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Structure–Activity Relationship for Dihydromyricetin as a New Natural Antioxidant in Polymer

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ABSTRACT: Dihydromyricetin (DMY), a natural product, was used as antioxidant in ethylene vinyl acetate copolymer (EVA) in our laboratory. Oxidation induction temperature (OIT) of EVA with the antioxidant of DMY and effect of pH on OIT was measured by means of differential scanning calorimeter (DSC). The mechanism on that antioxidation of DMY in EVA is produced on basis of determination of UV–Vis absorption, nuclear magnetic resonance (NMR) and the experiment of reacting the pyrogallic (PA) and DMY, respectively, with the DPPH. The result shows that the ortho-trihydroxyl group (B ring) in DMY has mainly antioxidative activity and hydroxyl group of 7-position (A ring) not only displayed reversibility to acidic or alkaline medium but also affected in some extent the antioxidative ability in EVA. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: EVA; dihydromyricetin; natural antioxidant; radical scavenging activity

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INTRODUCTION

Dihydromyricetin (DMY) (Figure 1) from the stems and leaves of *Ampelopsis* grossedentata, is one kind of flavonoids which has many pharmacological functions on organism, such as relieving cough, removing sputum, inhibiting hypertension, protecting liver, absorbing ultraviolet radiation,[1] antioxidation in food,[2] and antibacterial properties.[3,4]

With the rising of public awareness for environment protection, natural products as antioxidants become one of the most attractive research fields. The stabilizing effect of synthetic Vitamin E (DL-α-tocopherol) against oxidative degradation of polyolefins has been extensively studied over 10 years.[5] A good stabilizing activity of α-tocopherol in LDPE, HDPE, and PP is well documented in a number of papers, especially in the field of melt processing and food packaging.[6–10]

It is our first attempt to investigate the antioxidative properties of DMY in polymer. Taking the oxidation of ethylene-vinyl acetate copolymer (EVA) as an example we showed in an earlier paper[11] that DMY has a more efficient thermal antioxidant for polymers compared with Irganox 1010. Therefore, it should be possible to be used in polyolefin.

DMY shown a great antioxidant activity for small molecule in papers published. The most active H-donating hydroxyl groups were the ones attached to C4′[12] in DMY against lard oil oxidation. Using the UV–VIS spectrophotometric method, it was shown that hydroxyl groups (on the C ring) of the 3-position (flavonols) were positive effect on the radical scavenger activity; however, the hydroxyl group on the 7-position (A ring) was negative.[13] In this study, the activity of hydroxyl groups on three rings of DMY would further be determined by UV—Vis absorption, nuclear magnetic resonance (NMR) and the experiment of reacting the DMY with the DPPH in order to explain the antioxidant role of DMY in the system of ethylene-vinyl acetate copolymer (EVA).

EXPERIMENTAL

Materials

Dihydromyricetin (Purity >97.3%) was made in our laboratory. Ethylene vinyl acetate copolymer (EVA 260) made in Mitsui Polychemical Co. (Tokyo, Japan) was with 28 wt % vinyl acetate and 6.0 of melt flow rate. Pyrogallic (PA) and α, α-diphenyl-β-picrylhydraziyl radical (DPPH) were purchased from Aladdin Chemistry Company (Shanghai, China). All other reagents were analytical reagent grade and double-distilled water was used during the experiment.

Sample Preparation

A total of 25.0 mg of DMY was transferred to 25 mL volumetric flask, dissolved, and diluted to 25 mL with acetone. Samples of EVA with DMY were prepared by mixing EVA with DMY solution at proper ratio and in different pH. Then the EVA/DMY

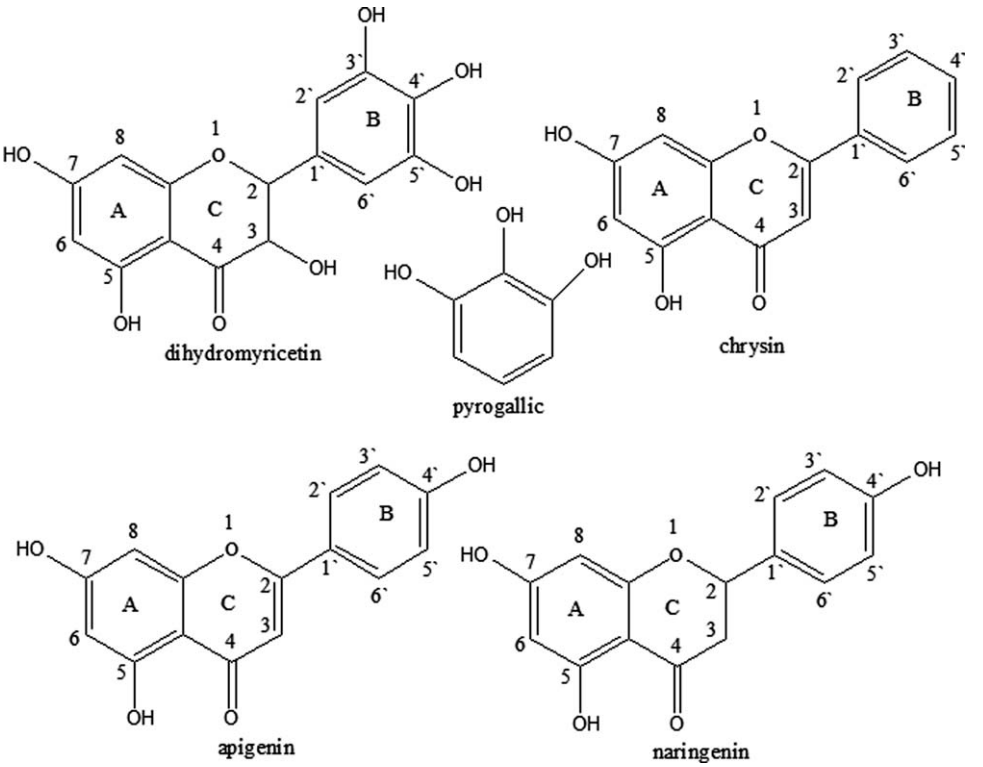


Figure 1. Structures of the compounds examined in this article.

samples were put into petri dish to evaporate the solvent and melted and mixed at 190°C at hot plate. The compositions of tested materials were listed in Table I.

Oxidation Induction Temperature (OIT)

Oxidation induction temperature of all samples was determined by differential scanning calorimeter (DSC) as the point in the thermogram where the onset of the decomposition was appeared.[11] The samples of 5–8 mg were placed in perforated aluminum pans with an air flow of 20 mL/min. Heating rate was 10°C/min. OIT was defined as the temperature when the heat flow started to change abruptly after melting peak.

UV–Vis Absorption Spectra

A 1.25 m*M* solution of the DMY in ethanol (95%) was prepared as a stock solution. It was diluted to 0.1 m*M* of concentration with the ethanol and used as the sample tested. The absorption spectra were obtained on an UV−8000 spectrophotometer (Metash, China) at room temperature with 1 cm silica cells.

NMR Spectroscopy

^{13}C spectra were obtained on a Bruker DRX-400 spectrometer in Fourier transform mode. Chemical shift values are reported using methanol-d_4.

DPPH Assay

The DPPH assay for radical scavenging activity was based on the methodology of Piras et al.[14] A 100 μL sample of an ethanol solution of each test compound was added to 3.9 mL of 47 μ*M* DPPH free radical in ethanol, vortexed, and kept in the dark for 30 min. The absorbance of the reaction mixture was then measured at 516 nm in order to determine the scavenger activity of tested compound. The experiments were then repeated at

Table I. EVA/DMY Samples Prepared Via Melt Compounding

Sample number	DMY (wt %)	Irganox 1010 (wt %)	pH[a]
EVA0	0	0	-
EVA1	0	0.1	-
EVA2	0.1	0	-
EVA3	0.1	0	9
EVA4	0.1	0	9 to 5
EVA5	0.1	0	5

[a]Different pH (pH = 5 and 9) adjusted with hydrochloric acid and sodium hydroxide.

Table II. OIT of Nonstabilized and Stabilized EVA at 0.1 wt % of DMY and Irgnox 1010

	1	2	3
EVA0	225	224	226
EVA1	246	244	245
EVA2	273	275	271
EVA3	225	221	223
EVA4	235	235	231
EVA5	265	263	260

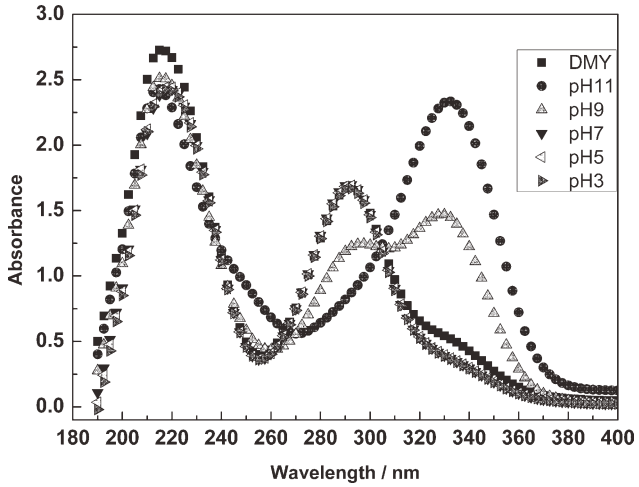


Figure 2. UV–Vis absorption spectra of DMY under different pH condition.

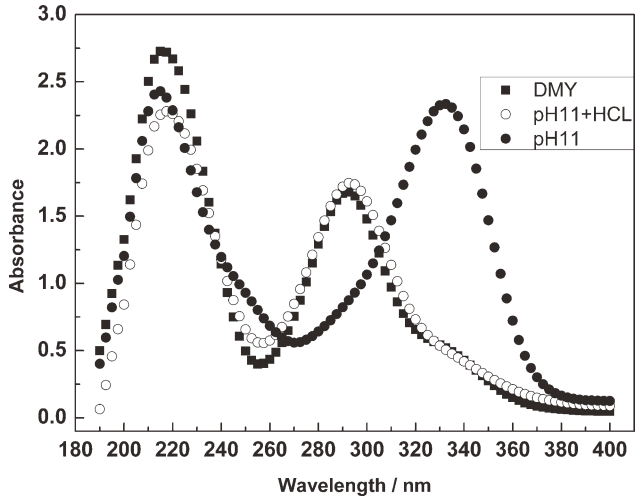


Figure 4. UV–Vis absorption spectra of DMY (pH = 11) acidification.

different concentrations in order to calculate the amount required to scavenge 50% of DPPH radicals (IC_{50}).

RESULTS AND DISCUSSION

OIT Measurement

OIT is one of the most commonly used indicators of polymer stability both in academic and industrial environment,[15] being the preferred test to assess the oxidative stability of polyolefins. As the OIT is a relative measure of the degree or level of stabilization of the material tested, the higher the OIT, the more stable is the material.

To investigate antioxidative mechanism and antioxidative activity of DMY in EVA, the values of OIT under nonisothermal measurements in DSC were put in the Table II for a series of samples of pure EVA, EVA with DMY in different pH condition, and Irganox 1010. The value of OIT for EVA with DMY was higher than that of EVA without DMY and even higher than that stabilized by Irganox 1010 which is commonly used as antioxidant in polyolefins. It is recognized that the DMY in EVA possessed good antioxidative activity under atmosphere. When the sample of EVA was mixed with DMY under alkaline or acid the value of OIT is quite different. The value of OIT of EVA with DMY in alkaline was almost as same as pure EVA. However, it has been obtained that the OIT of EVA stabilized with DMY increases as the pH changed from 9 to 5. The antioxidant activity of DMY in acidic condition in EVA might be recovered partly. This means that the antioxidant activity of DMY strongly depends on pH. Active H-donating hydroxyl groups of DMY changed to ionic bond of negative oxygen ion when treated with alkali, whereas active H-donating hydroxyl groups of DMY remain in acidic condition. This is why DMY lose stabilization role in alkaline case.

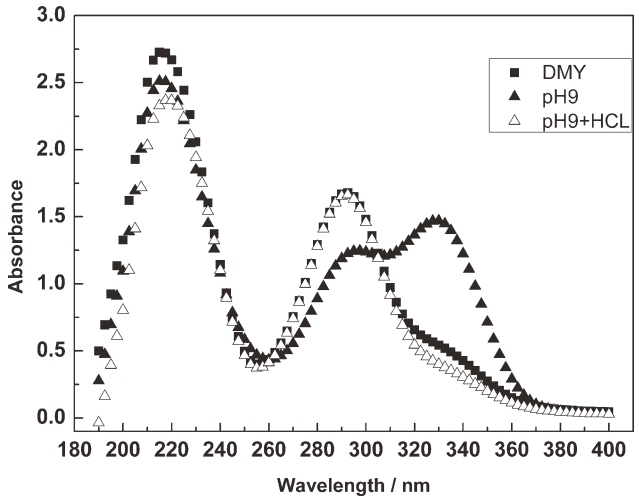


Figure 3. UV–Vis absorption spectra of DMY (pH = 9) acidification.

UV–Vis Absorption Spectra and NMR

DMY consists of 15 carbon atoms and possesses six hydroxyl groups. There is a number of discussion and contradiction regarding the structure-antioxidant activity relationships of

Table III. The ^{13}C-NMR Data for DMY (1), and pH = 9–10 (2) and pH= 5–6 (3)

	1	2	3
C-2	85.3	84.8	85.1
C-3	73.7	73.4	73.6
C-4	198.3	194.8	198.3
C-5	165.3	165.3	165.1
C-6	97.3	99.7	97.3
C-7	168.7	180.1	168.6
C-8	96.2	98.9	96.3
C-9	164.4	164.1	164.3
C-10	101.8	100.4	101.8
C-1′	134.9	134.9	134.8
C-2′	108.0	108.0	108.0
C-3′	146.8	146.9	146.7
C-4′	129.1	129.6	129.0
C-5′	146.8	146.9	146.7
C-6′	108.0	108.0	108.1

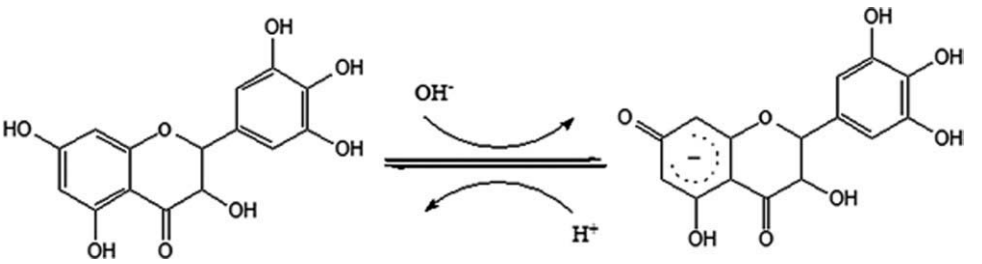


Figure 5. Proposed mechanisms for DMY/OH− and DMY/H+ reaction.

flavonoids for food. Antioxidant activity of natural flavonoids is governed by the number and location of their aromatic hydroxyl groups.[16] In contrast, in determining antioxidant activity of flavonoids, the number of hydroxyl groups is of negligible importance, and so is their position either in ring A or ring B.[17] The antioxidant activity of DMY is due to the ability to scavenge free radical. Structurally, the nature of substitutions on ring B has great influence on the antioxidant activity. To confirm the mechanism on antioxidant activity of DMY, UV–Vis absorption and nuclear magnetic resonance (NMR) were performed on DMY and treaded DMY, respectively.

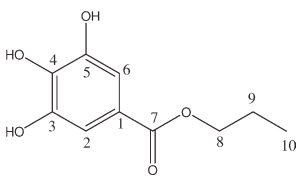
The use of UV–Vis absorption measurement in determining various structural features of flavonoid compounds is attractive because of its speed, simplicity, and economy of material.[18,19] The absorptions in the peak of 290 nm of UV–Vis spectra correspond to the A ring portion (benzoyl system, band II), and the absorptions in the peak of 330 nm correspond to B ring portion (cinnamoyl system, band I).[20] This lack of conjugation of B rings with other one result in a small band I in UV spectra of DMY.

For confirming the effect of acid–base on DMY, the absorption spectra of DMY under different pH were recorded and presented in Figures 2−4. It is noteworthy that a bathochromic shift of the band II occurs upon pH range from 9 to 11. In particular, there is, to a certain extent, a bathochromic shift which is observed at pH 9. As can be seen from Figure 2, the band II in the spectra is associated mainly with absorption in the A ring of the DMY, and that a bathochromic shift of this band in the presence of sodium hydroxide is caused by the ionization of the 7-hydroxyl group. Figures 3 and 4 show the absorption spectra of DMY at pH 9 and pH 11, acidified, respectively, with hydrochloric acid. The position and shape of band II remained almost unchanged for the DMY at pH < 7. The conclusion may be drawn that A—ring is highly sensitive to alkali.

In a previous study, in the ^{1}H-NMR of DMY, the individual signals of the hydroxyl groups of DMY were not observed.[21] In ^{13}C-NMR, however, all the resonances of 15 carbon atoms had been observed and assigned.[22] Therefore, in order to evaluate the change of the structure of DMY in different pH, the chemical shifts of 15 carbon atoms in the ^{13}C-NMR spectrum are listed in Table III for DMY and DMY in different pH. It can be seen that C (7) is highly sensitive to alkali with a change of chemical shift from 168.7 to 180.1, and turn back original station of chemical shift when the acid was added. For other carbon linked with hydroxyl group, the difference in chemical shifts in the ^{13}C-NMR spectrum was small. This shows that hydroxyl group linked with C (7) should form ionic bond of negative oxygen ion in alkali condition. This causes the shift of band II (ring A) at peak of 290 nm to a position of 330 nm in alkali condition in UV spectra of DMY.

A hypothesis is put forward (as shown in Figure 5) to explain the shift of the band II in UV–Vis absorption spectra and change of the chemical shifts of C (7) in ^{13}C-NMR. After DMY

Table IV. The ^{13}C-NMR Data for Propyl Gallate(1), and pH = 9–10 (2) and pH = 5–6 (3)

Structure		1	2	3
	C-1	121.7	120.5	121.7
	C-2	110.0	109.9	110.1
	C-3	146.5	146.7	146.3
	C-4	139.7	141.3	139.6
	C-5	146.5	146.7	146.3
	C-6	110.0	109.9	110.1
	C-7	168.6	169.1	168.8
	C-8	67.3	67.3	67.5
	C-9	23.2	23.1	23.0
	C-10	10.8	10.8	10.8

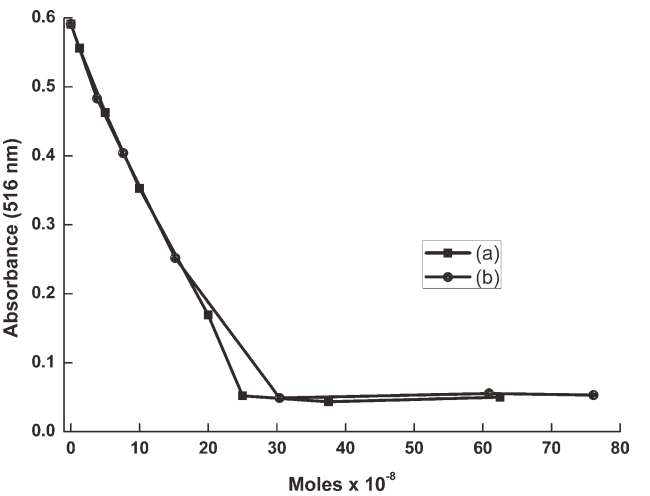


Figure 6. 516 nm absorbance value of a DPPH solution after 40 min exposure to increasing quantities of DMY (a) and PA (b).

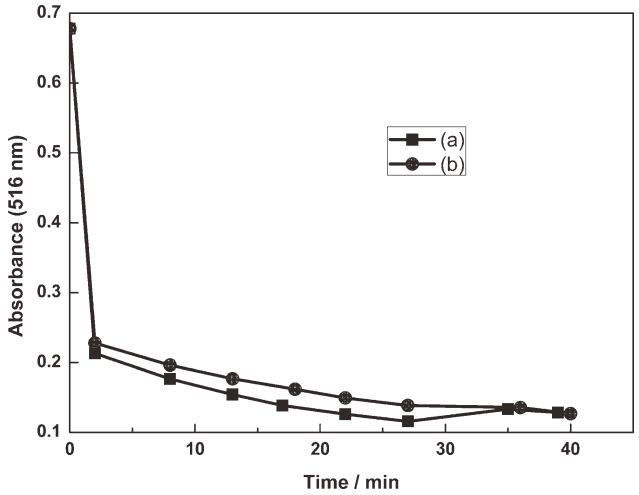


Figure 7. 516 nm DPPH absorbance values after different times of incubation with DMY (a) and PA (b).

react with alkali the hydroxyl groups on C (7) of the A-ring donate 7-hydrogen ion and form a negative oxygen ion. The bathochromic shift of DMY in UV spectra is caused by conjugation of the A-ring and negative oxygen ion in alkalized DMY. With the increase of pH more negative oxygen ions on C (7) are formed. The band II at 290 nm gradually moved to 330 nm until its disappearance at pH = 11. The chemical shifts of carbons on B-ring of DMY in alkali do not shift obviously in conformity with the reference sample propyl gallate (Table IV). This means that Alkali do not result in change of the hydroxyl groups on the B-ring but influence the scavenger activity of the ortho-trihydroxyl group.

DPPH Assay

The stable α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical was used as a probe for testing the ability of scavenging free radical of antioxidant DMY. In its radical form, DPPH shows

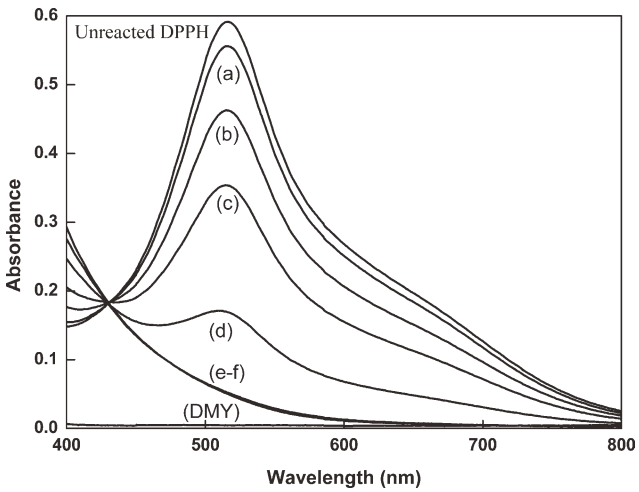


Figure 8. UV–Vis (400–800 nm) absorbance spectra of DMY/DPPH after the achievement of the steady state; DMY moles: (a) = 0.12×10^{-7} (b) = 0.5×10^{-7} (c) = 1.0×10^{-7} (d) = 2.0×10^{-7} (e) = 2.5×10^{-7} (f) = 6.3×10^{-7}.

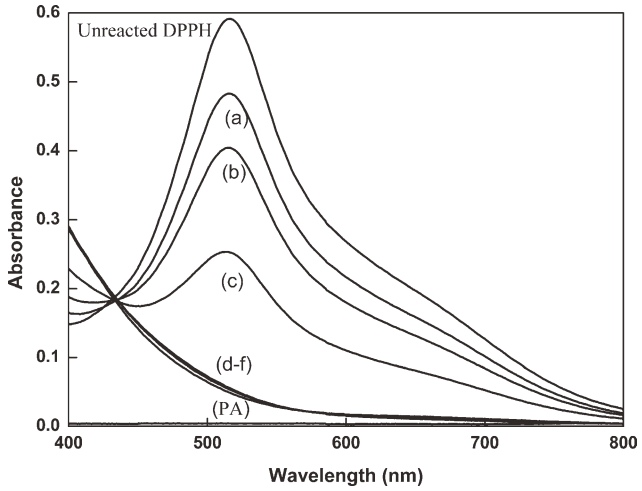


Figure 9. UV–Vis (400–800 nm) absorbance spectra of PA/DPPH after the achievement of the steady state; PA moles: (a) = 0.38×10^{-7} (b) = 0.76×10^{-7} (c) = 1.5×10^{-7} (d) = 3.0×10^{-7} (e) = 6.1×10^{-7} (f) = 7.6×10^{-7}.

an absorbance peak at 516 nm, which progressively weaken as DPPH react with antioxidant.[23] So reducing absorbance of the DPPH at 516 nm could correlate the scavenger activity of the tested antioxidant. The absorbance at 516 nm is tested, respectively, for the solution of different concentrations of the tested antioxidant in a fixed amount of DPPH in order to estimate the 50% inactivation concentration (IC_{50}) (Figure 6).

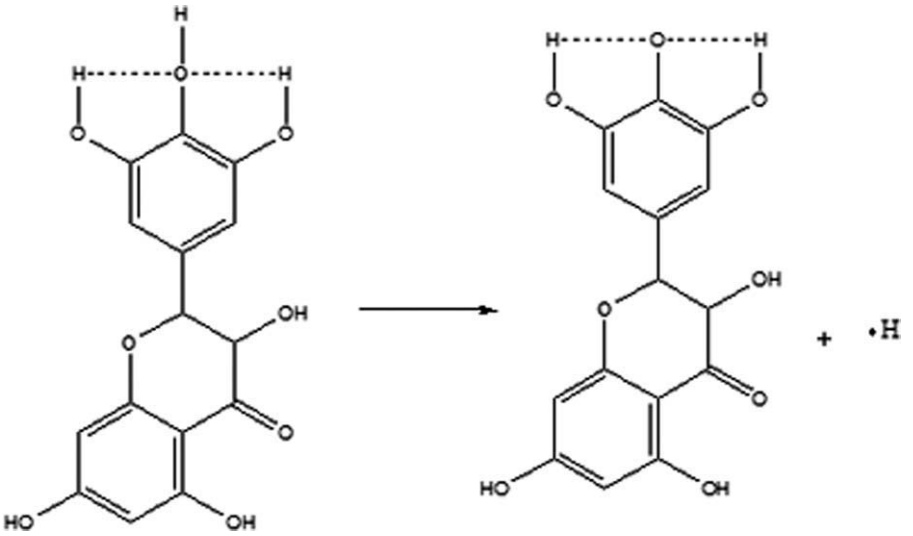
By using a five-parameter logistic equation,[14] the IC_{50} values were estimated to be 5.4 μ*M* for DMY and 5.2 μ*M* for PA revealing a reduction of 4.4 mole of DPPH per DMY mole and 4.5 mole of DPPH per PA mole. For getting the reaction kinetics of both DMY and PA with DPPH, the absorbance values at 516 nm were monitored at different time with solution containing 3.0×10^{-7} mol of the tested antioxidant and 1.1×10^{-7} mol of DPPH (Figure 7).

As shown in Figure 7, the reaction of both DMY and PA with DPPH was completed after 30 min of incubation. After an overnight incubation, the steady state of the reaction of both DMY/DPPH and PA/DPPH is achieved in the UV–Vis spectra (Figures 8 and 9). Moreover, it can be noted that the reactivity of DMY reacted with DPPH is a relatively similar to PA.

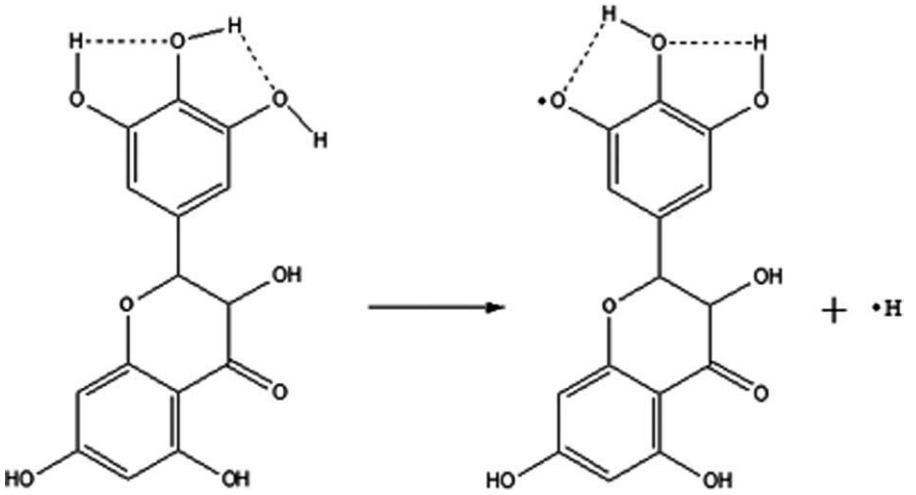
As increasing the antioxidant moles, the steady state progressively decrease in absorbance value at 516 nm. It is notable that reduction of the absorbance at 516 nm for DMY is same as PA.

Table V. DPPH Radical Scavenging Abilities of DMY and Polyphenols

Compound	DPPH : compound
DMY	4.4 : 1
Pyrogallic (PA)	4.5 : 1
	4.9 : 1[24]
Chrysin	0[24]
Apigenin	0[24]
Naringenin	0[14]



Scheme 1. Proposed mechanism for DMY by donation the center hydrogen atom.



Scheme 2. Proposed mechanism for DMY by donation the two sides hydrogen atom.

Comparing DPPH radical scavenging abilities of DMY and PA with polyphenols of chrysin, apigenin, and naringenin in the Table V, it is confirmed that the ortho-trihydroxyl groups in ring B play important role in the scavenger activity.

Schemes 1 and 2 are assumed for explaining the result. Two of hydrogen in three O—H (in the center or two sides) of the ortho-trihydroxyl groups in ring B form two hydrogen bonds. A remainder easily loses a hydrogen atom to form a radical as the radical share the electron in two H-bonds. It is also possible to explain why DMY has high antioxidant activity, in which hydroxyl groups in 3′and 5′position in DMY should have antioxidant activity besides the hydroxyl group in 4′.[25]

CONCLUSIONS

The antioxidant activity of the DMY depended on the pH values. DMY treated with sodium hydroxide had a negative effect on the OIT of EVA and recovery with the decrease of pH value. The hydroxyl group on the 7-position (A ring) formed ionic bond of negative oxygen ion in alkali condition. Through the kinetics of the reaction between the antioxidant and the DPPH radical, it is confirmed that the ortho-trihydroxyl group in B ring might play a key role in radical scavenging activity. On the base of the result, the mechanism of radical scavenging activity of DMY was proposed.

ACKNOWLEDGMENTS

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