4-OCH_3	0.101±0.003	0.194±0.005
9	Naphthyl	0.144±0.017	0.204±0.009
10	Dioxim	0.139±0.008	0.181±0.010
11	Trolox	0.146±0.011	0.167±0.005
12	BHA	0.152±0.006	0.227±0.008
13	BHA	0.141±0.007	0.215±0.011

Table 3. DPPH· free radical scavenging activity (IC_{50}: μg/mL) of oximes. BHA. BHT and Trolox.

Compounds	R	DPPH· free radical scavenging activity, IC_{50}
1	H	170.47±5.14
2	Cl	144.21±8.20
3	CH_3	104.09±4.26
4	OCH_3	44.43±2.54
5	3,4-diCl	48.16±4.07
6	3,4- diCH_3	43.50±3.17
7	3-Cl-4-CH_3	116.67±6.82
8	3Cl-4-OCH_3	62.68±8.19
9	Naphthyl	46.53±4.08
10	Dioxim	42.91±2.27
11	Trolox	59.50±7.41
12	BHA	57.18±3.52
13	BHA	39.11±5.06

Table 4. Ferrous ion (Fe^{2+}) chelating activity (%) of oximes and the standard antioxidants BHA. BHT and trolox at different concentrations (5 and 50 μM).

		Reducing power. Abs	
Compounds	R	5 μM	50 μM
1	H	52.03±1.18	61.08±2.04
2	Cl	70.58±2.10	81.90±1.29
3	CH3	69.23±1.88	80.76±5.10
4	OCH3	72.85±2.60	80.99±1.30
5	3,4-diCl	72.39±3.41	82.80±2.27
6	3,4- diCH_3	70.58±5.10	81.67±3.78
7	3-Cl-4-CH3	72.85±1.28	60.40±1.10
8	3Cl-4-OCH_3	70.81±4.1	82.12±1.91
9	Naphthyl	70.13±3.47	82.12±1.97
10	Dioxim	66.74±2.44	81.22±0.28
11	Trolox	74.66±1.74	73.30±2.84
12	BHA	72.85±1.84	73.52±2.86
13	BHA	37.03±4.53	54.16±1.78

Metal chelating activity

The production of highly ROS (reactive oxygen species) is also catalyzed by free iron through Haber–Weiss reaction ($O_2^{\cdot}$ +$H_2O_2 \rightarrow O_2 + OH^- + OH^{\cdot}$) [52]. Iron has the most important lipid pro-oxidant. It is known that the Fe^{+2} accelerates lipid peroxidation by breaking down hydrogen and lipid peroxides forms by Fenton free radicalic reaction;

($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$). Fe^{+2} ion can form complexes with ferrozine. In the presence of chelating agents, the complex formation is prevented, resulting in a decrease in the red color of the complex. Measurement of color reduction allows determination of metal chelating activity. In this assay, oximes are interfered with the formation of Fe^{2+} and Fe^{3+}-complex since they have chelating activity to capture Fe^{2+} ion before ferrozine (oxime + $Fe^{2+} \rightarrow$ oxime-Fe^{2+} complex). The oxime ligands are good chelating agents for metal ions [1, 27]. The metal chelating effects of the oximes were found as concentration-dependent. As can be seen in Table IV, at the lower concentration, the metal chelating activity was almost similar with the standards. But at the higher concentration the most of oximes showed, significantly ($p< 0.01$) higher metal chelating activity than standards.

These results indicate the good metal chelating capacity of oximes and reduction of the catalysis of lipid peroxidation by the transition metals.

Table 5. Superoxide anion scavenging activity (%) of oximes and the standard antioxidants BHA, BHT and trolox at different concentrations (5 and 50 μM).

		Superoxide anion scavenging activity, %	
Compounds	R	5 μM	50 μM
1	R: H	52.03±2.45	61.08±5.21
2	R: Cl	70.58±3.47	81.90±8.40
3	R:CH3	69.23±1.47	80.76±4.81
4	R:OCH3	72.85±2.51	80.99±1.77
5	R: 3,4-diCl	72.39±3.47	82.80±2.82
6	R: 3,4- diCH_3	70.58±5.44	81.67±3.41
7	R: 3-Cl-4-CH3	72.85±2.88	60.40±5.47
8	R: 3Cl-4-OCH_3	70.81±2.41	82.12±3.97
9	R: Naphthyl	70.13±2.91	82.12±3.64
10	R: Dioxim	66.74±2.45	81.22±2.55
11	Trolox	74.66±2.87	73.30±7.28
12	BHA	72.85±4.15	73.52±5.61
13	BHA	68.55±3.11	76.69±3.75

Superoxide anion scavenging activity

Numerous biological reactions generate superoxide anion which is a highly toxic species. Superoxide anions are a precursor to active free radicals that have potential of reacting with biological macromolecules and thereby inducing tissue damage [53]. Superoxide has also been accelerated to directly initiate lipid peroxidation [54]. Superoxide anion plays an important role in the formation of other ROS such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative damage in lipids, proteins, and DNA [55]. Superoxide anion formed by the reaction between dissolved oxygen with PMS/NADH coupling, is reduced NBT in this system. In this method, superoxide anion is reduced by the yellow dye (NBT) to produce the blue formazan (NBT-H_2) which can measured spectrophotometrically at 560 nm. The test system is used for superoxide generation by PMS/NADH system and reducing superoxide by NBT [47, 56]. It can be said the reaction occurs in two principal stages. The first is the oxidation of NADH by PMS to produce superoxide (1).

$$2NADH + 2O_2 \xrightarrow{PMS} 2NAD + 2O_2^{-\cdot} + 2H^+ \quad (1)$$

The second is the reduction of superoxide by NBT:

$$NBT + 2O_2^{-\cdot} + 2H^+ \longrightarrow NBT\text{-}H_2 + 2O_2 \quad (2)$$

Overall, the stoichiometry of the reaction is as follows reaction:

$$2NADH + NBT + 2O_2 \xrightarrow{PMS} NBT\text{-}H_2 + 2NAD + 2O_2 \quad (3)$$

Antioxidants are able to inhibit producing superoxide by PMS/NADH system and this activity can be monitored by

Table 6. Hydrogen peroxide scavenging activity (%) of oximes and the standard antioxidants BHA, BHT and trolox at different concentrations (5 and 50 μM).

		Hydrogen peroxide scavenging activity, %	
Compounds	R	5 μM	50 μM
1	H	13.33±2.89	7.76±3.41
2	Cl	37.04±5.21	37.29±2.47
3	CH3	43.29±4.87	42.74±2.16
4	OCH3	42.39±2.08	35.96±1.99
5	3,4-diCl	24.29±2.54	29.89±3.91
6	3,4- diCH_3	26.43±2.08	34.88±2.41
7	3-Cl-4-CH3	44.63±2.55	36.73±1.34
8	3Cl-4-OCH_3	2.56±2.08	3.89±3.74
9	Naphthyl	19.71±2.54	40.79±3.11
10	Dioxim	30.67±2.74	41.03±2.73
11	Trolox	30.67±5.07	33.67±1.57
12	BHA	28.04±2.41	34.75±2.66
13	BHA	27.76±2.21	31.53±1.31

decreasing blue NBT-H_2 formation [58]. The decreasing of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. Table 5 shows the inhibition percentage of superoxide radical generation by 5 and 50 μM concentration by oximes and standards. The inhibition of superoxide radical generation by oximes, standards were found statistically significant (p<0.05). As can be seen in Table 5, the percentage inhibition of superoxide anion generation of oximes was higher than standards except for **1** and **10**, at 50 μM and similar with standards at 5 μM concentrations.

Hydrogen peroxide scavenging activity

Hydrogen peroxide forms *in vivo* by many enzymes such as superoxide dismutase and hydrogen peroxide is a precursor to produce the hydroxyl radical (·OH). The hydroxyl radical (·OH) in the cells can easily cross cell membranes at specific sites, react with most bio-molecules and furthermore cause tissue damage and cell death [35]. Thus, removing ·OH is very important for the protection of living, pharmaceutical and food systems.

Table 4 shows the percentage hydrogen peroxide scavenging effect by the oximes and comparison with the effect of standards (trolox, BHT and BHA) at the dose of 5 and 50 μM. Compounds **2, 3, 4,** and **7** showed appreciable hydrogen peroxide scavenging activity higher than standards at lower dose. **2, 3, 4, 6, 7, 9** and **10** showed higher hydrogen peroxide scavenging activity than standards at higher dose, not significantly.

Conclusion

The results of this study clearly indicate that the oximes have a powerful antioxidant capacity against various antioxidant systems *in vitro* assays and the capacity dependent on the concentrations. They were shown to have better antioxidant and radical scavenging activity than the standards used. Therefore, they can be potentely useful as food additive material preventing oxidation, for foods or pharmaceuticals.

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