Antioxidant and structure–activity relationships (SARs) of some phenolic and anilines compounds


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Abstract The scavenging behavior of a series of phenolic and anilines compounds toward H$_2$O$_2$ and DPPH was examined. The efficient concentration (EC$_{50}$) was calculated for all compounds under investigation by using H$_2$O$_2$-scavenging activity assay. The antiradical efficiency (AE) and EC$_{50}$ were calculated for all investigated compounds by applying DPPH scavenging activity assay. Wide differences among compounds in each series and between the two series were observed. In H$_2$O$_2$-scavenging activity assay, the anilines series were more active than the phenolic series due to the reduction properties of the anilines compounds. While in the DPPH scavenging activity, the phenolic compounds were more active than the anilines compounds due to the lower bond dissociation energies (BDE) of O–H than that of N–H. So, the phenolic compounds were comparatively easier to lose H atom than anilines. The antioxidant activity related to the compound structure was found to be dependable on the number of the included active group (OH or NH$_2$). The more active compound is the more included active groups. The position of the active groups also plays an important role of structure–antioxidant relationship activity. The ortho position was found to be the more active one, due to its ability to form intramolecular hydrogen bonding (iHB), followed by para position and then meta position of compounds.

Introduction

Antioxidants have become a topic of increasing interest recently. A literature search revealed that the number of publications on antioxidants and oxidative stress has nearly quadrupled in the past decade (1684 in 1993; 6510 in 2003). Antioxidants are compounds that, in low concentration, can prevent biomolecules (proteins, nucleic acids, polyunsaturated lipids, and sugars) from undergoing oxidative damage through free radical mediated reactions. They can inhibit oxidizing chain reactions in several ways, including direct quenching of reactive oxygen species, inhibition of enzymes, and chelating of metal ions (Fe$^{2+}$, Cu$^+$). Their beneficial effects are related to diseases in which oxidative processes are remarkable, i.e., atherosclerosis, coronary heart disease, certain tumors, and
aging itself (Luximon-Rama et al., 2003; Toyokuni et al., 2003; Caia et al., 2004; Romani et al., 2004).

Dietary antioxidants, including polyphenolic compounds, vitamins E and C, and carotenoids, are believed to be the effective nutrients in the prevention of these oxidative stress related diseases (Ames et al., 1995; Kaur and Kapoor, 2001). There is increasingly growing market for nutraceuticals and functional food. Products containing nutraceuticals have reached a worldwide estimated value of $65 billion (Lachance, 2002).

Clinical trials and epidemiological studies have established an inverse correlation between the intake of fruits, cereals, and vegetables (dietary antioxidants) and the occurrence of diseases such as inflammation, cardiovascular disease, cancer, Alzheimer’s, and aging-related disorders (Halliwell, 1992; Willet, 2001).

In fact, a diet rich in fruits, vegetables, cereals, and olive oil can prevent cardiovascular diseases and certain forms of cancer. The major antioxidant components of these common foods are the phenolic compounds. Their antioxidant activity seems to be related to their molecular structure, more precisely to the presence and number of hydroxyl groups, and to double bond conjugation and resonance effects (Rice-Evans et al., 1996).

Recently, a quantum–mechanical investigation has shown that the antioxidant action of flavonols is related to radicals showing a planar conformation that allows extended electronic delocalization between adjacent rings (Russo et al., 2000).

Recently, the antioxidant properties of some diary amines in the benzo [b] thiophene series were reported by evaluation of their free radical scavenging activity and reducing power, and it was possible to establish some structure–activity relationships based on the position of arylamination (either on the benzene or on the thiophene ring) and in the presence of different substituents on both rings (Ferreira et al., 2006). It was possible to establish some structure–activity relationships (SARs) based on the presence and position of different substituents in the phenyl ring (1 or 2 OMe and C–N), in the presence of a pyridine ring and on the position of its nitrogen atom relative to the N–H bond (Queiroz et al., 2007).

Two main mechanisms by which antioxidants can play their protective role have been proposed. In the first mechanism, the free radical removes a hydrogen atom from the antioxidant (ArOH) that itself becomes a radical:

\[ R^+ + \text{ArOH} \rightarrow RH + \text{ArO}^- \]

In this mechanism, the bond dissociation energy (BDE) of the O–H bonds is an important parameter in evaluating the antioxidant action, because the weaker the OH bond the easier will be the reaction of free radical inactivation.

In the second mechanism (the one-electron transfer), the antioxidant can give an electron to the free radical becoming itself a radical cation:

\[ R^+ + \text{ArOH} \rightarrow R^- + \text{ArOH}^{2+} \]

According to the second mechanism, the lower of the ionization potential (IP), the easier is the electron abstraction. So, the calculation of BDEs and IPs includes interesting information about the efficiency and the activity of the phenolic antioxidants (Wright et al., 2001).

This study aims to evaluate the antioxidant activity of some phenolic and anilines compounds by two different methods; \( H_2O_2 \)-scavenging activity and DPPH scavenging activity assays. The efficient concentration (EC_{50}) and antiradical efficiency (AE) were calculated for the tested compounds. The comparison between EC_{50} values and AE values were established to evaluate the most active group of compounds and the most active compound among each group. Based on the above-mentioned, the relationships between the chemical structure and the antioxidant activity of the selected compounds were also elucidated.

Materials and methods

Materials

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Aldrich company. Ethanol 95%, Methanol, hydrogen peroxide 30%, and phenol were purchased from El-Nasr pharmaceuticals company. 4-Aminophenol, 3-aminophenol, 2-aminophenol, catechol, resorcinol, hydroxyquinone, aniline, o-phenylenediamine, and p-phenylenediamine were laboratory pure reagents.

Preparation of antioxidants stock solutions

A solution (100 mM) of each tested compounds (phenol, 4-aminophenol, 3-aminophenol, 2-aminophenol, catechol, resorcinol, hydroquinone, aniline, o-phenylenediamine) was prepared in ethanol 95%. p-Phenylenediamine was dissolved in ethanol 95% to give 50 mM solution. Total volume of the solutions was 25 ml.

Methods of chemical analysis

Determination of hydrogen peroxide scavenging

The ability of tested compounds to scavenge hydrogen peroxide was determined according to the method of (Ruch et al., 1989). The reaction mixture had contained 1 ml of hydrogen peroxide solution (35.4 mM) and different concentrations of tested compounds (from 0.0425 mM to 4 mM). Total volume of the reaction mixture was 3 ml. Absorption of hydrogen peroxide at 230 nm was determined within 3 min against a blank solution that contained tested compound in ethanol without hydrogen peroxide.

\[
\% \text{ scavenging of hydrogen peroxide effect} = \left( \frac{As}{Ac} - 1 \right) \times 100
\]

where As = absorbance of sample. Ac = absorbance of control (hydrogen peroxide solution in ethanol without sample).

Determination of DPPH scavenging

The ability of tested phenolic compounds and aniline compounds to scavenge DPPH radical was determined according to the method of Hatano et al. (1988). The reaction mixture had contained 0.1 ml of DPPH radical solution (5 mM) and different concentration of tested compounds (from 0.0026 mM to 83 mM). Total volume of the reaction mixture was 3 ml. Absorption of DPPH radical at 515 nm was determined after 10 min against a blank solution that contained only methanol.

\[
\% \text{ DPPH radical scavenging activity} = \left( \frac{(Ac - As)}{Ac} \right) \times 100
\]

where As = absorbance of sample. Ac = absorbance of control (DPPH radical solution in methanol without sample).
According to Ordoudi et al. (2006), Kinetic parameter EC50 (efficient concentration of the antioxidant necessary to decrease the initial DPPH radical concentration by 50%) was calculated. Also, \( T_{EC50} \) (reaction time needed to reach the steady state at EC50) was determined. Finally, the calculated EC50 and \( T_{EC50} \) values were used to calculate the antiradical index AE (antiradical efficiency) as follows:

\[
AE = \frac{1}{(EC_{50} \times T_{EC50})}
\]

**Results and discussion**

**Results**

**Hydrogen peroxide scavenging activity**

*Phenolic compounds.* As indicated in Fig. 1, phenolic compounds under investigation showed a strong concentration-dependent scavenging of H2O2. The obtained results were confirmed by the EC50 value “efficient concentration” value (also called the half maximal inhibitory concentration) IC50 value) which is defined as the concentration of substrate that causes 50% loss of the DPPH activity (Molyneux, 2004).

The EC50 of the tested phenolic compounds increased in the following order: 4-aminophenol < 2-aminophenol < catechol < 3-aminophenol < hydroquinone < resoricinol and phenol. Phenol was at the top of all tested concentrations.

*Aniline compounds.* The ability of anilines compounds to act as antioxidant using hydrogen peroxide was determined at different concentrations, and the EC50 was calculated for all compounds. As shown in Fig. 2, anilines compounds showed different hydrogen peroxide scavenging activity and it was increased by increasing their concentrations.

The tested anilines compounds could be arranged according to their EC50 value as the following order: aniline < 4-aminophenol < 2-aminophenol < catechol < 3-aminophenol < hydroquinone < resoricinol and phenol. Phenol was at the top of all tested concentrations.

**DPPH scavenging activity**

*Phenolic compounds* DPPH radical has been widely used to evaluate the antioxidant properties of natural products (Toyokuni et al., 2003; Romani et al., 2004). DPPH was used as the free radical source, since it simulates reactive oxygen and nitrogen species affecting biological systems (Arnao, 2000). In addition, free radical scavenging is generally the accepted mechanism for antioxidants inhibiting lipid oxidation (Brand-Williams et al., 1995).

Antiradical efficiency (AE) is an expression of results that takes into accounts both stoichiometry (in terms of EC50) and time to reach steady state (\( T_{EC50} \)). This parameter provides an indirect means to consider that low \( T_{EC50} \) and low amounts of a potent antioxidant are needed to prevent auto oxidation of free radical mediated oxidation of a lipid substrate (Sanchez-Moreno et al., 1998). The AE value is the result of combination of kinetic and static approaches to characterize the antioxidant efficiency of a molecule (Huang et al., 2005; Roginsky and Lissi, 2005). Instead of stoichiometric factors (EC50) which are most often used to describe differences in the scavenging potential of mono- and poly-phenol acid derivatives, though other stoichiometry aspects may be used, e.g., rate of reaction (Frankel and Meyer, 2000).

The antioxidant activity of phenolic compounds was tested by measuring their capacity to scavenge DPPH radical. Efficient concentration (EC50), \( T_{EC50} \) (reaction time needed to reach the steady state at EC50), and antiradical efficiency (AE) were determined. The obtained data showed a wide variation between the tested phenolic compounds in their antioxidant activity. All tested phenolic compounds have the ability to quench DPPH radical in a concentration-dependent manner.

The AE value of the tested phenolic compounds was increased in the following order: catechol > 2-aminophenol > 4-aminophenol > hydroquinone > 3-aminophenol > resoricinol and phenol at the top of all tested concentrations. The AE value of phenol and resoricinol was near to zero so did not appear in Fig. 3.

*Anilines compounds* The antioxidant activity of selected anilines compounds was determined by DPPH assay. Different parameters were calculated (EC50, \( T_{EC50} \), and AE). The tested anilines compounds had different antioxidant activity as shown in Fig. 4. The antioxidant activity of the tested anilines was increased by increasing their concentration.

The AE value of the tested anilines compounds was decreased in the following order: 2-aminophenol > o-phenylenediamine > 4-aminophenol > p-phenylenediamine > 3-aminophenol. Aniline was at the bottom of all tested concentrations, as the AE value of aniline was near to zero so did not appear in Fig. 4.
Discussion

$H_2O_2$-scavenging activity

Mechanism of $H_2O_2$-scavenging activity could be explained according to Wettasinghe and Shahidi (1999). The decomposition of hydrogen peroxide into water may occur according to the following reaction:

\[ H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O \]

Phenolic compounds

The phenolic derivatives, diphenol and aminophenol, recorded higher antioxidant activity than their parent compound. The obtained results indicated that the aminophenol in 2 and 4 positions showed the highest $H_2O_2$-scavenging activity. Phenols had a strong antioxidant activity, where amino-substituted phenol was in general more potent than hydroxyl-substituted ones (Iwatsuki et al., 1995). Our obtained results concerning the $H_2O_2$-scavenging activity of phenolic compounds could be supported by the first antioxidant mechanism proposed by Wright et al. (2001).

The differences in $H_2O_2$-scavenging activity of phenolic compounds are probably affected by the nucleophilicities of the corresponding phenoxy radicals which are formed in a slightly alkaline medium. Phenoxy radicals generated may be stabilized through resonance and/or intramolecular hydrogen bonding as in the ortho position substituent (catechol and 2-aminophenol).
The number of hydroxyl groups and the aromatic ring substitution pattern are all important associated factors. In other words, the \( \text{H}_2\text{O}_2 \)-scavenging activity is largely dependent on the hydrogen-donating ability of phenolic compounds and the stability of phenoxyl radicals formed after the dehydrogenation. When the hydrogen atoms of the aromatic ring are substituted by electron-donating groups (hydroxyl group), the nucleophilicity of the phenoxyl radical would be increased, enhancing its \( \text{H}_2\text{O}_2 \)-scavenging activity. The ortho and para position substitution with another hydroxyl group is also another factor increasing the stability of the phenoxyl radical (Ma et al., 2011).

Scavenging of hydrogen peroxide by PPE (polyphenolic extract) may be attributed to their phenolic nature, which can donate electrons to \( \text{H}_2\text{O}_2 \), thus neutralizing it to water (Saurav and Kannabiran, 2012).

Since phenolic compounds are good electron donators, they may accelerate the conversion of \( \text{H}_2\text{O}_2 \) to \( \text{H}_2\text{O} \). Phenolic compounds are known as powerful chain breaking antioxidants. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups and may contribute directly to antioxidative action (Wagh et al., 2012).

**Anilines compounds**

In contrast, our results revealed that the aromatic amines (anilines) had a strong antioxidant activity, where amino-substituted phenol was in general more active than amino-substituted ones (Iwatsuki et al., 1995). By comparing between the tested anilines compounds, results showed aniline (the parent compound) is the most active one as antioxidant. This result could be explained according to the second mechanism explained by Wright et al. (2001), where aniline had a lower \( \text{IP} \) value, so it has the easiest electron abstraction property. The substitution with electron donor groups (\( \text{NH}_2 \) or \( \text{OH} \)) gave a negative effect on \( \text{IP} \) value, so the \( \text{H}_2\text{O}_2 \)-scavenging activity decreases.

**Comparison between phenolics and anilines antioxidant activity**

Anilines were more active in \( \text{H}_2\text{O}_2 \)-scavenging than phenols. The most active compound is aniline due to its lower \( \text{IP} \) value (Wright et al., 2001). The second mechanism according to Wettasinghe and Shahidi (1999) equation and our result, was more favorable mechanism to scavenging \( \text{H}_2\text{O}_2 \). So, the compound was easier to lose electron which resulted in more \( \text{H}_2\text{O}_2 \)-scavenging activity. On the other hand, the compound which is easier to lose hydrogen atom (based on the first mechanism) will lead to less \( \text{H}_2\text{O}_2 \)-scavenging activity.

**DPPH scavenging activity**

The DPPH assay was believed to involve hydrogen atom transfer reaction as in the following equation (Mohammadpour et al., 2012).

**Phenolic compounds**

Based on the aforesaid, the most active compound as antioxidant is the one possesses more than one active group (e.g., \( \text{NH}_2 \) or \( \text{OH} \)) in ortho position. So, the most active antioxidant compound is catechol, which possesses two hydroxyl groups in ortho position (Valgimigli et al., 2008). The first chain carrying peroxyl radical was being trapped by H-atom transfer from the labile phenolic O–H and the second by reaction with the resultant phenoxyl radical. Catechols are able to trap two peroxyl radicals under most conditions as follows:

**The reaction mechanism of catechol as antioxidant**

explained by Wright et al. (2001), where aniline had a lower \( \text{IP} \) value, so it has the easiest electron abstraction property. The substitution with electron donor groups (\( \text{NH}_2 \) or \( \text{OH} \)) gave a negative effect on \( \text{IP} \) value, so the \( \text{H}_2\text{O}_2 \)-scavenging activity decreases.

So, the reaction mechanism of 2-aminophenol could be explained as follows:
The high antioxidant activity of these compounds is due to the presence of di-active groups (OH or NH₂) in the 1,2 position in their molecular structure. This structure feature has the ability to form an intramolecular hydrogen bonding (iHB). The H atom which is not involved in this bond will then be abstracted by free radicals, resulting in a stable phenoxy radical (Ordoudi et al., 2006).

Then, 4-aminophenol, hydroquinone, and p-phenylenediamine were in the second order as antioxidant. These compounds have the same substituent position. The reaction mechanism of hydroquinone could be explained according to Valgimigli et al. (2008) as follows:

The lower stoichiometric factors observed for hydroquinones and the dependence of these values on experimental conditions have been explained through a chain transfer reaction, wherein a peroxyl radical is quenched by the hydroquinone, but the resultant semiquinone radical reacts with O₂ to generate hydroperoxyl, which can carry on an oxidation chain. As a result, no net oxidation chains are broken and the stoichiometric factor is zero. The actual stoichiometric factor would then depend on the competition between the reaction of the semiquinone radical with O₂ and its reaction with a peroxyl radical, the kinetics of which may depend on experimental conditions, such as the solvent and the rate of initiation (steady state concentration of peroxyl radicals).

On the other hand, the amino group in the para position in 4-aminophenol had played an important role in increasing the antioxidant activity than hydroquinone. Since, the amino group was an electron donor group. Thus, the peroxyl radical was more stable.

So, the reaction mechanism of 4-aminophenol could be explained as follows:
In the third order, 3-aminophenol has a mediate antioxidant activity. 3-aminophenol has an electron donor group (NH₂) but in meta position. This position decreases the effect of the amino group on the stability of the resultant phenoxyl radical, so the antioxidant activity of 3-aminophenol was decreased.

In the fourth order, resorcinol has low antioxidant activity. The presence of the OH group in meta position has a slight positive effect on the antioxidant activity in comparison with phenol which has no antioxidant activity.

**Aniline compounds**

From the above-mentioned results, anilines compounds had antioxidant activity but there were wide differences among them. The most active anilines as antioxidant were 2-aminophenol then o-phenylenediamine in the first order. As previously mentioned, the formation of intramolecular hydrogen bonding (iHB) had played an important role to increase the antioxidant activity of the 1,2 di-active groups (OH or NH₂) isomer. We had explained the mechanism of 2-aminophenol according to Valgimigli et al. (2008), also the mechanism of o-phenylenediamine could be explained as follows:

Then, 4-aminophenol and p-phenylenediamine were in the second order as antioxidant. These compounds have the same substitution position. As, the mechanism of 4-aminophenol was discussed before, the reaction mechanism of p-phenylenediamine could be explained as follows:

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**The reaction mechanism of 4-aminophenol as antioxidant**

\[
\text{4-aminophenol} + \text{ROO}^- \rightarrow \text{amine radical} + \text{ROOH}
\]

**The reaction mechanism of o-phenylenediamine as antioxidant**

\[
\text{o-phenylenediamine} + \text{ROO}^- \rightarrow \text{amine radical} + \text{ROOH}
\]

**The reaction mechanism of p-phenylenediamine as antioxidant**

\[
\text{p-phenylenediamine} + \text{ROO}^- \rightarrow \text{amine radical} + \text{ROOH}
\]
As the previous, 3-aminophenol was in the third order then aniline came in the fourth order.

Comparison between phenolics and anilines

Phenolic compounds were more active as antioxidant than anilines compounds in DPPH scavenging assay. The most active compound was catechol. The highest antioxidant activity of phenolics is due to the lower bond dissociation energies (BDE) of OH than that of NH₂. According to Wright et al. (2001), Mohammadpour et al. (2012) equation and our results first mechanism was more favorable mechanism to scavenging DPPH. In this mechanism, the bond dissociation energies (BDE) of the O–H bonds is an important parameter in evaluating the antioxidant action, because the weaker the OH bond the easier will be the reaction of free radical inactivation. 

According to Wright et al. (2000) showed that the bond dissociation energies of OH in catechol was 68.8–72.6 kcal/mol while it was 70.4–70.7 kcal/mol in 2-aminophenol. Catechol comparatively has the lowest bond dissociation energies of OH, so it’s easier to lose H atom than 2-aminophenol, which is followed by o-phenylenediamine. The antioxidant activity followed an inverse dependence on the magnitude of the phenolic bond dissociation energies of OH. The key mechanism of the chain-breaking action was attributed to hydrogen atom transfer (HAT) from the phenolic OH to peroxyl radicals (Amorati et al., 2006).

Conclusion

Phenolic compounds are known as powerful chain breaking antioxidants. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups and may contribute directly to antioxidative action. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans (Sawant et al., 2009). In this study, the antioxidant activity of two different compound groups was investigated by H₂O₂-scavenging activity assay and DPPH scavenging activity assay. Anilines comparatively have higher H₂O₂-scavenging activity of all tested compounds due to the presence of the amino group. So, the lower IP of the amino group had played a role in increasing the H₂O₂-scavenging activity of anilines. In general, the H₂O₂-scavenging activity is dependent on electron transfer (ET) and supported by the second mechanism of antioxidant, which was suggested by Wright et al. (2001). However, the H₂O₂-scavenging activity is largely dependent on the chemical structure of compound, where the H₂O₂-scavenging activity was affected with the kind, number and position of active group (OH or NH₂) and the kind, number and position of the substituted group. In general, the presence of amino group and electron donor group at ortho or para position has a negative effect on the H₂O₂-scavenging activity.

In DPPH scavenging activity assay, there are wide differences among tested compounds. In contrast, the phenolic compounds were more active than anilines due to lower value of bond dissociation energies (BDE) of OH where the DPPH scavenging activity was dependent on hydrogen atom transfer (HAT) and supported by the first mechanism of antioxidant according to Wright et al. (2001). Also, the DPPH scavenging activity was related to the number of the active group, as the compounds have more than one active group, they become more active. In addition, the intramolecular hydrogen bond (iHB) has an active role for stability of phenoxyl radicals formed after dehydrogenation. This is so clear in the results of catechol and 2-aminophenol. So, the ortho position substitution is more active as antioxidant than the para position.

Notice that the chemical structure of the aminophenol compounds was involved the two active different groups (OH and NH₂); aminophenols have antioxidant activity in both assays. But the question here is “Which of the two active groups is responsible for their activity in each assay?” The answer will be very clear when we combine the antioxidant mechanism of each assay and our results, whereas the H₂O₂-scavenging activity depends on the second mechanism and the compound ability to lose electron (ET), so the amino group in this assay is the responsible group for aminophenol antioxidant activity. In contrast, in the DPPH scavenging activity depends on the first mechanism and the compound ability to hydrogen atom transfer (HAT), so the hydroxyl group in this assay is the responsible group for aminophenol antioxidant activity.

References


