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RESEARCH ARTICLE

Antioxidant activity of taxifolin: an activity–structure relationship

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Abstract

Taxifolin is a kind of flavanonol, whose biological ability. The objectives of this study were to investigate the antioxidants and antiradical activities of taxifolin by using different *in vitro* bioanalytical antioxidant methods including DMPD$^{\bullet+}$, ABTS$^{\bullet+}$, $O_2^{\bullet-}$, and DPPH$^{\bullet}$-scavenging effects, the total antioxidant influence, reducing capabilities, and Fe^{2+}-chelating activities. Taxifolin demonstrated 81.02% inhibition of linoleic acid emulsion peroxidation at 30 μg/mL concentration. At the same concentration, standard antioxidants including trolox, α-tocopherol, BHT, and BHA exhibited inhibitions of linoleic acid emulsion as 88.57, 73.88, 94.29, and 90.12%, respectively. Also, taxifolin exhibited effective DMPD$^{\bullet+}$, ABTS$^{\bullet+}$, $O_2^{\bullet-}$, and DPPH$^{\bullet}$-scavenging effects, reducing capabilities, and Fe^{2+}-chelating effects. The results obtained from this study clearly showed that taxifolin had marked antioxidant, reducing ability, radical scavenging and metal-chelating activities. Also, this study exhibits a scientific shore for the significant antioxidant activity of taxifolin and its structure-activity insight.

Keywords

Antioxidant activity, radical scavenging, structure–activity insight, taxifolin

History

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Introduction

Oxidation process is the electrons transfer between two atoms and stands for a required part of aerobic life and our metabolism. Although problems may occur when the electron flow becomes disconnect, generating free radicals and reactive oxygen species (ROS). A free radical can be described as a molecule or molecular fragment including one or more unpaired electrons[1]. The existence of unpaired electrons usually confers a considerable degree of reactivity upon a free radical. Those radicals derived from molecular oxygen represent the most important class of such species generated in living organisms[2]. Recently, there has been an intensive interest in the role of ROS in clinical and experimental medicine. ROS includes non-free radical species like ozone (O_3), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and free radical varieties like hydroxyl radicals (OH$^{\bullet}$), superoxide anion radicals ($O_2^{\bullet-}$), peroxyl radicals (ROO$^{\bullet}$), hydroperoxyl radicals (HOO$^{\bullet}$)[3,4]. Free radicals may be occurring by living cells during pathophysiological and biochemical processes as well as due to environmental pollutants, radiation, chemicals, and toxins[5]. ROS can occur in different ways. ROS (i) are generated during irradiation by X-rays, UV light, and γ-rays; (ii) are present as pollutants in the atmosphere; (iii) are products of metal-catalyzed reactions; (iv) are produced by macrophages and neutrophils during inflammation process; and (v) are by-products of mitochondria-catalyzed electron transport reactions and other biochemical mechanisms[1].

A normal cell has convenient prooxidant–antioxidant equilibrium. Although this equilibrium can be changed towards the prooxidants when production of ROS is gone up enormously or when ranges of antioxidants are decreased. This stage is entitled as oxidative stress[1,6]. As our understanding of the free radicals role in human diseases has increased, antioxidants have attracted broader interest because of their role in inhibiting ROS-relayed reactions and their help in protecting the human body against damage by ROS[7]. Antioxidant is a compound that prevents or retards the oxidation of substrates even if this compound is available in substantially lower concentration than the oxidized substrates. Also, they have features that safeguard the person from free radicals and effects of ROS[8,9]. In recent years, interest has remarkably increased in identify alternate safe and innate source materials of food antioxidants, and the search for natural antioxidants, particularly of plant basis[3,10]. Also, restricting the use of synthetic antioxidants has leaded to an incremental interest of natural antioxidant sources. Therefore, there is an increasing way in consumer priorities based on native antioxidants, all them have given driving force to the initiatives to detect native source material of antioxidants[4,11–13].

Vegetables are rich in term of secondary plants metabolites including flavonoids and other pigments. It was reported that growing consumption of vegetable has been widely growing because of theirs. Most of phytochemicals in vegetables such as phenolic acids β-carotene, tocopherols, flavonol, ascorbate, and polyphenols are identified for their biological activity including antioxidant properties[1].

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According to several clinical, epidemiological, and *in vitro* studies, phenolic compounds reduce the risk of various degenerative diseases including neurodegenerative diseases, cancer, osteoporosis, diabetes, and cardiovascular diseases due to their antioxidant properties[14,15]. Also, phenolic compound had a wide spectrum of biological activities[16–29].

Flavonoids are a main class of phenolic compounds and secondary plant metabolites generally located in leaves as water-soluble glycosides in vacuoles of plant cell[14]. They are widely distributed in plants, fulfilling many functions. Flavonoids are not only present in plants as constitutive agents but are also gathered in plant tissues in response to microbial attack. They are the most important plant pigments for different functions including flower coloration, producing yellow or pigmentation in petals designed to attract pollinator animals[30].

Taxifolin (3,5,7,3,4-pentahydroxy flavanone or dihydroquercetin), a flavononol subclass of flavonoids, are abundant in citrus fruits and onion. Taxifolin is interesting potential component of dietary supplements or antioxidant-rich functional food[31].

In this study, we spectrophotometrically determined the antioxidant and antiradical activities of taxifolin using by different bioanalytical methods. Also, another goal of the current study was to explain the structure and antioxidant or antiradical activities of taxifolin.

Materials and methods

Chemicals

Taxifolin, methionine, riboflavin, linoleic acid, butylated hydroxyanisole, butylated hydroxytoluene, *N,N*-dimethyl-*p*-phenylenediamine, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), 2,9-dimethyl-1,10-phenanthroline, nitroblue tetrazolium, 1,1-diphenyl-2-picryl-hydrazyl, 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine, trichloroacetic acid, and α-tocopherol were purchased commercially from Sigma-Aldrich GmbH, Sternheim, Germany. Ammonium thiocyanate was obtained from Merck. All other chemicals used were of analytical grade and attained from either Sigma-Aldrich (Sternheim, Germany) or Merck (Darmstat, Germany).

Total antioxidant activity of taxifolin

For the determination of total antioxidant activity of taxifolin and positive controls, the ferric thiocyanate method (FTM) was used[32,33]. This method, in other words inhibition of linoleic acid emulsion (LAE), was described previously[34–36]. The preparation of stock solution and LAE was defined in our previous studies[1,37]. The peroxides consisted through peroxidation of linoleic acid will oxidize Fe^{2+} to Fe^{3+}, which generates a complex with thiocyanate that has a maximum absorbance at 500 nm[38]. The assay steps were renewed every 5 h up to arriving a high scale. In linoleic acid emulsion, the percent inhibition of lipid peroxidation (ILP) was computed by the following equation:

$$\mathrm{ILP}\,(\%) = [100 - (A_s/A_c) \times 100]$$

where A_C is the absorbance value of the control reaction, which includes only phosphate buffer and LAE. In the existing solution, the absorbance value of taxifolin and other tested samples is called A_S[39,40].

Fe^{3+}-reducing antioxidant power assay

For the determination of Fe^{3+}-reducing ability of taxifolin, $Fe^{3+}(CN^-)_6$–$Fe^{2+}(CN^-)_6$ reduction method was used[41,42]. In brief, different concentrations of taxifolin (10–30 µg/mL) in 0.75 mL of deionized H_2O were added with 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%). Then, the solution was incubated at 50 °C during 20 min. After incubation period, 1.25 mL of trichloroacetic acid (TCA) was added (10%). Finally, 0.5 mL of $FeCl_3$ (0.1%) was transferred to this mixture and the absorbance value was enrolled at 700 nm in a spectrophotometer[3,10].

Cupric ions (Cu^{2+})-reducing power-CUPRAC assay

Cupric ions (Cu^{2+})-reducing power was used as a second reducing method for taxifolin. Cu^{2+}-reducing capability was done according to the method described by Apak et al.[43] with slight modification[44]. Thus, 0.25 mL of $CuCl_2$ solution (0.01 M), 0.25 mL of ethanolic neocuproine solution (7.5×10^{-3} M) and 0.25 mL of NH_4Ac buffer solution (1 M) were transferred to a test tube, which contains a taxifolin at different concentrations (10–30 µg/mL). The total volume was completed with distilled H_2O to 2 mL and shaken vigorously. The absorbance of samples was recorded at 450 nm after 30 min.

Ferric ions (Fe^{2+})-chelating activity

Ferric ions (Fe^{2+})-binding ability of taxifolin was determined according to the method of Dinis et al.[45] with minor modification[46,47]. Fe^{2+} capacity of taxifolin was spectrophotometrically recorded at 562 nm. In brief, to 0.1 mL of $FeCl_2$ (0.6 mM) were added taxifolin at different concentrations (10–20 µg/mL) in methanol (0.4 mL). Then, the reaction was started by the 0.1 mL of ferrozine solution addition (5 mM). The solution was mixed and incubated at room temperature for 10 min. Finally, the absorbance value of the mixture was quantified spectrophotometrically at 562 nm versus blank sample[48].

Superoxide anion radical-scavenging activity

Superoxide radicals ($O_2^{\bullet-}$)-scavenging activity of taxifolin was performed in accordance with the methodology of Zhishen et al.[49] with slightly adjustment[50]. $O_2^{\bullet-}$ is produced in methionine, riboflavin, enlightens system, and determined by the come down to NBT to generate blue formazan (NBT^{2+}). All mixtures were prepared in phosphate buffer (0.05 M, pH 7.8). The photo-induced reactions were realized by using the fluorescent lamps (20 W). The mixture was enlightened at 25 °C for 40 min. The photochemically reduced riboflavin produced $O_2^{\bullet-}$, which reduced NBT to NBT^{2+}. The absorbance was spectrophotometrically measured at 560 nm[51,52].

DPPH•-scavenging activity

DPPH• radical-scavenging activity of taxifolin was performed according to the previous studies[53,54]. In brief, fresh solution of DPPH· (0.1 mM) was prepared in ethanol. To a solution 1.5 mL of taxifolin in ethanol was added to 0.5 mL of this solution (10–30 µg/mL). These mixtures were mixed vigorously and incubated in dark for 30 min. Finally, the absorbance value was recorded at 517 nm in a spectrophotometer[55,56].

$ABTS^{\bullet+}$-scavenging activity

$ABTS^{\bullet+}$-scavenging activity of taxifolin was done using the spectroscopic method of Re et al.[57]. The ABTS radical cation ($ABTS^{\bullet+}$) was acquired by reacting 7 mM solution of ABTS with 2.45 mM $K_2S_2O_8$. Prior to assay, the ABTS radical cation solution was diluted with ethanol to an absorbance of 0.750 ± 0.05 at 734 nm. Then, 1 mL of $ABTS^{\bullet+}$ solution was supplement to 3 mL of taxifolin and control solutions. The extent of decolorization is calculated as percentage reduction of absorbance[58].

DMPD$^{\bullet+}$-scavenging activity

DMPD radical-scavenging ability of taxifolin was done according to the method described by Fogliano et al.[59]. DMPD (0.1 M) was prepared by dissolving 0.2 g of DMPD in 10 mL of deionized H_2O. Then 1 mL of this solution was supplement to acetate buffer (100 mL, 0.1 M, and pH 5.3). DMPD$^{\bullet+}$ was obtained by adding 0.2 mL of $FeCl_3$ solution (0.05 M). Different concentrations of taxifolin and positive controls (10–30 µg/mL) were transferred in test tubes. Distilled water was added up to complete 0.5 mL of volume and the absorbance value was measured at 505 nm after ten min.

Percentage of chelating or scavenging effects was computed using the following equation:

$$\text{Scavenging effect}\,(\%) = [1 - (A_s/A_c)] \times 100$$

where A_C is the absorbance value of control and A_S is the absorbance value of sample[60].

Statistical analysis

Each experiment was implemented in three times. The acquired data were enrolled as mean ± standard deflection and analyzed by SPSS (version 11.5 for Windows 2000, SPSS Inc., Chicago, IL). One-way analysis of type ANOVA was implemented by procedures. Considerable distinctions between means were identified by Duncan's Multiple Range tests, and $p<0.05$ was seen as important and $p<0.01$ as very important.

Results and discussion

Polyphenols contain one of the most commonly occurring and ubiquitous groups of plant metabolites and represent an integral part of human diet[2]. Flavonoids are a class of plant secondary metabolites and the most important group of polyphenols. It was reported that flavonoids are the most common polyphenolic group in the human diet. Recently, interest in phenolic compounds, in general, and flavonoids in particular has increased greatly owing to their antioxidant capacity and their possible beneficial food and pharmaceutical applications in human health[61].

Antioxidant activity, which reflected the ability of plant extracts or pure compounds, inhibits the oxidation process[62–64]. It was demonstrated that addition of taxifolin inhibits oxidation of linoleic acid emulsion (LAE) by ferric thiocyanate method (FTM). As shown in Figure 1, taxifolin showed greater antioxidative potency. In the current study, the first used antioxidant method is FTM. These methods determine the quantity of peroxides produced throughout the first steps peroxidation of lipid. Lipid oxidation occurs in food and pharmaceutical and is one of the major anxiety both food and pharmaceutical technologies[65,66]. It is in charge of nutritional and pharmaceutical qualities and safeties caused by the formation of secondary, potentially toxic compounds. This situation is very important to human health protection and also economically important[67]. Taxifolin demonstrated effective inhibition of lipid peroxidation in the LAE. The impression of 30 µg/mL concentration of taxifolin on the lipid peroxidation of LAE is demonstrated in Figure 1 and Table 1. It was identified to be 81.02%. Otherwise, the positive controls like trolox, α-tocopherol, BHT, and BHA demonstrated 88.57, 73.88, 94.29, and 90.12% peroxidation of LAE at the above-mentioned concentration, respectively. During the formation of linoleic acid autoxidation, peroxides occur rapidly. These outcomes expressly showed that taxifolin had the greatest antioxidant activity compared with α-tocopherol (73.88%), but lower than BHA (90.12%), BHT (94.29%), and trolox (88.57%).

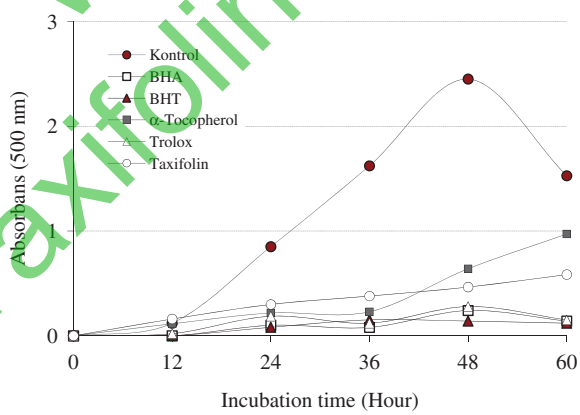


Figure 1. Total antioxidant activity of taxifolin and standards like trolox, α-tocopherol, BHT, and BHA at the same concentration (30 µg/mL) assayed by the ferric thiocyanate method in the linoleic acid system. The control value reached a maximum 60 h (BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene).

Lipid peroxidation is a free-radical chain reaction, and ROS can quicken this process. The mechanistically approaches of the spectrophotometric analysis of lipid hydroperoxides based on the oxidation of Fe^{2+} to Fe^{3+} ions and following chelation of the latter by thiocyanate (SCN^-) are thought. The FTM measures the amount of peroxide generated along the first steps of lipid peroxidation. In this test, hydroperoxides produced from linoleic acid supplement to the reaction solution, which has oxidized in air through the experimental period, were immediately evaluated. Thiocyanate and $FeCl_2$ react with one another to generate $Fe(SCN^-)_2$ by the agency of hydroperoxides[1,68].

The reducing capability of a bioactive compound can be calculated by means of the direct reduction of $Fe[(CN)_6]_3$ to $Fe[(CN)_6]_2$[69]. In this technic, the presence of reductants like taxifolin would result in the reduction of Fe^{3+} to Fe^{2+}. Addition of free Fe^{3+} to the reduced product brings about the formation of the intensive Perl's Prussian blue complex, $Fe_4[Fe(CN^-)_6]_3$, which has a strong absorbance at 700 nm[70]. The Fe^{3+} reducing assay gets advantage of an electron chain reaction where a ferric salt is utilized as an oxidant. Also, the yellow color of the tested mixture changes to diverse tons of green and blue according as the reducing ability of taxifolin.

As seen in Table 2 and Figure 2(A), taxifolin demonstrated potent Fe^{3+} reducing capability and these diversities were statistically seen to be considerable important ($p<0.01$). The reducing capacity of taxifolin, trolox, α-tocopherol, BHT, and BHA increased constantly when the concentration of sample was increased. Reducing capacity of taxifolin and standard compounds exposed the following order: BHT (3.080, r^2: 0.981) ≈ BHA (3.006, r^2: 0.979) ≥ Taxifolin (2.847, r^2: 0.960) > trolox (1.787, r^2: 0.969) > α-tocopherol (1.204, r^2: 0.967) at the above-mentioned concentration. The results proved that taxifolin had marked Fe^{3+}-reducing ability.

Copper is a vital component for several endogenous antioxidant enzymes. On the other hand, free radicals have been proposed to play a role in the carcinogenesis process[71]. Hence, the effects of dietary copper levels on the development of cancer have been investigated[72]. The CUPRAC method is a simple, rapid, selective, cost-effective, steady, and versatile antioxidant assay useful for a wide variety of polyphenols, as well as for thiols, synthetic antioxidants, and vitamins C and E. Also, this chromogenic redox reaction is conducted at a pH (7.0) and the method allows measuring antioxidants including thiol like glutathione and non-protein thiols. CUPRAC reactions are essentially complete within 30 min[43].

Cupric ions (Cu^{2+}) reducing power of 30-µg/mL concentration of taxifolin and standard reducing agents is shown in Table 2

Table 1. Determination of half maximal concentrations (IC_{50}) of taxifolin and standards belonging to Fe^{2+} chelating, $DPPH^{\bullet}$, $ABTS^{\bullet+}$, $DMPD^{\bullet+}$, and $O_2^{\bullet-}$ scavenging assays.

Antioxidants	Inhibition of lipid peroxidation (%)*	Fe^{2+} chelating†	$DPPH^{\bullet}$ scavenging†	$ABTS^{\bullet+}$ scavenging†	$DMPD^{\bullet+}$ scavenging†	$O_2^{\bullet-}$ scavenging†
BHA	90.12	30.13	86.63	12.30	231.01	34.65
BHT	94.29	46.51	86.64	12.32	–‡	69.31
α-Tocopherol	73.88	19.81	13.86	01.01	–‡	93.01
Trolox	88.57	99.01	06.93	12.62	173.25	46.51
Taxifolin	81.02	34.61	77.00	0.83	231.04	09.91

*Percentage of lipid peroxidation inhibition on linoleic acid emulsion values determined after incubation period (60 h).
†Hydrophobic antioxidants such as α-tocopherol or BHT did not show activity in this assay.
‡They were determined as μg/mL.

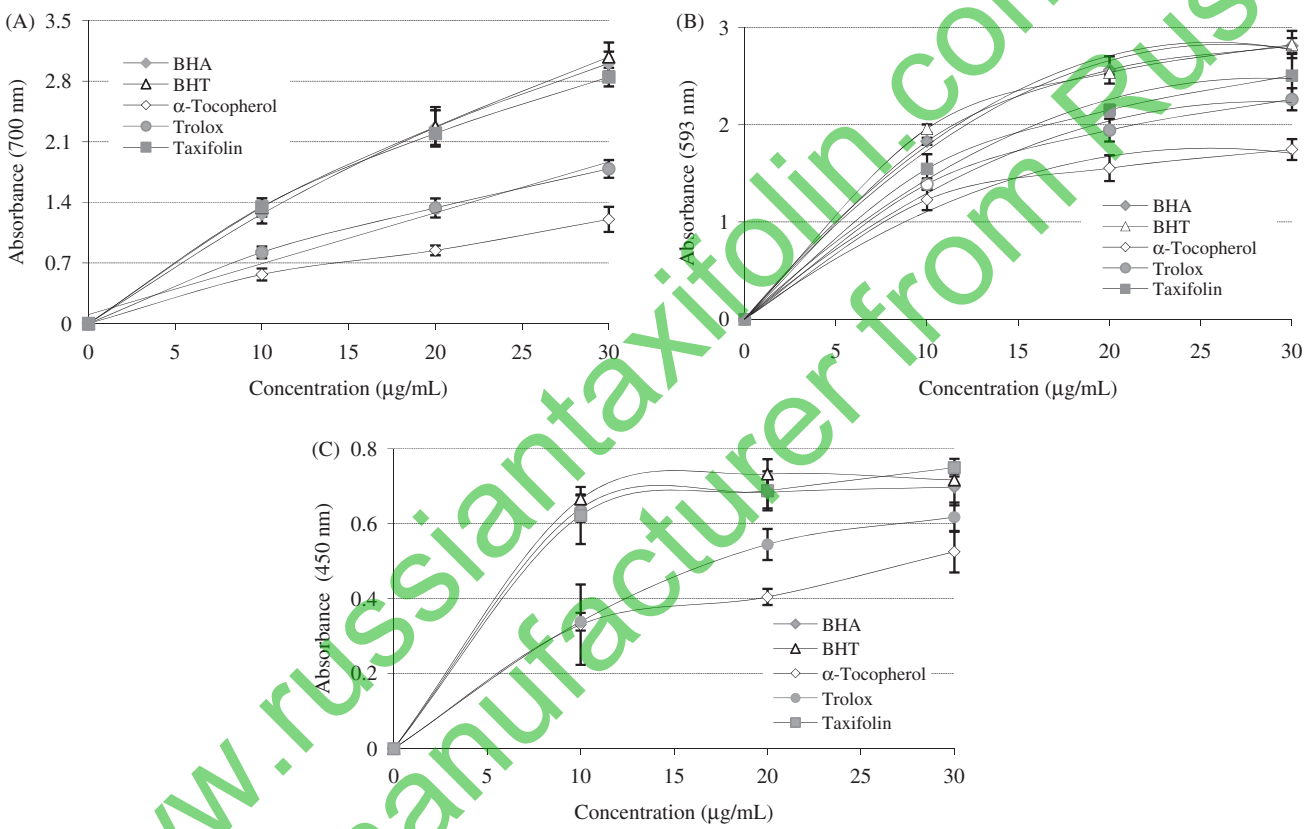


Figure 2. Reducing power of taxifolin. (A) $Fe^{3+} \rightarrow Fe^{2+}$ reductive potential of different concentrations (10–30 μg/mL) of taxifolin (r^2: 0.960) and reference antioxidants. (B) Cu^{2+}-reducing ability of different concentrations (10–30 μg/mL) of taxifolin (r^2: 0.956) and reference antioxidants. (C) TPTZ-$Fe^{3+} \rightarrow$TPTZ-Fe^{2+} reductive potential of different concentrations (10–30 μg/mL) of taxifolin (r^2: 0.993) and reference antioxidants (BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene).

and Figure 2(B). A positive relationship was found between the Cu^{2+} reducing power and different concentration of the taxifolin (r^2: 0.956). It was detected that Cu^{2+}-reducing capacity of taxifolin was addict to concentration (10–30 μg/mL). Cu^{2+}-reducing capability of taxifolin and standard reducing agents at the same concentration (30 μg/mL) showed the following order: taxifolin (0.750 ± 0.24, r^2: 0.956) > BHT (0.717 ± 0.17, r^2: 0.963) > BHA (0.698 ± 0.49, r^2: 0.951) > trolox (0.618 ± 0.39, r^2: 0.990) > α-tocopherol (0.525 ± 0.55, r^2: 0.968).

The FRAP assay measures the ferric ions (Fe^{3+})–ferrous ions (Fe^{2+})-reducing ability of the substance and was initially proposed for the measurement of total antioxidant capacity The $Fe^{3+}(CN^{-})_6$ reduction method measures the antioxidant effect of any molecule as reducing capability in the reaction. As can seen in Table 2 and Figure 2(C), taxifolin had the most effective reducing capacity using the $Fe^{3+}(CN^{-})_6$ reduction, Fe^{3+}-TPTZ, and Cu^{2+}-reducing ability when class with the standards including trolox, α-tocopherol, BHT, and BHA[58]. According to results obtained from FRAP assay (Table 1) reducing power of taxifolin and standard compounds decreased in the following order: BHT (2.826 ± 0.140, r^2: 0.987) ≈ BHA (2.811 ± 0.082, r^2: 0.996) > taxifolin (2.507 ± 0.136, r^2: 0.993) > trolox ($2.263 \pm 0.114 r^2$: 0.994) > α-tocopherol (1.745 ± 0.108, r^2: 0.983). In this method, higher absorbance values indicate higher of reducing ability Fe^{3+}–TPTZ complex. Also, the highest reduction ability was observed for the phenolic BHA. Benzie and Strain reported that the FRAP assay offers a well-known index of antioxidant, or reducing, potential of samples or pure compounds. At low pH values, a Fe^{3+}–TPTZ complex is reduced to the Fe^{2+}–TPTZ, which has maximum absorption at 593 nm with intense blue color of reaction medium[12].

Ferrozine, which reacts with divalent Fe to form a stable magenta complex species, can create complexes with Fe^{2+}. Fe^{2+} ions are the most efficient pro-oxidants in pharmacology systems

and food[72]. In the presence of Fe^{2+}-chelating compounds, Ferrozine–Fe^{2+} complex formation is broken down, resulting in a decrease in the red color of the Ferrozine–Fe^{2+} complex[58]. The data seen from Table 1 and Figure 3 display that taxifolin has a strong capability to bind Fe^{2+}. It is submitting that its main action as a peroxidation inhibitor may be involved to its Fe^{2+}-linking capacity[71]. Taxifolin prohibited the formation of the ferrous–ferrozine complex. Taxifolin is able to catch ferrous ion before ferrozine. It can convert Fe^{2+} ions into insoluble metal complexes or generate sterically hindrance, which can prevent the interactions between metals and lipid intermediates[73]. The chemical structure of taxifolin and its three metal binding sites is given in Figure 4. It may probably chelate three ferrous ions (Fe^{2+}) by the agency of its hydroxyl and carbonyl groups. It was known that biological active molecules with structures including functional groups like C–OH and C=O can bind Fe^{2+} ions. Additionally, the compounds with molecules containing two or more of the following functional groups: –S–, –O–, –OH, –SH, C=O, $–NR_2$, –COOH, and $–H_2PO_3$ in favor of structure–function configuration. In this way, the biological active compounds can easily chelate Fe^{2+} ions[1,74–76]. In our prior study, it was demonstrated that L-Carnitine, which is required for the transport of fatty acids from the cytosol into the mitochondria during the breakdown of lipids, similarly chelated Fe^{2+} ions thanks to the hydroxyl and carbonyl groups. In a slightly different, it was specified that curcumin, natural phenols that are responsible for the yellow color of turmeric, bounded Fe^{2+} ions thanks to the hydroxyl and carbonyl groups[49]. In a similar vein, L-adrenaline, which used for the treatment of a number of conditions including anaphylaxis, cardiac arrest, and superficial bleeding, bounded Fe^{2+} ions with hydroxyl and amine groups[12]. Taxifolin has the following characteristics: 4′,5′-dihydroxy group in ring B, or 4-keto and 3-hydroxy groups in C ring, or 4′-keto groups in C ring and 5,7-dihydroxy group in A ring have the metal-chelated properties (Figure 4). In this study, we demonstrated that a taxifolin molecule linked to three ferrous ions (Fe^{2+}) by the agency of hydroxyl and carbonyl groups. Lately, Fiorucci et al. have demonstrated that quercetin, a plant pigment with a molecular structure like or derived from flavone, complexioned metal ions in the same way[77]. Their common structural feature is the diphenylpropane moiety, which consists of two aromatic rings linked through three carbon atoms that together generally form an oxygenated heterocyclic. Also, it was reported that different metals have different binding affinities of the flavonoids[78]. Thus, for example, iron has the highest binding affinity for the 3-OH group of ring C, then the catechol group ring B, and at the end of 5-OH group of ring A, while the copper ions bind to the first ring catechol group B[79].

Table 2. Determination of reducing power of taxifolin by $K_3[Fe(CN)_6]$ reduction and FRAP methods, cupric ions (Cu^{2+}) reduction capacity by Cuprac and FRAP methods*.

	Fe^{3+}–Fe^{3+} reducing		Cu^{2+}–Cu^{+} reducing		Fe^{3+}–TPTZ reducing	
Antioxidants	IC_{50} (μg/mL)	R^2	IC_{50} (μg/mL)	R^2	IC_{50} (μg/mL)	R^2
BHA	3.006 ± 0.135	0.981	0.698 ± 0.49	0.951	2.811 ± 0.082	0.996
BHT	3.080 ± 0.168	0.979	0.717 ± 0.17	0.963	2.826 ± 0.140	0.987
α-Tocopherol	1.204 ± 0.145	0.967	0.525 ± 0.55	0.968	1.745 ± 0.108	0.983
Trolox	1.787 ± 0.102	0.969	0.618 ± 0.39	0.990	2.263 ± 0.114	0.994
Taxifolin	2.197 ± 0.108	0.960	0.750 ± 0.24	0.956	2.507 ± 0.136	0.993

*Expressed as absorbance values.

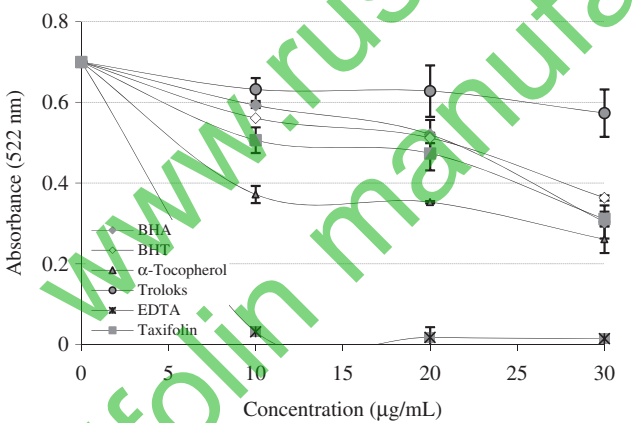
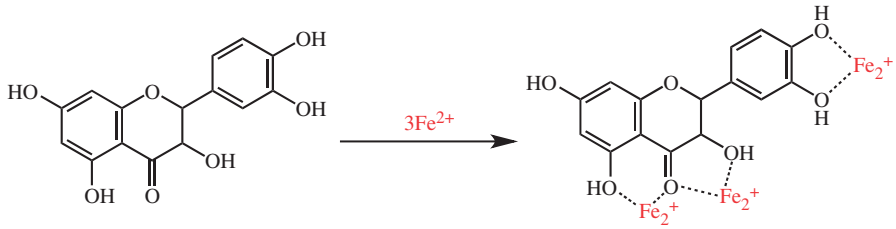


Figure 3. Comparison of Fe^{2+}-chelating activity of taxifolin (r^2: 0.942) and standards like trolox, EDTA, α-tocopherol, BHT, and BHA at the concentrations of 10–20 mg/mL (BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene).

Figure 4. Possible places on taxifolin for chelating the transition metal ions such as Fe^{2+} in the process of lipid peroxidation.

In this regard, taxifolin had also effective metal ions-chelating effect. The distinction between taxifolin at different concentrations (10–30 μg/mL) and control value was fixed to be statistically important ($p < 0.01$, Table 1 and Figure 3). The half maximal inhibitory concentration (IC_{50}) belonging to taxifolin and standard metal chelator compounds including EDTA demonstrated the following order: EDTA (IC_{50} value: 4.41 μg/mL, r^2: 0.940) < α-tocopherol (IC_{50} value: 19.81 μg/mL, r^2: 0.934) < BHA (IC_{50} value: 30.13 μg/mL, r^2: 0.923) < taxifolin (IC_{50} value: 34.61 μg/mL, r^2: 0.945) < BHT (IC_{50} value: 46.51 μg/mL, r^2: 0.945) < trolox (IC_{50} value: 99.01 μg/mL, r^2: 0.918). These results clearly introduce that the Fe^{2+} ions-chelating effect of taxifolin was higher than trolox and BHT, parallel to BHA, but lower that of α-tocopherol. IC_{50} represents the concentration of a metal chelator that is required for 50% chelation *in vitro*. Lower IC_{50} values indicate higher metal chelation capacity. The results show that taxifolin, being a strong chelating agent, forms highly stable complexes with Fe^{2+}.

DPPH• becomes a steadier diamagnetic molecule after acceptation of an electron (e^-) or hydrogen radical (•H) from an antioxidant compound[67]. DPPH radicals scavenging of taxifolin are summarized in Table 1 and Figure 5(A). It is well known that a radical can be stabilizing by the agency of resonance structure of the phenolic compounds. Chemically, it is an ester formed from quinic acid and two units of caffeic acid. Taxifolin has two aromatic rings, which had two phenolic groups (–OH) at the *meta*- and *para*- positions with respect to each other. It is this order of



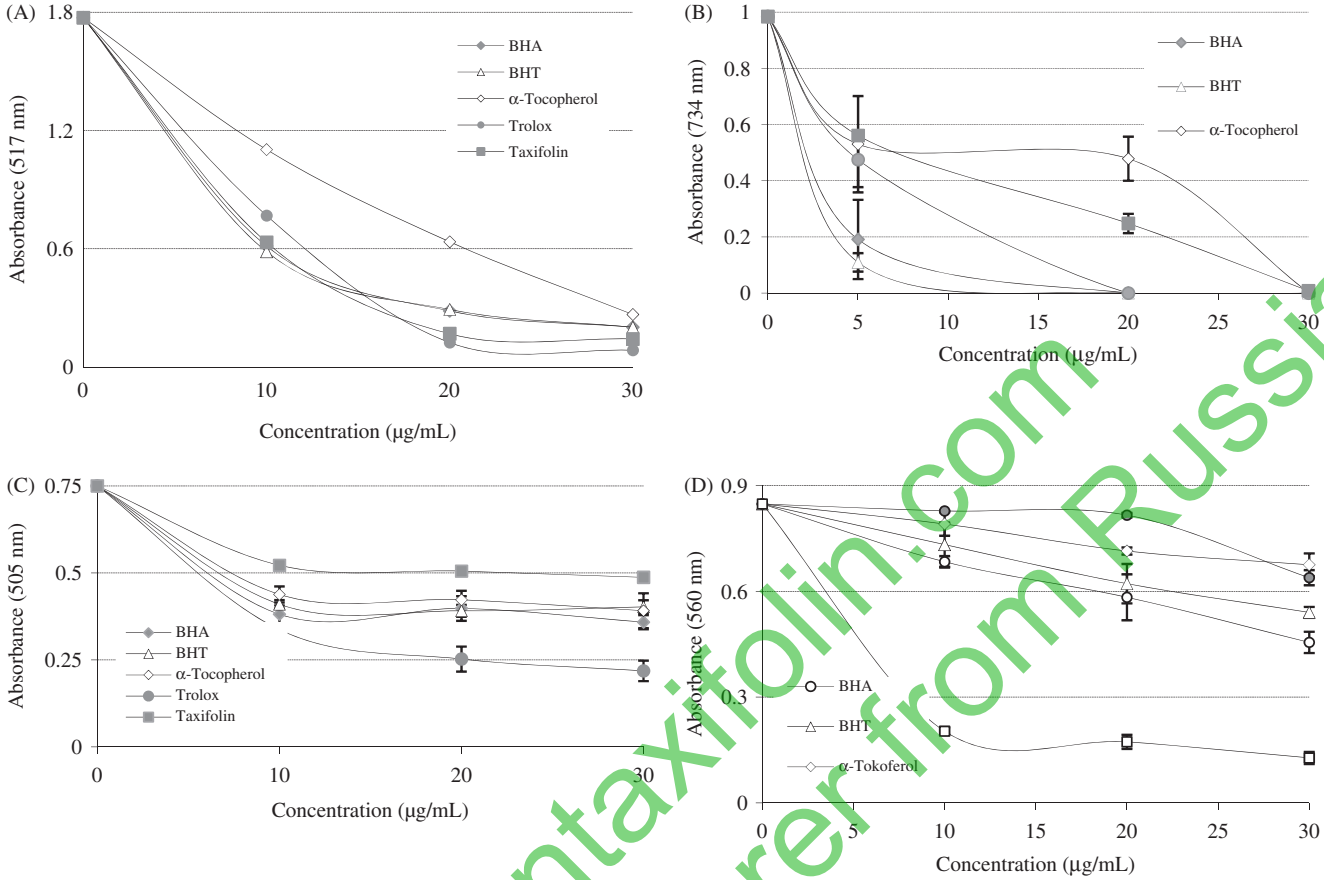


Figure 5. Radical-scavenging activity of taxifolin. (A) DPPH free radical-scavenging activity of different concentrations (10–30 μg/mL) of taxifolin (r^2: 0.931) and reference antioxidants. (B) ABTS radical-scavenging activity of different concentrations (10–30 μg/mL) of taxifolin (r^2: 0.925) and reference antioxidants. (C) DMPD radical-scavenging activity of different concentrations (10–30 μg/mL) of taxifolin (r^2: 0.907) and reference antioxidants. (D) Superoxide anion radical-scavenging activity of different concentrations (10–30 μg/mL) of taxifolin (r^2: 0.982) and reference antioxidants (BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; DPPH•, 1,1-diphenyl-2-picryl-hydrazyl free radical; ABTS•+, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid; DMPD•+, *N,N*-dimethyl-*p*-phenylenediamine radical).

arrangement, which is the chief determinant for the strong antioxidant capacity of phenolic compounds[80]. It was demonstrated, by studying eight phenolic and analogous compounds, that the antioxidant activity of a molecule increases with increase in the number of hydroxyl groups attached to the aromatic ring[81]. Also, a positive correlation was further obtained between the number of –OH groups attached to the aromatic ring and the antioxidant activity as well as the antiradical activity of phenolic acids[80,82]. Structure–activity research studies have shown that the radical-scavenging efficiency of phenolic compounds is dependent on the presence of –OH groups as well as steric freedom[83]. Especially, the *para*-substituted –OH group was found to be highly efficient in radical scavenging. The nature and the position of the substituents with respect to –OH also affect the activity of polyphenols. Previously, the role of –OH groups at the *ortho*-position in the antioxidant activity of phenolic acids has also been described[84]. The extra –OH group in trihydroxy phenolic acid provides greater stability along with higher antioxidant activity. Also, –OH groups not only affect antioxidant ability by intramolecular hydrogen bonding (Figure 6)[84–86], but they also tend to stabilize the antioxidant radical formed. It was reported that the –OH group *ortho*- to phenol tends to stabilize the radical formed resulting in a lower hydrogen bond dissociation enthalpy and hence an increased antioxidant capacity[84]. Separation of a hydrogen atom from monophenolic and diphenolic –OH may be readily. As can be seen in Figure 7, oxygen radicals, which formed in the first level, and supported by the aromatic ring are made

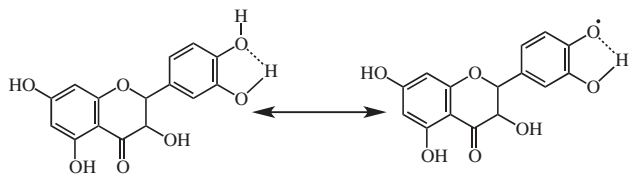


Figure 6. Stabilization of radicals by phenol group of taxifolin.

profitable. The stability of a radical is measured with possible intermediate stages. The stability of a radical is depending on the number of intermediates. The first radical is stabilized by occurred hiperconjugation. As shown in Figure 7, depending on the nature of the molecule, more than one mechanism can be written from most stable structure toward the unprofitable radical formation. The arrangement phenolic aromatic markedly influences the antioxidant activity of phenolic acids. Accordingly, several factors such as the number and the position of –OH group, the presence of other functional groups and their position with respect to –OH groups have been shown to affect the antioxidant and antiradical activities. Taxifolin scavenged a DPPH radical on phenolic groups. The reasons of powerful radical-scavenging activity of taxifolin molecule are its conjugation structures and resonance stability of both phenolic rings (Figure 5A). The stabilization of radicals by phenol group of taxifolin is given in Figure 7. Two radicals are possible by the abstraction of a

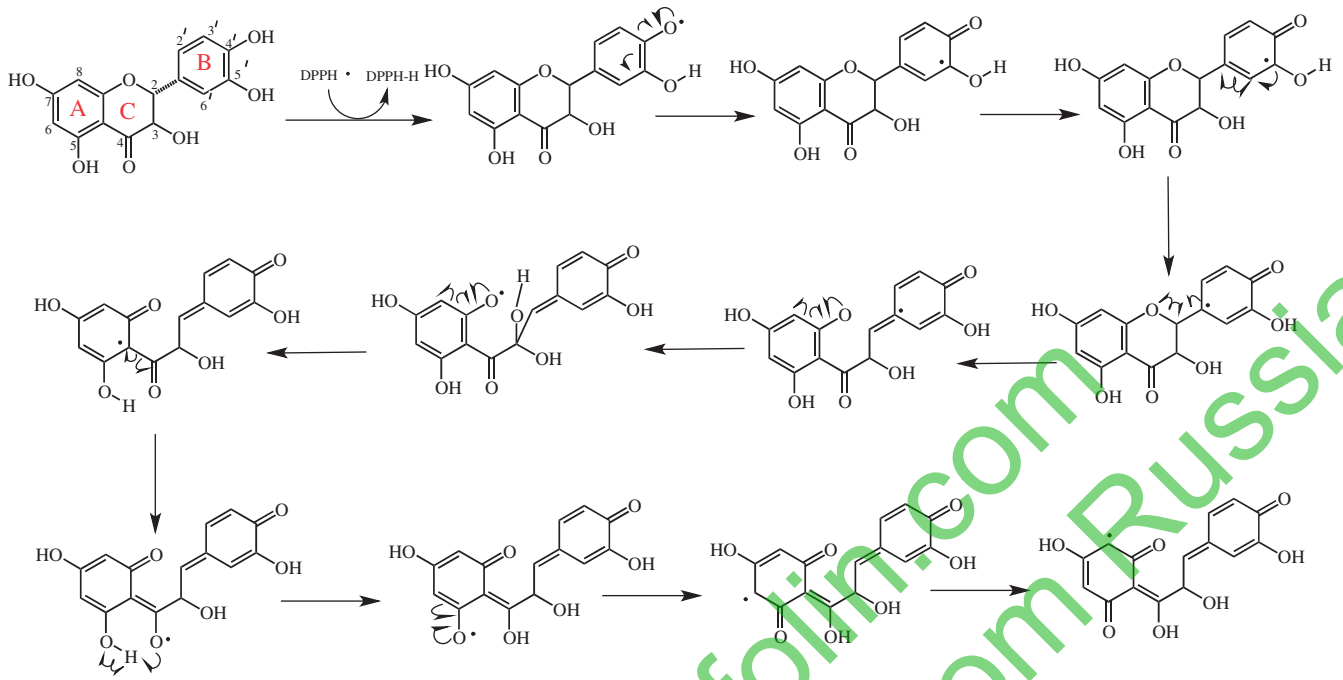


Figure 7. The proposed reaction scheme between DPPH free radicals and taxifolin.

hydrogen atom from the corresponding *orto-* or *meta-* hydroxyl groups from taxifolin.

Antiradical potential of taxifolin is evaluated by DPPH$^{\bullet}$ radicals. DPPH is a dark-colored crystalline powder composed of stable free-radical molecules (DPPH$^{\bullet}$). It is a stable free radical and converts to a stable diamagnetic molecule by accepting an electron or hydrogen radical. It has an absorbance band at 517 nm, which disappears upon reduction by an antiradical compound[87]. It is based on the reduction of a DPPH solution in alcohol in the source of a hydrogen donating antioxidant, owing to the formation of the non-radical form of DPPH-H[88]. Taxifolin has the ability to convert the steady radical DPPH to the yellow-colored DPPH-H. Table 1 shows half maximal radical-scavenging concentrations (IC_{50}) of taxifolin and the reference chemical agents like BHA, BHT trolox, and α-tocopherol. IC_{50} values were found as 77.00 µg/mL for taxifolin (r^2: 0.938), 6.93 µg/mL for trolox (r^2: 0.945), 13.86 µg/mL for α-tocopherol (r^2: 0.973), 86.64 µg/mL for BHT (r^2: 0.931), and 86.63 µg/mL for BHA (r^2: 0.938). Therefore, DPPH radical-scavenging effects of studied antioxidants increased in the order of trolox > α-tocopherol taxifolin ≥ BHA ≈ BHT (Table 1 and Figure 5A). A lower EC_{50} value demonstrates a higher DPPH·-scavenging activity.

Another improved technique for the determination of radical scavenging is ABTS$^{\bullet+}$ scavenging activity. The generation of ABTS$^{\bullet+}$ defined here includes the directly production of the blue/green ABTS$^{\bullet+}$ chromophore thanks to the reaction between ABTS and $K_2S_2O_8$. ABTS$^{\bullet-}$, the oxidant, was produced by the agency of $K_2S_2O_8$ oxidation of $ABTS^{2-}$ and the radical cation is calculated spectrophotometrically. This is a direct production of a steady form of radical to create a blue-green ABTS$^{\bullet+}$ chromophore previous to the reaction with antioxidants[89]. ABTS$^{\bullet+}$ cation can be prepared by running distinct oxidants like permanganate (MnO_4^-), chromate (CrO_4^{2-}), and perchlorate (ClO_4^-). In this sense, the oxidizing agent can be called an oxygenation reagent or oxygen-atom transfer agent. Results acquired using $K_2S_2O_8$ as oxidant show that the occurrence of $K_2S_2O_8$ increases the ratio of ABTS$^{\bullet+}$. These radicals were generated in ABTS/$K_2S_2O_8$ system[1,90].

Efficient radical cation scavenging activity was seen on the tested compounds. As shown in Table 1(B), taxifolin is an efficient ABTS$^{\bullet+}$ scavenger in a concentration-dependent manner (10–30 µg/mL, r^2: 0.957). The EC_{50} value for taxifolin in this analysis was 0.83 µg/mL (r^2: 0.937). It is seen that concentration of ABTS$^{\bullet+}$ ($p < 0.01$) declines substantially owing to the scavenging capability at all taxifolin concentrations. Moreover, EC_{50} values for trolox, α-tocopherol, BHT, and BHA were found to be 12.62 µg/mL (r^2: 0.986), 1.01 µg/mL (r^2: 0.835), 12.30 µg/mL (r^2: 0.852), and 12.32 µg/mL (r^2: 0.797), respectively. ABTS$^{\bullet+}$-scavenging efficacy of taxifolin and standards on the ABTS$^{\bullet+}$ increased in the following order: taxifolin ≥ α-tocopherol > BHA ≈ BHT ≈ trolox (Table 1 and Figure 5B). As well as in DPPH free radical scavenging activity, a lower EC_{50} value indicates a higher ABTS$^{\bullet+}$ scavenging activity.

The basic guideline of DMPD$^{\bullet+}$ scavenging assay is that when it have been used at acidic pH and in convenient oxidant solution, DMPD can form a steady and colored DMPD radical cation. DMPD$^{\bullet+}$ had a maximum absorbance at 505 nm[91]. Taxifolin can transfer a hydrogen atom to DMPD$^{\bullet+}$, quench the color, and produce a solution decoloration. This reaction is rapid and the end point, which is steady, is taken as a measure of the antioxidative competence. Also, this assay shows the talent of radical hydrogen-donors to scavenge the single electron from DMPD$^{\bullet+}$. Actually, radical cation forms slowly and because of this reason absorbance value increases continuously[91]. On the contrary of the ABTS$^{\bullet+}$ scavenging method, DMPD$^{\bullet+}$-scavenging procedure guarantees a very steady end point. This is quite significant when a large-scale screening is necessary. It was shown the main drawback of DMPD$^{\bullet+}$-scavenging procedure is the fact that its sensitivity and renewability significantly decreased when hydrophobic antioxidants like BHT or α-tocopherol were used. Therefore, these positive controls were not suitable for using in DMPD$^{\bullet+}$-scavenging assay[1,92,93].

The principle of the DMPD assay is that at an acidic pH and in the presence of a suitable oxidant solution DMPD can form a stable and colored radical cation (DMPD$^{\bullet+}$)[93,94]. As in the previous both DPPH· and ABTS$^{\bullet+}$ radical-scavenging methods,

taxifolin was an efficient $DMPD^{\bullet+}$-radical scavenging in a concentration-dependent manner (10–30 µg/mL, r^2: 0.951). The EC_{50} value for taxifolin was found as 231.04 µg/mL (r^2: 0.951). This value was found as 173.25 µg/mL (r^2: 0.979) for trolox, and 231.01 µg/mL (r^2: 0.954) for BHA (Table 1 and Figure 5C). It was reported that hydrophobic antioxidants such as α-tocopherol or BHT did not show activity in this assay[95–98]. There was an important decline ($p < 0.05$) in the $DMPD^{\bullet+}$ concentration owing to the scavenging ability at all taxifolin concentrations. Within different concentrations of taxifolin could not be determined crucial differences in $ABTS^{\bullet+}$-scavenging potential.

Based on superoxide anion radical-scavenging ($O_2^{\bullet-}$) activity results, it was found that $O_2^{\bullet-}$ scavenging activity of taxifolin is more than that of BHA, BHT, α-tocopherol, and trolox. As seen in Table 1 and Figure 5(D), the IC_{50} value of $O_2^{\bullet-}$ radical scavenging of taxifolin was found to be 9.91 µg/mL (r^2: 0.943). Conversely, IC_{50} values of BHA, BHT, trolox, and α-tocopherol were found as 34.65 µg/mL (r^2: 0.995), 69.31 µg/mL (r^2: 0.994), 93.01 µg/mL (r^2: 0.991), and 46.51 µg/mL (r^2: 0.947), respectively. When the EC_{50} value is lower, O_2^--scavenging activity increases. These resultants demonstrated that when compared with O_2^--scavenging activity, in general, chemical agents' taxifolin had the highest activity, and these distinctions were found to be statistically important.

Conclusion

Phenolic structures comprise a diverse group of molecules classified as secondary metabolites in plants that have a large range of structures and functions. Taxifolin is effective polyphenolic compounds and a food and nutritional supplements. A greater number of potential clinical applications have been reported. Taxifolin was found to be a powerful antioxidant and antiradical activities in different *in vitro* bioassays when compared with standard antioxidant compounds. As discussed above, taxifolin can be used for minimizing or preventing lipid oxidation in food or pharmaceutical products, delaying the formation of toxic oxidation products, maintaining nutritional quality, and prolonging the shelf life of food or pharmaceutical materials.

Declaration of interest

The authors declare no conflict of interest. S. H. Alwasel would like to extend his sincere appreciation to the Research Chairs Program at King Saud University for funding this research.

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