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Artikel Asli/*Original Article*

Screening and Evaluation of Antioxidant Activities of Selected Naphthalene Compounds

(Penyaringan dan Penilaian Aktiviti Antioksidan Sebatian Naftalena Terpilih)

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ABSTRACT

The antioxidant activities of twelve naphthalene compounds containing (E)-1-((3-iodophenylimino)methyl) naphthalen-2-ol (NAPH1), (E)-1-((3-bromophenylimino) methyl) naphthalen-2-ol (NAPH2), E)-1-((4-bromo-2-(trifluoromethoxy) phenylimino) methyl) naphthalen-2-ol, (E)-1-((4-bromo-2-(trifluoromethoxy) phenylimino) methyl)naphthalen-2-ol (NAPH3), (E)-1-((2-methoxy-5-(trifluoromethyl) phenylimino) methyl) naphthalen-2-ol (NAPH4), (E)-1-((naphthalen-2-ylimino) methyl) naphthalen-2-ol (NAPH5), (E)-1-((2-bromo-3-methylphenylimino) methyl) naphthalen-2-ol (NAPH6), (E)-N-((2-ethoxynaphthalen-1-yl)methylene)-3-methylaniline (NAPH7), (E)-4-ethoxy-N-((2-ethoxynaphthalen-1-yl) methylene) aniline (NAPH8), (E)-N-((2-ethoxynaphthalen-1-yl) methylene) naphthalen-1-amine (NAPH9), (E)-1-(2,5-difluorophenyl)-N-((2-ethoxynaphthalen-1-yl) methylene) methanamine (NAPH10), (E)-N-((2-ethoxynaphthalen-1-yl) methylene)-4-fluoroaniline (NAPH11), (E)-N-((2-ethoxynaphthalen-1-yl)methylene)-2-ethylaniline (NAPH12) were investigated in vitro by antioxidant activity (phosphomolybdenum assay), reducing power, H_2O_2 scavenging activity, metal chelating effects and lipid peroxidation. Scavenging activities of the naphthalen compounds were tested against 1,1-diphenyl-2-picrylhydrazyl, hydroxyl and superoxide anion radicals. Most of them are potent antioxidant, radical superoxide anion scavengers and in vitro inhibition of lipid peroxidation. The compounds; NAPH5, NAPH10 and NAPH12 were found to exhibit promising antioxidant profiles at 10 and 50 mM concentrations. Among these, NAPH5 at higher concentration was the most active compound in inhibiting lipid peroxidation as shown in the homogenates of kidney, heart and spleen. The presented results validate that NAPH5, NAPH10 and NAPH12 can be possessed as a source of antioxidant potential and a rich source of synthetic antioxidant for medicinal or foods.

Keywords: Schiff bases; naphthalene; antioxidant activity; radical scavenging activity; inhibition of lipid peroxidation

ABSTRAK

Aktiviti antioksidan 12 sebatian naftalena yang merangkumi (E)-1-((3-iodofenilimino)metil) naftalen-2-ol (NAPH1), (E)-1-((3-bromofenilimino) metil) naftalen-2-ol (NAPH2), (E)-1-((4-bromo-2-(trifluorometosi) fenilimino) metil) naftalen-2-ol, (E)-1-((4-bromo-2-(trifluorometosi) fenilimino) metil) naftalen-2-ol (NAPH3), (E)-1-((2-metosi-5-(trifluorometil) fenilimino) metil) naftalen-2-ol (NAPH4), (E)-1-((naftalen-2-fenilimino) metil) naftalen-2-ol (NAPH5), (E)-1-((2-bromo-3-metilfenilimino) metil) naftalen-2-ol (NAPH6), (E)-N-((2-etosinaftalen-1-il) metilena)-3-metilanilina (NAPH7), (E)-4-etosi-N-((2-etosinaftalen-1-il) metilena) anilina (NAPH8), (E)-N-((2-etosinaftalen-1-il) metilena) naftalen-1-amina (NAPH9), (E)-1-(2,5-difluorofenil)-N-((2-etosinaftalen-1-il) metilena) metanamina (NAPH10), (E)-N-((2-etosinaftalen-1-yl) metilena)-4-fluoroanilina (NAPH11), (E)-N-((2-etosinaftalen-1-yl)metilena)-2-etilanilina (NAPH12) telah dikaji in vitro dengan menilai aktiviti antioksidan (asai phosphomolybdenum), kuasa penurunan, aktiviti pemangsa H_2O_2, kesan pengkelatan logam dan pengoksidaan lipid. Aktiviti pemangsa sebatian naftalena diuji ke atas 1,1-difenil-2-pikrilhidrazil, hidroksil and bahan radikal anion superoksida. Kebanyakan daripada sebatian tersebut adalah bahan antioksidan yang poten, pemangsa radikal anion superoksida and pehalang pengoksidaan lipid in vitro. Sebatian sperti NAPH5, NAPH10 and NAPH12 didapati menunjukkan profil antioksidan yang memberangsangkan pada kepekatan 10 and 50 mM. Antaranya, NAPH5 pada kepekatan yang lebih tinggi merupakan sebatian yang paling aktif dalam menghalang pengoksidaan lipid seperti yang ditunjukkan dalam homogenat ginjal, jantung dan limpa. Hasil kajian mengesahkan NAPH5, NAPH10 and NAPH12 boleh dijadikan sumber bahan antioksidan yang berpotensi dan juga sumber berguna kepada bahan antioksidan sintetik untuk tujuan perubatan atau pemakanan.

Kata kunci: Alkali Schiff; naftalena; aktiviti antioksidan; aktiviti pemangsa radical; penyekatan peroksidasi lipid

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INTRODUCTION

Schiff bases which are formed by condensation of primary carbonyl and amines compounds (aldehydes and ketones) and their transition metal complexes are very important organic compounds in medicinal and pharmaceutical field because of their biological applications including antibacterial, antitumor activity, antifungal, antioxidant, anti-inflammatory, anti-HIV, antihypertensive, anticonvulsant, herbicidal, antifilarial, insecticidal, anthelmintic and schistosomicidal activities because of their three-dimensional structural profiles and cationic character. It has been shown that azomethine linkage (C = N) of the schiff base provides an opportunity for the stupendous biological effect such as antifungal, antibacterial, antitumor and herbicidal activities (Taha et al. 2011). Schiff bases including naphthalenes are classified as a benzenoid polycyclic aromatic hydrocarbon including two fused benzene rings and do not contain heteroatoms or carry substituents (Tamer et al. 2014). Currently, schiff-bases have been investigated as scavenging free radicals of reactive oxygen species to be developed as potential antioxidants. The free radical scavenging effects of the schiff base may be due to its imino group and substituent extending free radical delocalization (Ekennia et al.; Shrivastava et al. 2017).

Antioxidant activity is a very important parameter used to characterize different chemical materials. Therefore, the antioxidant molecules have become one of the major areas of scientific research. They are extensively investigated for their capacity to protect organism and cell from damage caused by oxidative stress (Carocho & Ferreira 2013). The scientists become more interested in newly synthesized antioxidant compounds that could prevent harmful effects of oxidative processes or scavenge the radicals on cell. The discovery of novel antioxidants has gained a lot of attention in the last years by many researchers based on their potential activities in controlling or preventing the occurence of destructive diseases such as aging, cancer disorders and cardiovascular (Nandagokula et al. 2013). Recently, the newly synthesized compounds incorporating oxadiazole ring have been of widespread attention due to their diverse pharmacological properties such as antitumor activities, anti-inflammatory antimicrobial and antianalgesic (Al-Amiery 2012). Trolox, butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ) and butylated hydroxyanisole (BHA) in fat and oily foods were used as antioxidant supplements to prevent oxidative degradation. It was found that the standard antioxidants may cause carcinogenesis and liver damage in laboratory animals (Taha et al. 2011). Therefore, we attempted to determine whether naphthalene derivatives exhibit the antioxidant activity in the in vitro model assays related to doses and also evaluated the effects of heterocyclic substituents (phenyl, naphthyl, ethoxynaphthyl) on their activities.

The report of literature survey reveals that there is no information available about the antioxidant properties of naphthalene derivatives. The main goal of the study was to evaluate the total antioxidant, reducing power, inhibition level of lipid peroxidation (LP), metal chelating, superoxide anion ($O_2^{\cdot-}$), hydroxyl radical (•OH), H_2O_2 and free radical (DPPH•) scavenging activities of naphthalene derivatives and also to compare the activities of these compounds with BHA, BHT, TBHQ, trolox and vitamin E commonly used in the pharmaceutical industry at different concentrations.

MATERIALS AND METHODS

CHEMICALS

3-(2-pyridyl)-5,6-bis (4-phenyl-sulphonic acid)-1,2,4-triazine (ferrozine), ethylenediaminetetraacetic acid (EDTA), tween-20, sodium phosphate, ammonium molybdate, $K_3Fe(CN)_6$, thiobarbituric acid (TBA), malondialdehyde (MDA) in phenazine methosulphate (PMS), nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium (NBT), 1,1-diphenyl-2-picryl-hydrazl (DPPH•), ferrous chloride, $Na_2S_2O_3$, L-ascorbic acid, K_2HPO_4, KH_2PO_4, KI, $FeSO_4$, vitamin E, tert-butylhydroquinone (TBHQ), BHA, BHT and trolox were purchased from Sigma–Aldrich (Steinheim, Germany). All other chemicals in the assays used were research grade.

SYNTHESIS OF THE NAPHTHALENE DERIVATIVES

The naphthalene derivatives, NAPH1 (Akbal et al. 2012b), NAPH2 (Akbal et al. 2012a), NAPH3 (Kargılı et al. 2013), NAPH4 (Alpaslan et al. 2015), NAPH5 (Tamer et al. 2014), NAPH6 (Yıldırım 2012), NAPH7 (Yıldırım et al. 2016), NAPH8 (Yıldırım 2012), NAPH9 (Yıldırım 2012), NAPH10 (Pekdemir et al. 2013), NAPH11 (Yıldırım 2012) and NAPH12 (Kargılı et al. 2012) were synthesized, and their antioxidant, radical scavenging activities and inhibition of lipid peroxidation were evaluated in the current study (Table 1).

ASSAY OF TOTAL ANTIOXIDANT

The total antioxidant activity of the naphthalene samples and standards (10 and 50 mM) was evaluated following the method given by Prieto et al. (1999) based on the reduction of Mo(VI) to Mo(V) by the naphthalenes or standards and the subsequent formation of specific green phosphate/Mo(V) compounds. A reagent solution (28 mM sodium phosphate, 0.6 M sulfuric acid and 4 mM ammonium molybdate) were mixed with 0.3 ml of naphthalene compounds and standards. All samples were incubated in water bath at 95°C for 90 min and cooled down to 25°C. The absorbance of the green phosphomolybdenum complex was recorded at 695 nm. The higher the absorbance of naphthalene derivatives the more effective the antioxidant compound was.

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ASSAY OF REDUCING POWER

In this test, the reductant compounds cause the reduction of the Fe^{3+}/ferricyanide complex to the Fe^{2+} form and the presence of Fe^{2+} can be monitored by measuring the absorbance at 700 nm (Oyaizu 1986). One ml samples (10-50 µM) were mixed with 0.2 M phosphate buffer (2.5 ml, pH 6.6) and 2.5 ml of 1% $K_3Fe(CN)_6$ and the mixture was incubated in a water bath at 50°C for 20 min. Then, 2.5 ml of 10% TCA was added. The 2.5 ml of the mixture was mixed with 2.5 ml water and 0.5 ml of 0.1% $FeCl_3$. It is indicated that high absorbance of the sample had good reducing power under the reaction condition.

TABLE 1. Structures of the naphthalene derivatives used

Compounds	Structure	Compounds	Structure
NAPH1; (E)-1-((3-iodophenyl-imino)methyl)-naphthalene-2-ol		NAPH7; (E)-N-((2-ethoxy-naphthalene-1-yl)-methylene)-3-methylaniline	
NAPH2; (E)-1-((3-bromo-phenylimino)methyl)-naphthalene-2-ol		NAPH8; (E)-4-ethoxy-N-((2-ethoxynaphthalene-1-yl)methylene)aniline	
NAPH3; (E)-1-((4-bromo-2-(trifluoromethoxy)-phenylimino)methyl)-naphthalene-2-ol		NAPH9; (E)-N-((2-ethoxy-naphthalen-1-yl)-methylene)naphthalene-1-amine	
NAPH4; (E)-1-((2-methoxy-5-(trifluoromethyl)-phenylimino)methyl)-naphthalene-2-ol		NAPH10; (E)-1-(2,5-difluoro-phenyl)-N-((2-ethoxy naphthalene-1-yl)-methylene)methanamine	
NAPH5; (E)-1-((naphthalen-2-ylimino)methyl)-naphthalene-2-ol		NAPH11; (E)-N-((2-ethoxy naphthalene-1-yl)-methylene)-4-fluoro-aniline	
NAPH6; (E)-1-((2-bromo-3-methylphenylimino)-methyl)naphthalene-2-ol		NAPH12; (E)-N-((2-ethoxy-naphthalene-1-yl)-methylene)-2-ethylaniline	

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ASSAY OF METAL CHELATING

The ferrous ion chelating activity of naphthalene derivatives and standard antioxidants was assessed as described by Dinis (Dinis et al. 1994). Ferrozine can form complex with ferrozine-Fe^{2+}, effectively. In the presence of other chelating compounds, this complex is disrupted. Therefore, the intensity of specific red colour of the complex allows the prediction level of the chelating activity. Briefly, different concentrations of samples were added to a solution of 2 mM $FeCl_2$ (0.05 ml) and left for incubation at 25°C. The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml) and the mixture was shaken vigorously and incubated at 25°C for 10 min. The absorbance of the solution was measured at 562 nm. The activity was calculated by the formula; The metal chelating activity (%) = $[(A_c\text{-}A_s)/A_c] \times 100$ from which A_c is the absorbance of control and A_s is the absorbance of the sample.

ASSAY OF SUPEROXIDE ANION SCAVENGING

The estimation of superoxide anion ($O_2^{\cdot-}$) scavenging activity was based on the assay described in literature (Nishikimi et al. 1972). Briefly, $O_2^{\cdot-}$ is generated in PMS-NADH systems by oxidation of NADH and evaluated by adding NBT. In this experiment, 1.0 ml of sample, 1.0 ml of NBT solution (156 mM NBT) and 1.0 ml of NADH solution (468 mM) were mixed in a test tube. The reaction was initiated by adding 100 ml of PMS solution (60 mM PMS) to the mixture. The mixture was incubated at 25°C for 5 min and absorbance was recorded at 532 nm. The activity was calculated according to the following formula: Inhibition (%) = $[(A_c\text{-}A_s) / A_c] \times 100$ where A_c is the absorbance of the control and A_s is the absorbance of the sample.

ASSAY OF FREE RADICAL SCAVENGING

The electron donation activity of the naphthalene derivatives was measured by bleaching of the purple-coloured solution of DPPH· according to the technique reported by Blois with a slight modification (Blois 1958). In this assay, the bleaching rate of a stable free radical (DPPH·) was monitored at a characteristic wavelength (517 nm) in the presence of naphthalene derivatives. The solutions of naphthalenes or standard antioxidants (10-50 µM) were added to 0.5 ml of DPPH· (0.1 mM). The mixtures were incubated at 25°C and the absorbance was recorded at 517 nm. The free radical scavenging activity was calculated by the given formula: Inhibition (%) = $(A_c - A_s) / A_s \times 100$ where A_s is the absorbance of the control and A_c is the absorbance in the presence of the sample.

ASSAY OF H_2O_2 SCAVENGING

The H_2O_2 scavenging activity of the naphthalene derivatives was measured according to slightly modified method given in the reference (Zhao et al. 2006). Briefly, 10 and 50 µM concentration of samples were mixed with 1 ml of H_2O_2 (0.1 mM), 1 ml of 100 µl ammonium molybdate (3%), 10 ml of H_2SO_4 (2 M) and 7 ml of KI (1.8 M), respectively. The mixed solution was titrated with $Na_2S_2O_3$ (5 mM) until the yellow color disappeared. The H_2O_2 scavenging activity was calculated according to the following formula:

Inhibition (%) = $(V_c - V_s) / V_c \times 100$ where V_c and V_s are volumes of the control and the sample, respectively.

ASSAY OF HYDROXYL RADICAL SCAVENGING

The scavenging activity of peroxide by the naphthalene derivatives was determined by slightly modified method given by Smirnoff & Cumbes (1989). In this assays, hydroxyl radical was produced by mixing H_2O_2 and $FeSO_4$ at room temperature. The mixture composed of 1 ml of $FeSO_4$ (1.5 mM), 0.7 ml of H_2O_2 (6 mM), 0.3 ml sodium salicylate (20 mM) and naphthalenes compounds (10-50 µM). The samples were incubated at room temperature for 1 h. The absorbance of the hydroxylated salicylate was measured at 562 nm against a blank. The hydroxyl scavenging activity was calculated using the following equation: Inhibition (%) = $[1\text{-} (A_c - A_s) / A_2] \times 100$ where A_c and A_s are the absorbances of the control and the sample, respectively.

ASSAY OF LIPID PEROXIDATION

The lipid peroxidation (LP) level of naphthalene compounds was determined by using the rat liver, lung, kidney, brain, heart and spleen homogenates in the presence of $FeCl_2$-ascorbic acid (Varshney & Kale 1990). Male Albino Wistar rats (200-220 g) were fed with a standard laboratory pellet and allowed to drink tap water ad libitum. The rats were starved for 24 h prior to execution by decapitation under anaesthesia. After applying heparin to the liver, the organs were perfused with ice-cold 0.9% NaCl (+ 4°C) to cleanse the blood and to eliminate any possible effects due to diurnal variation, immediately. The liver, lung, kidney, brain, heart and spleen were immediately removed and washed with ice-cold distilled water (+ 4°C) and homogenized with 0.15 M Tris KCl (pH 7.4) in an all-glass homogenizer. The homogenate of each organ was centrifuged at + 4°C for 25 min at 15.000 × g to obtain cytosolic fraction. The protein contents of cytosolic fractions were determined at 660 nm using BSA as the standards (Lowry et al. 1951). The LP level was measured by estimation of TBA as the level of nmoles of MDA formed per mg protein at 532 nm. An appropriate concentration of cytosolic sample was mixed with 0.15 M Tris KCl (pH 7.4), 30% TCA and 52 mM TBA in a final volume of 3.0 ml. The mixture was incubated in water bath at 90°C for 45 min, cooled immediately in an ice bath, and centrifuged at 5000 × g. The absorbance of clear supernatant solution was measured at 532 nm. MDA was used as standard. The results were defined as nmoles of MDA formed/mg cytosolic protein.

STATISTICAL ANALYSIS

The data were presented as mean ± standard deviation (S.D.). Statistical analysis was conducted using SPSS (17.0) software. The results of activities were peformed using one-way ANOVA and followed by Tukey's HSD test with $\alpha = 0.05$. All assays were performed in triplicate.

RESULTS AND DISCUSSION

The antioxidant activities of NAPH1-12 were evaluated by several *in vitro* methods at various concentrations in order to compare the results with different standards and to establish the relationships between structure and antioxidant activity for each assay (Table 1-6).

ANTIOXIDANT ACTIVITY

This method is routinely applied in the samples to evaluate the total antioxidant capacity (Prieto et al. 1999). The results indicated that naphthalene derivatives had the highiest antioxidant activity and exhibited the concentration and substitutent effects on the antioxidant activities (Govindhan et al. 2015). The heterocyclic ligands effectively pulling electron in the naphthalene rings had an effective increasing on antioxidant activity (Bala et al. 2013). The results of the preliminary qualitative antioxidant activity of all the tested compounds were as listed in Table 2. Different concentration of naphthalene derivatives and standards exhibited significant increased in the antioxidant activities ($p < 0.05$). It is revealed that NAPH3, 4, 5 and 6 exhibited the potential antioxidant activity. Among these, NAPH6 appears to show the best antioxidant when compared with BHA, trolox, BHT and TBHQ. The increasing of the naphthalene functional group numbers as electron acceptor phenyl and naphthyl groups indicated the best antioxidant activity. The ligands inductively pulling electron from the naphthalene ring exhibited increasing on total antioxidant activity.

The order of activity was VIT E > NAPH6 > NAPH5 > NAPH4 > NAPH3 > BHA > NAPH8 > NAPH1 > NAPH12 > NAPH2 > NAPH10 > trolox > BHT > NAPH11 > TBHQ > NAPH7 > NAPH9 in terms of phosphomolybdenum reduction potential at 50 μM (Table 2).

REDUCING POWER

The presence of reductants causes the reduction of the Fe^{3+}-ferricyanide complex to the Fe^{2+} in conformity with the measurements of the blue-color such as antioxidant substances in the naphthalene derivatives (Matsushita et al. 2011). An increase in absorbance of the reaction mixture may indicate an increase in the reducing capacity due to an increase in the formation of the complex, $Fe_4[Fe(CN^-)_6]_3$). The reducing power has been shown to exert antioxidant action by donating a hydrogen atom to break the free radical chain (Kiokias & Gordon 2004). The results of reducing power of of the naphthalenes, BHA, BHT, TBHQ, trolox and VIT E were evaluated at the concentrations of 10 and 50 μM, asdepicted in the Table 2. Their reducing power capacities were found to be dose dependent except for the reducing power of NAPH2, NAPH6 and NAPH9. Due to the presence of substituted naphthyl containing hydroxy and naphthalene rings (electron withdrawing group), NAPH5 exhibited much better reducing power and had a higher absorbance value when compared to the other napthalene derivatives and standards. Table 2 shows that the reducing power capacity of samples in decreasing order order: NAPH5 > NAPH12 > BHA > BHT > trolox > NAPH8 > VIT E > TBHQ > NAPH11 > NAPH9 > NAPH10 > NAPH1 > NAPH3 > NAPH4 > NAPH7 > NAPH6 > NAPH2 at higher concentration ($p < 0.05$). While reductive properties of NAPH5 and NAPH12 were higher than the standard antioxidants at 50 μM, reductive capabilities of NAPH5 and NAPH12 were lower than the standards at 10 μM.

FREE RADICAL SCAVENGING ACTIVITY

DPPH• scavenging assay is a standard assay for evaluating free radical scavenging activity in a rapid technique compared with other assays of specific compounds (Polterait 1997). DPPH• is a stable, deep purple color with free radical widely used to evaluate radical scavenging activity. The antioxidant reacts with DPPH• free radical (i.e., by providing hydrogen atom or by electron donation, conceivable), converting it to colorless 1,1-diphenyl-2-picrylhydrazine ($DPPH_2$) when it is present in the medium. The degree of discoloration indicates the radical scavenging activity of the antioxidant in terms of their hydrogen donating property (Charef et al. 2015). Accordingly, scavenging capacity of naphthalene derivatives were measured following the decrease of absorbance at 517 nm. Percentage activity of naphthalenes at different concentrations were recorded and compared with the standards. The scavenging activity of all naphthalene derivative compounds were calculated (Table 3) and found to be concentration-dependent significantly ($p < 0.05$). All synthesized naphthalene analogues exhibited lower scavenging activity than the standard antioxidants and showed moderate to excellent scavenging activities. The increasing order of free radical scavenging activity of synthesized analogues was as follows: trolox > TBHQ > VIT E > BHA > NAPH7 > NAPH10 > BHT > NAPH12 > NAPH8 > NAPH1 > NAPH4 > NAPH9 > NAPH11 > NAPH6 > NAPH3 > NAPH5 > NAPH2 at 50 μM. On the basis of this observation, it can be suggested that the presence of electron-donating groups in the naphthalene moiety have an important influence on the quality of their radical scavenging activities at 10 μM and 50 μM. Among them, NAPH7 and NAPH10 compounds exhibited dominant free radical scavenging activities compared to BHT due to the presence of more electron donating groups.

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METAL CHELATING ACTIVITY

Iron accelerates lipid peroxidation by breaking down hydrogen and lipid peroxide forms (Haber & Weiss 1934). In the presence of antioxidant chelating agents, the ferrozine-Fe^{2+} complex formation is prevented by the formation of the red color complex (Dinis et al. 1994). Changes in the color intensity allows the estimation of metal chelating capacity. Naphthalene derivatives interfered with the formation of Fe^{3+} or Fe^{2+}-complex since they have the chelating activity to capture Fe^{2+} before ferrozine (Turan et al. 2016). Metal chelating activity of naphthalen-2-ol and 2-ethoxynaphthalen containing phenylimino (NAPH1-6) and substituted aniline or naphthalyl (NAPH7-12) were compared. The activities of the naphthalenes were found to be concentration-dependent (Table 3). The metal chelating activity was lower than standards at 50 μM, signifigantly ($p < 0.05$) and the naphthalene ligands could be effective chelating agents for metal ions. The increasing order of the metal chelating activity of the samples was EDTA > BHT > trolox > BHA > VIT E > TBHQ > NAPH12 > NAPH10 > NAPH11 > NAPH9 > NAPH1 > NAPH8 > NAPH7 > NAPH2 > NAPH3 > NAPH5 > NAPH6 > NAPH4. The 2-ethoxynaphthalen analogues (NAPH10-12) and substituted naphalene (NAPH9) exhibited better metal chelating activity than the other synthesized compounds.

TABLE 2. Total antioxidant activity by phosphomolybdenum method and reducing power of the naphthalene derivatives and standards at 10 and 50 μM. Each value represents mean ± SD, ($n = 3$), where * corresponds to $p < 0.05$.

Samples		Antioxidant activiy*, 695 nm		Reducing power*, 700 nm	
		10 μM	50 μM	10 μM	50 μM
Compound	NAPH1	0.156 ± 0.010	0.179 ± 0.010	0.112 ± 0.001	0.115 ± 0.003
	NAPH2	0.178 ± 0.002	0.175 ± 0.012	0.097 ± 0.001	0.070 ± 0.011
	NAPH3	0.181 ± 0.006	0.184 ± 0.016	0.115 ± 0.001	0.113 ± 0.020
	NAPH4	0.183 ± 0.058	0.185 ± 0.002	0.092 ± 0.001	0.108 ± 0.024
	NAPH5	0.163 ± 0.005	0.188 ± 0.004	0.117 ± 0.002	0.217 ± 0.013
	NAPH6	0.149 ± 0.002	0.189 ± 0.005	0.102 ± 0.007	0.094 ± 0.022
	NAPH7	0.144 ± 0.012	0.156 ± 0.007	0.114 ± 0.003	0.104 ± 0.021
	NAPH8	0.156 ± 0.018	0.181 ± 0.014	0.116 ± 0.067	0.157 ± 0.014
	NAPH9	0.164 ± 0.013	0.156 ± 0.012	0.179 ± 0.012	0.121 ± 0.012
	NAPH10	0.188 ± 0.014	0.175 ± 0.010	0.115 ± 0.041	0.118 ± 0.022
	NAPH11	0.144 ± 0.006	0.169 ± 0.007	0.111 ± 0.012	0.123 ± 0.030
	NAPH12	0.164 ± 0.011	0.177 ± 0.013	0.133 ± 0.060	0.195 ± 0.011
Positive control	BHA	0.184 ± 0.005	0.184 ± 0.041	0.134 ± 0.018	0.194 ± 0.034
	BHT	0.178 ± 0.015	0.171 ± 0.012	0.176 ± 0.024	0.181 ± 0.025
	TBHQ	0.154 ± 0.006	0.167 ± 0.022	0.129 ± 0.021	0.151 ± 0.014
	Trolox	0.164 ± 0.022	0.173 ± 0.009	0.160 ± 0.011	0.168 ± 0.009
	VIT E	0.182 ± 0.002	0.191 ± 0.010	0.169 ± 0.008	0.167 ± 0.023

Abbreviations: BHA; Butylated hydroxyanisol, BHT; Butylated hydroxytoluene, TBHQ; t-butyl-hydroxyquinone, VIT E; Vitamin E

SUPEROXIDE ANION SCAVENGING ACTIVITY

$O_2^{\cdot -}$ generated by metabolic reactions in cells are the precursor to active free radicals that have the power to induce tissue damage and react with biological macromolecules. $O_2^{\cdot -}$ radicals directly initiate lipid peroxidation, (Wickens 2001). Therefore, it plays an important role in the formation of many reactive oxygen species such as singlet oxygen, hydroxyl radical and hydrogen peroxide which induce oxidative damage in proteins, lipids and DNA (Pietta 2000). The antioxidants are able to inhibit the production of $O_2^{\cdot -}$ and the NBT-H_2 formation is monitored via blue colored product (Dinis et al. 1994). The inhibiting percentage of $O_2^{\cdot -}$ generation by naphthalenes and standards (10 and 50 mM) was given in Table 4. The decreasing order of $O_2^{\cdot -}$ scavenging activity was TBHQ > trolox > BHA > NAPH5 > NAPH12 > VIT E > BHT > NAPH4 > NAPH3 > NAPH6 > NAPH11 > NAPH8 > NAPH2 > NAPH10 > NAPH7 > NAPH9 > NAPH1, ($p < 0.05$) (Table 4). The compound NAPH5 bearing two naphthalyl groups (electron donating group) at trans position exhibited higher superoxide scavenging activity.

H_2O_2 SCAVENGING ACTIVITY

H_2O_2 is formed *in vivo* by antioxidant enzymes and behaves as a precursor to produce $^{\cdot}OH$. The $^{\cdot}OH$ radicals react with most biomolecules, able to cross cell membrane, cause tissue damage and cell death (Oktay et al. 2003). The

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percentage of H_2O_2 scavenging ability by the naphthalenes and the standards (BHA, TBHQ, BHT, trolox and VIT E) at 10 and 50 mM were as shown in Table 4. NAPH1, NAPH3 and NAPH7 exhibited higher H_2O_2 scavenging activity than the standards at 50 mM, ($p < 0.05$). The scavenging activity values of H_2O_2 were in the following order: NAPH1 > NAPH3 > NAPH7 > BHA > TBHQ > NAPH8 > trolox > NAPH10 > NAPH11 > NAPH2 > NAPH12 > BHT > NAPH9 > NAPH5 > VIT E > NAPH4 > NAPH6. NAPH1 (60%) and NADP3 (59%) bearing iodophenylimino and trifluoromethoxyphenylimino groups have significantly enhanced scavenging activity towards H_2O_2.

·OH SCAVENGING ACTIVITY

The ·OH, known as the most reactive of oxygen radicals can severely damage biomolecules such as proteins, lipids and nucleic acids (Yoshikawa et al. 1997). The activity of naphthalenes at different concentrations was assessed by their competitive ability with salicylic acid for ·OH radical in the ·OH generating-detecting system. The scavenging activity was found to be higher in NAPH10 comparing to the other naphthalene derivatives and to be in the order of NAPH10 > BHA > BHT > TBHQ > VIT E > trolox > NAPH11 > NAPH12 > NAPH9 > NAPH7 > NAPH1 > NAPH8 > NAPH4 > NAPH6 > NAPH3 > NAPH5 > NAPH2 at 50 mM (Table 4), ($p < 0.05$). NAPH10 compound dominated ·OH radical scavenging activity compared to the standards due to its substituted difluoro group (electron withdrawing) at para position. The coupling of two difluoro groups and substituted ethoxynaphthyl enhanced the scavenging activity. The other naphthalenes exhibited moderate ·OH scavenging activity at the two different doses.

TABLE 3 Free radical scavenging (DPPH·) and metal chelating (Fe^{2+}) activity (10 and 50 mM) of the naphthalene derivatives and standards (TBHQ, BHA, BHT, trolox and VIT E) at 10 and 50 mM. Each value represents mean ± SD, ($n = 3$), where * corresponds to $p < 0.05$.

Samples		Free radical scavenging activity*, %		Metal chelating activity*, %	
		10 µM	50 µM	10 µM	50 µM
Compound	NAPH1	65.49 ± 3.04	69.39 ± 0.21	52.81 ± 0.16	58.37 ± 0.08
	NAPH2	62.23 ± 2.86	65.25 ± 0.24	53.90 ± 1.37	55.05 ± 0.21
	NAPH3	65.29 ± 2.81	66.33 ± 0.65	27.76 ± 1.09	53.15 ± 0.60
	NAPH4	66.11 ± 3.38	69.11 ± 0.03	3.45 ± 0.55	9.84 ± 0.38
	NAPH5	60.69 ± 3.15	65.51 ± 0.07	15.30 ± 11.05	26.30 ± 0.42
	NAPH6	67.83 ± 3.56	66.50 ± 0.25	1.32 ± 0.46	12.99 ± 0.46
	NAPH7	76.43 ± 2.73	78.42 ± 0.01	58.47 ± 0.49	56.00 ± 0.28
	NAPH8	70.45 ± 2.80	73.90 ± 0.23	61.32 ± 0.32	56.23 ± 0.78
	NAPH9	68.51 ± 2.73	68.95 ± 0.09	59.82 ± 0.34	60.23 ± 1.70
	NAPH10	76.02 ± 2.72	76.22 ± 0.35	49.00 ± 1.86	61.62 ± 0.50
	NAPH11	67.01 ± 2.99	66.53 ± 0.18	54.98 ± 1.43	60.34 ± 0.93
	NAPH12	68.17 ± 3.02	74.00 ± 0.22	7.56 ± 0.51	61.85 ± 0.30
Positive control	BHA	80.55 ± 2.96	94.46 ± 0.10	85.96 ± 4.45	86.35 ± 0.38
	BHT	71.36 ± 2.89	74.76 ± 0.05	74.68 ± 5.48	91.53 ± 1.42
	TBHQ	91.89 ± 2.91	96.25 ± 0.01	48.81 ± 0.91	62.21 ± 3.70
	Trolox	96.15 ± 3.05	98.41 ± 0.04	82.60 ± 3.12	88.47 ± 6.00
	VIT E	77.80 ± 2.88	95.43 ± 0.17	62.21 ± 3.70	80.39 ± 4.58
	EDTA	-	-	81.28 ± 6.94	95.39 ± 3.71

Abbreviations: BHA; Butylated hydroxyanisol, BHT; Butylated hydroxytoluene, TBHQ; t-butyl-hydroxyquinone, VIT E; Vitamin E

ASSAY OF LIPID PEROXIDATION

A number of physical and chemical phenomena initiates the LP level which proceeds continuously in the presence of a suitable substrate until a scavenging defence mechanism occurs. Lipids and lipid-containing materials including phospholipids, cholesterol, polyunsaturated fatty acids, DNA and oxygen prone to peroxidation during processing and storage (Antolovich et al. 2002; Frankel 2014). LP, being a complex free radical chain process, involves an array of radicals and is measured by the amount of peroxide, the primary product of lipid oxidation, produced during the initial stages of oxidation. The effects of naphthalenes on LP level in the liver, lung, brain, kidney, spleen and heart homogenates were determined *in vitro* and the results are as shown in Tables 5 and 6. The extent of inhibition by the naphthalenes and standards were

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TABLE 4. $O_2^{\cdot-}$, H_2O_2 and $^{\cdot}OH$ scavenging activities of the naphthalene derivatives and standards (BHA, BHT, TBHQ, trolox and VIT E) at 10 and 50 mM. Each value represents mean ± SD, (n = 3), where * corresponds to $p < 0.05$.

	Samples	Superoxide scavenging activity*, %		H_2O_2 scavenging activity*, %		$^{\cdot}OH$ radical scavenging activity*, %	
		10 µM	50 µM	10 µM	50 µM	10 µM	50 µM
Compound	NAPH2	45.93 ± 0.91	53.61 ± 0.61	41.88 ± 0.83	52.83 ± 2.93	18.51 ± 1.36	22.95 ± 3.59
	NAPH3	47.92 ± 0.56	61.08 ± 0.45	54.13 ± 1.39	59.81 ± 1.62	20.81 ± 1.41	30.22 ± 0.38
	NAPH4	59.71 ± 0.73	62.35 ± 0.55	12.50 ± 2.20	23.08 ± 0.97	24.35 ± 2.00	36.57 ± 1.31
	NAPH5	28.88 ± 1.73	69.72 ± 1.14	44.04 ± 1.67	49.23 ± 0.80	21.30 ± 1.85	28.99 ± 3.01
	NAPH6	46.89 ± 1.75	59.19 ± 0.82	11.06 ± 4.33	15.86 ± 0.88	25.46 ± 2.28	35.20 ± 0.40
	NAPH7	45.37 ± 0.85	49.60 ± 1.11	52.81 ± 0.83	54.88 ± 2.03	45.67 ± 1.34	53.35 ± 2.83
	NAPH8	50.57 ± 1.06	54.40 ± 0.75	43.37 ± 1.67	53.85 ± 1.44	43.84 ± 1.69	47.16 ± 3.82
	NAPH9	41.84 ± 0.94	47.73 ± 0.76	46.92 ± 2.71	51.15 ± 1.67	35.49 ± 0.45	55.47 ± 0.99
	NAPH10	44.78 ± 0.69	53.29 ± 0.45	47.12 ± 0.83	52.88 ± 0.83	60.79 ± 1.85	79.06 ± 1.33
	NAPH11	50.53 ± 1.30	56.35 ± 0.78	48.56 ± 0.83	52.88 ± 0.83	47.76 ± 3.86	69.12 ± 2.43
	NAPH12	50.06 ± 0.94	69.50 ± 0.55	46.44 ± 4.23	51.63 ± 1.44	48.45 ± 1.75	67.50 ± 0.56
Positive control	BHA	61.56 ± 1.33	70.15 ± 1.81	47.77 ± 0.83	54.33 ± 2.20	74.18 ± 0.01	77.22 ± 0.61
	BHT	65.78 ± 1.29	66.76 ± 2.20	50.00 ± 1.73	51.44 ± 3.53	70.77 ± 0.87	73.19 ± 0.15
	TBHQ	74.63 ± 0.72	83.82 ± 0.16	44.85 ± 1.44	54.33 ± 0.83	65.10 ± 8.93	71.68 ± 0.17
	Trolox	72.01 ± 0.48	75.99 ± 0.61	49.85 ± 1.44	53.52 ± 1.44	60.74 ± 3.09	69.71 ± 4.07
	VIT E	57.16 ± 0.92	68.70 ± 1.71	45.12 ± 2.73	47.67 ± 2.20	64.86 ± 3.37	71.54 ± 0.77

Abbreviations: BHA; Butylated hydroxyanisol, BHT; Butylated hydroxytoluene, TBHQ; t-butyl-hydroxyquinone, VIT E; Vitamin E

TABLE 5. The effects of the naphthalene derivatives of the LP level on liver, kidney and brain. Each value represents mean ± SD, (n = 3), where * corresponds to $p < 0.05$.

	Samples	LP*,**, liver		LP*,**, kidney		LP*,**, brain	
		10 µM	50 µM	10 µM	50 µM	10 µM	50 µM
Compound	NAPH1	0.81 ± 0.03	1.12 ± 0.01	2.49 ± 0.01	2.24 ± 0.05	3.19 ± 0.04	2.81 ± 0.13
	NAPH2	0.94 ± 0.03	1.20 ± 0.02	2.49 ± 0.07	1.91 ± 0.03	3.65 ± 0.14	1.49 ± 0.09
	NAPH3	1.45 ± 0.00	1.19 ± 0.03	3.64 ± 0.05	2.30 ± 0.04	3.27 ± 0.05	4.62 ± 0.37
	NAPH4	0.78 ± 0.03	0.87 ± 0.01	2.33 ± 0.01	2.99 ± 0.01	4.08 ± 0.04	2.91 ± 0.02
	NAPH5	0.80 ± 0.20	1.02 ± 0.03	2.18 ± 0.11	1.90 ± 0.05	3.24 ± 0.04	2.58 ± 0.02
	NAPH6	1.05 ± 0.06	0.89 ± 0.01	3.05 ± 0.02	3.90 ± 0.17	3.74 ± 0.08	4.23 ± 0.24
	NAPH7	1.62 ± 0.00	0.99 ± 0.01	2.56 ± 0.02	2.71 ± 0.07	3.80 ± 0.06	3.25 ± 0.13
	NAPH8	0.82 ± 0.02	0.94 ± 0.03	1.93 ± 0.01	5.31 ± 0.03	2.72 ± 0.19	2.88 ± 0.02
	NAPH9	0.85 ± 0.04	0.50 ± 0.01	2.58 ± 0.04	2.43 ± 0.02	4.38 ± 0.36	2.23 ± 0.04
	NAPH10	0.83 ± 0.06	0.71 ± 0.01	2.17 ± 0.01	2.78 ± 0.02	2.78 ± 0.04	2.66 ± 0.10
	NAPH11	0.91 ± 0.02	0.60 ± 0.01	2.77 ± 0.02	3.15 ± 0.07	3.00 ± 0.12	2.15 ± 0.01
	NAPH12	0.53 ± 0.01	0.85 ± 0.00	3.29 ± 0.10	2.56 ± 0.31	3.09 ± 0.04	2.39 ± 0.04
Positive control	Trolox	1.59 ± 0.03	1.01 ± 0.01	8.54 ± 0.04	1.21 ± 0.08	2.55 ± 0.05	2.86 ± 0.07
	TBHQ	0.84 ± 0.01	0.66 ± 0.01	2.28 ± 0.01	2.14 ± 0.04	1.58 ± 0.01	3.92 ± 0.04
	BHT	4.40 ± 0.12	0.62 ± 0.00	2.23 ± 0.02	3.82 ± 0.88	3.07 ± 0.08	2.36 ± 0.01
	BHA	1.14 ± 0.08	1.10 ± 0.02	2.02 ± 0.02	2.63 ± 0.06	4.71 ± 0.13	4.08 ± 0.04
	VIT E	2.81 ± 0.03	0.53 ± 0.02	2.33 ± 0.05	2.99 ± 0.06	3.55 ± 0.06	3.94 ± 0,09

**nmole MDA formed/mg protein
Abbreviations: BHA; Butylated hydroxyanisol, BHT; Butylated hydroxytoluene, TBHQ; t-butyl-hydroxyquinone, VIT E; Vitamin E

remarkable at 10 and 50 mM, ($p < 0.05$). Most of the naphthalene derivatives had the highest lipid peroxidation inhibition activity at 50 µM and the results were found in the liver (NAPH9 < VIT E < NAPH11 < BHT < TBHQ < NAPH10 < NAPH12 < NAPH4 < NAPH6 < NAPH8 < NAPH7 < trolox < NAPH5 < BHA < NAPH1 < NAPH3 < NAPH2), in kidney (trolox < NAPH5 < NAPH2 < TBHQ < NAPH3 < NAPH1 < NAPH9 < NAPH12 < BHA < NAPH7 < NAPH10 < NAPH4 < VIT E < NAPH11 < BHT < NAPH6 < NAPH8), in brain (NAPH2 < NAPH11 < NAPH9 < BHT < NAPH12 < NAPH5 < NAPH10 < NAPH1 < trolox < NAPH8 < NAPH4 < NAPH7 < TBHQ < VIT E < BHA < NAPH6 < NAPH3), in lung (NAPH4 < NAPH6 < NAPH10 < NAPH12 < NAPH3 < VIT E < NAPH11 < NAPH1 < NAPH8 < Trolox < TBHQ < NAPH7 < NAPH2 < BHT < NAPH5 < BHA < NAPH9), in heart (NAPH5 < NAPH7 < NAPH3 < NAPH10 < BHA < TBHQ < NAPH8 < trolox < NAPH6 < NAPH2 < NAPH4 < NAPH9 < NAPH11 < NAPH1 < NAPH12 < BHT < VIT E) and in spleen (NAPH4 < NAPH5 < NAPH12 < trolox < NAPH9 < NAPH6 < NAPH2 < NAPH10 < NAPH11 < TBHQ < BHT < NAPH7 < NAPH8 < BHA < NAPH3 < VIT E < NAPH1) at 50 mM. The antioxidant, reducing power, metal chelating, ·OH radical, H_2O_2, superoxide and free radical scavenging effects of napthalene derivatives on antioxidant activity and inhibitory effect on the lipid peroxidation (liver, kidney, brain, lung, heart, spleen) were not similar. Due to physical structure and mode of oxidation in assay systems, it is quite difficult to evaluate different effects of synthesized napthalene derivatives. Among the naphthalene, two substituted naphthyl analogues NAPH5 and NAPH9 exhibited excellent inhibition of lipid peroxidation followed by trifluoromethyl, methoxyl and hydroxyl analogues NAPH4 and followed by NAPH2 at 50 µM concentration. The main role of antioxidants in the lipid medium is to delay or inhibit lipid peroxidation and to scavenge the peroxide radical to the hydro-peroxide before the radical chain propagation (Ates-Alagoz et al. 2006).

TABLE 6. The effects of the naphthalene derivatives of the LP (lipid peroxidation) activity on lung, heart and spleen homogenates. Each value represents mean ± SD, ($n = 3$), where * corresponds to $p < 0.05$.

	Samples	LP*,**, lung		LP*,**, heart		LP*,**, spleen	
		10 µM	50 µM	10 µM	50 µM	10 µM	50 µM
Compound	NAPH1	1.61 ± 0.02	1.62 ± 0.03	0.97 ± 0.04	1.15 ± 0.05	2.88 ± 0.04	7.28 ± 0.21
	NAPH2	1.90 ± 0.01	2.03 ± 0.12	1.73 ± 0.02	0.92 ± 0.02	2.57 ± 0.03	1.44 ± 0.05
	NAPH3	2.17 ± 0.02	1.44 ± 0.02	1.27 ± 0.04	0.53 ± 0.01	1.60 ± 0.04	2.61 ± 0.08
	NAPH4	1.57 ± 0.05	1.27 ± 0.03	0.99 ± 0.02	0.93 ± 0.03	1.09 ± 0.01	1.02 ± 0.01
	NAPH5	1.51 ± 0.04	2.11 ± 0.02	1.22 ± 0.01	0.47 ± 0.02	1.10 ± 0.06	1.09 ± 0.01
	NAPH6	2.76 ± 0.07	1.34 ± 0.03	1.40 ± 0.02	0.92 ± 0.21	1.94 ± 0.01	1.43 ± 0.04
	NAPH7	1.47 ± 0.08	1.85 ± 0.18	1.14 ± 0.05	0.51 ± 0.01	1.88 ± 0.01	2.00 ± 0.01
	NAPH8	1.41 ± 0.04	1.64 ± 0.02	1.32 ± 0.06	0.73 ± 0.03	3.44 ± 0.09	2.15 ± 0.01
	NAPH9	1.49 ± 0.01	3.33 ± 0.07	1.55 ± 0.04	0.96 ± 0.01	1.57 ± 0.01	1.33 ± 0.06
	NAPH10	1.65 ± 0.04	1.35 ± 0.01	1.21 ± 0.07	0.63 ± 0.02	2.01 ± 0.27	1.50 ± 0.01
	NAPH11	1.65 ± 0.04	1.48 ± 0.06	0.99 ± 0.01	1.07 ± 0.02	1.87 ± 0.04	1.52 ± 0.01
	NAPH12	1.48 ± 0.05	1.39 ± 0.04	1.16 ± 0.04	1.24 ± 0.00	1.64 ± 0.02	1.19 ± 0.04
Positive control	Trolox	1.32 ± 0.01	1.75 ± 0.09	1.27 ± 0.04	0.79 ± 0.02	1.13 ± 0.03	1.29 ± 0.03
	TBHQ	1.36 ± 0.03	1.83 ± 0.08	1.18 ± 0.04	0.67 ± 0.02	1.02 ± 0.08	1.59 ± 0.01
	BHT	1.29 ± 0.06	2.11 ± 0.06	1.19 ± 0.04	1.32 ± 0.03	2.29 ± 0.03	1.98 ± 0.01
	BHA	1.21 ± 0.04	2.28 ± 0.02	1.10 ± 0.09	0.66 ± 0.10	2.72 ± 0.01	2.54 ± 0.02
	VIT E	1.58 ± 0.16	1.44 ± 0.05	0.75 ± 0.01	1.35 ± 1.22	3.43 ± 0.01	2.87 ± 0.02

**nmole MDA formed/mg protein

Abbreviations: BHA; Butylated hydroxyanisol, BHT; Butylated hydroxytoluene, TBHQ; t-butyl-hydroxyquinone, VIT E; Vitamin E

CONCLUSION

The results of this investigation indicated that the naphthalene derivatives had high antioxidant activities against various *in vitro* antioxidant systems assays and their activities were concentration-dependent (Sashidhara et al. 2010; Süzen 2007). We have confirmed that naphthalene derivatives containing different substituents (phenyl, naphthyl, ethoxynaphthyl, etoxyl and hydroxyl) were found to have better antioxidant and inhibition of lipid peroxidation comparing to standards (VIT E, BHA, TBHQ, BHT and trolox) at similar dose. NAPH3, 4, 5 and 6 compounds made a reduction of improvement from Mo(VI) to Mo(V) in total antioxidant activity due to interacting with naphthalene-2-ol and phenyl in the naphthalene ring systems. These synthesized NAPH9

(liver), NAPH5 (kidney and heart), NAPH2 (brain) and NAPH4 (lung and spleen) were found to be most potent inhibitors of lipid peroxidation at 50 µM. The results of reducing power, metal chelating, free radical, superoxide and hydrogen peroxide scavenging activity at 10 and 50 µM showed that all tested napthalene derivatives were significantly reactive. Therefore, they can be considered to be effectively useful as potential materials modulating oxidation for pharmaceutical and render promising antioxidant compounds.

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