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ARTICLE

Photo-oxidation of some flavonoids with photochemically generated *t*-BuO• radicals in a *t*-BuOH water system using a kinetic approach

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As a dietary component, flavonoids are thought to have a variety of pharmacological and health-promoting properties in both in vivo and in vitro systems. This variety is attributed to their high antioxidant capacity, which in turn is associated with their free radical scavenging properties. In order to understand the mechanism of *t*-BuO• radical scavenging properties of some flavonoids, viz, quercetin (QU), apigenin (AP), daidzein (DA), genistein (GE), myricetin (MY), and kaempferol (KA), a kinetic study of photo-oxidation of these flavonoids with photochemically generated *t*-BuO• radicals in a *t*-BuOH water (2:1 v/v) system was carried out. The oxidation of flavonoids by *t*-BuO• radicals was performed spectrophotometrically by measuring the absorbance of quercetin (375 nm), apigenin (340 nm), daidzein (250 nm), genistein (263 nm), myricetin (255 nm), and kaempferol (375 nm) at their respective λ_{max}. The initial rates of oxidation of flavonoids increased with [flavonoid], [*t*-BuOOH], and light intensity. The quantum yields (φ) were considered from the initial rate of oxidation of flavonoids by *t*-BuO• radicals and measured light intensity at 254 nm. The order on [flavonoid] and [*t*-BuOOH] was found to be fractional, whereas the order on light intensity was found to be one. The products of the oxidation of flavonoids by *t*-BuO• radicals were identified using LC–MS and FTIR analysis.

KEYWORDS
flavonoids, oxidation products, photo-oxidation, quantum yields, t-BuO• radicals

1 | INTRODUCTION

Flavonoids are a large group of polyphenolic compounds with a benzo-γ-pyrone structure. Structurally, flavonoids are based on a 15-carbon skeleton consisting of two benzene rings (A and B) linked via a heterocyclic pyrane ring (C) (Figure 1). Structural variations[1] within the rings subdivide the flavonoids into several families, viz, flavonols, flavones, flavanols, isoflavones, catechins, anthocynidins, etc. A multitude of studies has suggested protective effects of flavonoids against many infectious (bacterial and viral diseases) and degenerative diseases such as cardiovascular diseases, cancers, and other age-related diseases.[2,3] Recent interest in these substances has been stimulated by the medical efficacies arising from the antioxidant activities of these polyphenolic compounds.[4–6]

The chemical properties of flavonoids depend on their structure, the degree of hydroxylation, other substitutions and conjugations, and the degree of polymerization.[3] It was suggested that the configuration, substitution, and an overall number of hydroxyl groups noticeably affect some of the mechanisms of antioxidant activity, such as radical scavenging and metal ion chelation ability.[7] The B ring hydroxyl configuration is the greatest significant contributing factor of

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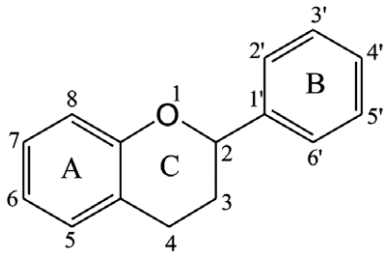

FIGURE 1 General structure of flavonoids

scavenging of reactive nitrogen species (RNS) and reactive oxygen species (ROS) because of its donation of hydrogen and an electron to hydroxyl, peroxyl, and peroxynitrite radicals, and the subsequent radicals produced can be delocalized over the flavonoid structure.[8] Various mechanisms of antioxidant action by flavonoids were suggested, of which scavenging ROS is considered one of the most prominent.

Flavonoids that are powerful antioxidants possess lower redox potentials and thus are thermodynamically able to reduce highly oxidizing free radicals, such as superoxide, peroxyl, alkoxyl, and hydroxyl radicals, mainly by hydrogen atom donation.[9,10] Manifestation, position, structure, and a total number of sugar moieties in flavonoids (flavonoids glycosides) play a major role in antioxidant activity. The mechanisms accountable for these effects are not clear but appear to be related to their antioxidant effect and ability to scavenge free radicals, which requires further investigation.

Alkoxyl radicals are produced on UV-A irradiation or metal-catalyzed decomposition of lipid hydroperoxides.[11,12] They have been reported to induce strand breaks in supercoiled pBR322 DNA[13] and may play a significant role in the tumor-promoting activity of peroxides.[14] Strand break formation and base oxidation have been observed as necessary modifications induced by the alkoxyl radicals in DNA damage.[15] *Tert-B*utyl hydroperoxide (*t*-BHP) is a recognized oxidant that has been used as a model oxygen-centered radical for the study of mechanisms of oxidative cell injury in mammalian cells and to investigate mechanisms of its interaction with polyphenols.[16,17] These free radicals readily cross cellular membranes and induct lipid peroxidation, damage protein

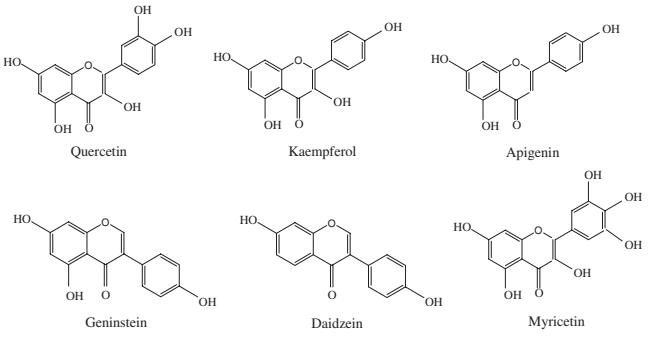

FIGURE 2 Structures of flavonoids studied

and DNA, affect cell membrane integrity, and result in cell injury in hepatocytes and rat liver.[18,19] Previous studies on the reactivity of *tert*-butoxyl radicals propose that these species mutually influence the anticipated attack on the base and the sugar moieties of DNA.[20,21] However, not many studies are available highlighting the alkoxyl (*t*-BuO$^\bullet$ radicals) radical scavenging activities of flavonoids. It is in this background that we have chosen *tert*-Butyl hydroperoxide (*t*-BuOOH) as the model peroxide in the present study, which results in *t*-BuO$^\bullet$ and $^\bullet$OH radicals on homolysis.

Although many structure–activity relationship (SAR) investigations have been performed on the antioxidant activity of flavonoids, only a few studies are available that focus on the kinetic model to assess the *t*-BuO$^\bullet$ radical scavenging abilities of flavonoids. Thus, a systematic mechanistic investigation has been conducted to focus on the interaction of some flavonoids (Figure 2), viz, quercetin (QU), apigenin (AP), daidzein (DA), genistein (GE), myricetin (MY), and kaempferol (KA), with *t*-BuO$^\bullet$ radicals using a kinetic approach. Particular attention is paid to SAR studies of six flavonoids regarding their chemical structure and radical scavenging properties. An effort has also been made to suggest a likely mechanism for oxidation of quercetin by *t*-BuO$^\bullet$ radicals based on the kinetic results and the products identified using LC–MS and FTIR analysis. Such studies have immense significance in understanding the molecular mechanisms underlying the synergistic interactions and help in exploring the possible role of flavonoids in cooperative (synergistic) interactions with other phytochemicals.

2 | MATERIALS AND METHODS

Quercetin, apigenin, daidzein, genistein, myricetin, and kaempferol were purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, and used as received. All solutions were prepared fresh using double-distilled water. *t*-BuOOH was used as received from Merck-Schuchardt of Germany and was estimated through the iodometric method.[22] The irradiations were performed at ambient temperature in a quantum yield reactor model QYR-20 supplied by Photophysics, England, attached to a 400 W, medium-pressure, mercury lamp. The quartz cuvette containing the sample was irradiated, the irradiations were interrupted at specific intervals of time, and the absorbance was noted. The light intensity equivalent to the irradiating wavelength (254 nm) was measured using peroxydisulphate chemical actinometry.[23] On photolysis, *t*-BuOOH was triggered at 254 nm to make OH and *t*-BuO radicals through the homolytic cleavage of –O–O– bond. The $^\bullet$OH radicals produced were scavenged by a sufficient concentration of *t*-BuOH present in the solvent mixture.[24]

The kinetic path of the reaction mixture enclosing flavonoid and *t*-BuOOH in *t*-BuOH–water (2:1 v/v) ratio, taken in

a specially designed path length of the quartz cuvette, is 1 cm, proper for both irradiations and absorbance determinations. The reaction mixture was purged with N_2 before irradiation. The absorbance measurements were taken at the $_{max}$ of flavonoid on a Systronics UV–Visible Double-beam spectrophotometer (model 2202). It is known that *t*-BuOOH is activated by the radical reaction of the absorption of light at 254 nm. However, some of the substrates may have strong absorption in this region. However, in the absence of *t*-BuOOH in the reaction mixture, they did not undergo any noticeable chemical change on shining the light. The light intensity at 254 nm was used to determine the quantum yields of oxidation of antioxidants under different experimental conditions. The initial rate of photo-oxidation of the flavonoid by *t*-BuOOH in a solvent mixture *t*-BuOH–water (2:1 v/v) ratio was calculated from the plots of absorbance versus time using the Microcal Origin (version 6.0) software program. The products of oxidation of flavonoids by *t*-BuO radicals were identified using Liquid Chromatography-Mass Spectrometry (LC–MS) and Fourier Transform Infrared (FTIR) analysis. For the product analysis, about 0.01 M of flavonoid solution in *t*-BuOH–water (2:1 v/v) ratio added to 0.1 M *t*-BuOOH solution was taken in a quartz cuvette and irradiated for 48 hr. The resultant solution was used for FTIR analysis and LC–MS.

The FTIR Spectrophotometer (Shimadzu IR Prestige21 Instrument) was used to record the FTIR spectra of a photo-oxidized mixture of the flavonoid with the *t*-BuOOH solution through the KBr pellet method. The scan was carried out in the range of 250–4000 cm^{-1}. LC–MS spectra of the samples were recorded using Shimadzu on column Ascentis Express C-18 (50 × 3.0 mm, 2.7 um), a mobile phase of acetonitrile +5% 0.025% aqueous trifluoroacetic acid with a flow rate of 1.2 mL/min (Gradient) connected to MS, with the ESI analyzer in positive/negative ion mode.

3 | RESULTS AND DISCUSSION

Flavonoids and their glycosides were found to perform as hydrophilic antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellents, and as UV-light filters and substrate for polyphenol oxidases. It is reported that flavonoids might account for a reasonable part of the health benefits associated with vegetable and fruit consumption.[25,26] The antioxidant nature of flavonoids is attributed to the effective ROS scavenging of radicals. This is due to their low redox potentials ($0.2 < E_o < 0.8$) depending on the arrangement of functional groups about the nuclear structure.[27,28] The free radical scavenging potential of flavonoids has been related to the stability of their radical species and is expected to increase by extended conjugation. Antioxidant capacity assays have mainly classified flavonoids as scavenging by electron transfer (ET) or by hydrogen atom transfer (HAT).[29]

The photo-oxidation of flavonoids by *t*-$BuO^{\bullet}$ radicals was carried out under different experimental conditions. The initial rates of oxidation of flavonoids were found to increase with an increase in [flavonoid], [*t*-BuOOH], and light strength in all the cases and are given in Tables 1 and 2. The quantum yields (φ) were estimated from the initial rate of oxidation of flavonoids by *t*-$BuO^{\bullet}$ radicals and measured light intensity at 254 nm, the wavelength at which *t*-BuOOH was activated to give *t*-$BuO^{\bullet}$ and $^{\bullet}OH$ radicals. The quantum yields of all the flavonoids were found to depend on [flavonoid] and [*t*-BuOOH] and were independent of light intensity and are given in Tables 3 and 4. The order on [flavonoid] and [*t*-BuOOH] was found to be fractional, whereas the order on light intensity was found to be one. The absorption spectra for the photo-oxidation of the flavonoids studied with *t*-$BuO^{\bullet}$ radicals at different time intervals are given in Figure 3.

TABLE 1 Effect of [flavonoid] and [*t*-BuOOH] on the rate of oxidation of flavonoid by *t*-$BuO^{\bullet}$ radicals in *t*-BuOH–water (2:1 v/v) system. Light intensity = 5.7549×10^{15} quanta/s, pH ~ 7.5, room temperature = 298 K

Concentration		Rate × 10^9 dm^3 mol^{-1} s^{-1}					
[Flavonoid] × 10^6 mol/dm^3	[*t*-BuOOH] × 10^3 mol/dm^3	MY	QU	KA	AP	GE	DA
1.0	5.0	—	11.944	11.178	—	0.3274	0.4150
2.0	5.0	0.5069	16.527	15.921	0.2807	0.3741	0.4603
3.0	5.0	—	19.097	18.834	—	—	—
5.0	5.0	1.0138	27.083	26.578	0.5526	0.6055	0.6238
8.0	5.0	1.7778	—	32.655	0.8157	0.9330	1.1507
10.0	5.0	2.9166	42.632	39.837	1.2543	1.9333	2.0079
20.0	5.0	6.1458	50.000	49.322	2.0438	2.3505	2.6984
40.0	5.0	12.826	—	—	3.3508	—	—
1.0	1.0	—	5.5556	—	—	—	—
1.0	2.0	—	9.027	—	—	—	—
1.0	10.0	—	22.564	—	—	—	—
10.0	10.0	5.3889	—	47.967	2.0350	2.1620	4.3650
10.0	15.0	6.6944	—	57.310	2.2807	2.4851	5.7143

TABLE 2 Effect of light intensity on the rate of oxidation of flavonoid by *t*-BuO$^{\bullet}$ radicals in *t*-BuOH–water (2:1 v/v) system. [*t*-BuOOH] = 5.0×10^{-3} mol/dm^3, [flavonoid] = 10.0×10^{-6} mol/dm^3 and [QU] = 1.0×10^{-6} mol/dm^3, pH ~ 7.5, room temperature = 298 K

Light intensity × 10^{-15} quanta/s	Rate × 10^9 dm^3 mol^{-1} s^{-1}					
	MY	QU	KA	AP	GE	DA
8.4379	5.0000	12.500	49.783	2.5614	2.6959	2.7778
5.7549	2.9166	11.944	39.837	1.2543	1.9333	2.0079
3.2375	1.7778	10.069	28.945	0.7894	1.0003	1.0317

Previous studies on the reactions of flavonoids with free radicals have correlated the number and position of hydroxyl groups and the extension of conjugation to the efficiency of flavonoids as antioxidants.[27–30] Three structural requirements were found to be important: (a) the *ortho*-dihydroxy (catechol) structure in the B ring; (b) the 2,3-double bond, in conjugation with the 4-oxo function; and (c) the presence of both 3- and 5-OH groups. Apart from these structural requirements, the number and position of hydroxyl substituents on the flavonoid molecule, the existence of glycosides, and the overall degree of conjugation are important in determining their activities. A common flavonoid that meets the above three criteria is QU, showing the highest antioxidant capacity. Based on a low dipole moment, quantum mechanical calculations and a significant deviation from the A-to-B dihedral angle are necessary for high antioxidant efficiency for flavonoids.[31] It has been indicated that the hydroxylation of ring B is the most important feature for the activity as it stabilizes the radical formed at the ring B. If there are no hydroxyl groups on ring B, then the hydroxylation of ring A becomes important.[2,32,33]

Some of the SAR studies on polyphenolic compounds indicated that QU has a higher antioxidant capacity compared to the other polyphenols.[34–36] Yang et al.[37]studied the antioxidant activities of flavonoids against lard oil oxidation using the Rancimat test. Based on the calculated protection factor (PF) values, the order of antioxidant activity was found to be QU > KA > DA ~ GE > AP. The antioxidant performance of flavonoids was explained by both ΔH_f (variance in the heat of formation of the phenolic antioxidant and its free radical produced after H-abstraction) and the several numbers of hydroxyl groups in the conjugated B and C ring system. Burda and Olezek[38] studied antioxidant and antiradical activities of flavonoids and found similar results wherein QU and KA showed higher antioxidant activity. Chung et al.[39] also predicted similar results from their DPPH free radical and inhibition of TPA-induced free radical formation with cultured HL-60 cells studies. Jovanovic et al.[27]proposed that the desirable electron-donating properties originate from the electron-donating hydroxy group in the C ring, which is conjugated to the catechol (B ring) radical through the 2,3-double bond. The kinetic results of Lin et al.[40] have indicated that the conjugation between the pyrone and catechol rings play an important role in the reactivity of flavonoids. However, the effectiveness of an antioxidant is determined by

TABLE 3 Effect of [flavonoid] and [*t*-BuOOH] on the quantum yield of oxidation of flavonoid by *t*-BuO$^{\bullet}$ radicals in *t*-BuOH–water (2:1 v/v) system. Light intensity = 5.7549×10^{15} quanta/s, pH ~ 7.5, room temperature = 298 K

Concentration		Quantum yield (φ)					
[Flavonoid] × 10^6 mol/dm^3	[*t*-BuOOH] × 10^3 mol/dm^3	MY	QU	KA	AP	GE	DA
1.0	5.0	—	0.00375	0.00350	—	0.00010	0.00013
2.0	5.0	0.00015	0.00518	0.00499	0.00008	0.00012	0.00014
3.0	5.0	—	0.00599	0.00591	—	—	—
5.0	5.0	0.00031	0.00850	0.00834	0.00017	0.00019	0.00019
8.0	5.0	0.00055	—	0.01025	0.00025	0.00029	0.00036
10.0	5.0	0.00091	0.01338	0.01250	0.00045	0.00060	0.00063
20.0	5.0	0.00192	0.01569	0.01548	0.00064	0.00073	0.00084
40.0	5.0	0.00402	—	—	0.00105	—	—
1.0	1.0	—	0.00174	—	—	—	—
1.0	2.0	—	0.00283	—	—	—	—
1.0	10.0	—	0.00708	—	—	—	—
10.0	10.0	0.00169	—	0.01506	0.00063	0.00067	0.00137
10.0	15.0	0.00210	—	0.01799	0.00071	0.00078	0.00179

TABLE 4 Effect of light intensity on the rate of oxidation of flavonoid by *t*-BuO$^{\bullet}$ radicals in *t*-BuOH-water (2:1 v/v) system. [*t*-BuOOH] = 5.0×10^{-3} mol/dm^3, [flavonoid] = 10.0×10^{-6} mol/dm^3 and [QU] = 1.0×10^{-6} mol/dm^3, pH ~ 7.5, room temperature = 298 K

Light intensity × 10^{-15} quanta/s	Quantum yield (ϕ)					
	MY	QU	KA	AP	GE	DA
8.4379	0.00107	0.00392	0.01066	0.00054	0.00057	0.00059
5.7549	0.00091	0.00375	0.01250	0.00045	0.00060	0.00063
3.2375	0.00099	0.00314	0.01615	0.00044	0.00055	0.00057

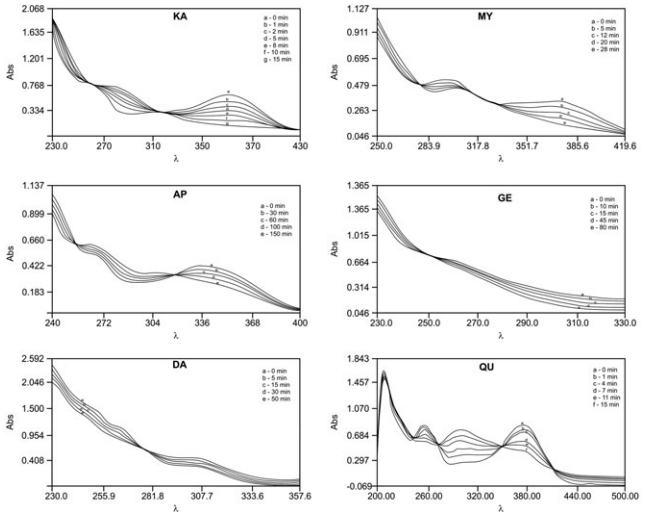

FIGURE 3 Absorption spectra of photo-oxidation of six flavonoids in the presence of *t*-BuOOH at different irradiation times in *t*-BuOH–water (2:1 v/v) medium at their corresponding λ_{max}[flavonoid] = 2.0×10^{-5} mol/dm^3, [*t*-BuOOH] = 5.0×10^{-3} mol/dm^3, pH 7.5, room temperature = 298 K, light intensity = 5.7549×10^{15} quanta/s. KA, kaempferol; MY, myricetin; AP, apigenin; GE, genistein; DA, daidzein and QU, quercetin

many factors, including its activation energy, rate constant, oxidation-reduction potential, bond dissociation energies (BDE), the stability of the radical intermediate, thermal stability, and hydrophobicity. In the present kinetic study, the order of the reactivity of flavonoids with *t*-BuO radicals was found to be QU > KA > MY > DA > GE > AP.

QU, a plant-derived aglycone form of flavonoid glycosides, satisfies all the structural features required to show powerful free radical scavenging ability and hence counteracts oxidative stress generated as a result of ROS, which contributes to the genesis of atherosclerosis, nervous disorders, obesity, etc.[41] In our kinetic study, QU was found to have the highest antioxidant property among all the six flavonoids studied.

KA is a flavonoid found in many edible plants and is commonly used in traditional medicine. Numerous preclinical studies have shown that KA has a broad variety of pharmacological activities, including antioxidant, antimicrobial, anticancer, cardioprotective, anti-osteoporotic, and antiallergic activities.[42,43] KA was found to demonstrate slightly less antioxidant activity toward *t*-BuO$^\bullet$ radicals compared to QU but was higher than other flavonoids in our present study. One of the possible reasons could be the presence of the 4-OH group in B ring instead of the catechol structure present in QU, which might have decreased its antioxidant power toward scavenging *t*-BuO radicals.

Compared to QU, MY is found in less common foods, viz, black currants, black grapes, cranberries, broad beans, etc, and is consequently consumed to a lesser extent. Most studies on the antioxidant activity of MY have shown that it is more efficient than QU as an antioxidant in oils, emulsions, and LDL oxidation, whereas the reverse order has also been found for oxidation in emulsions and few other investigations.[44–47] In the present study, MY showed antioxidant activity toward *t*-BuO radicals that was higher than AP but lower than other flavonoids studied. MY has the largest number of phenolic OH groups among all the six flavonoids studied but exhibits poor activity in scavenging *t*-BuO$^\bullet$ radicals. According to Rice-Evans et al., the presence of a third OH group in the B ring situated in *ortho* position to other OH groups in the B ring, as in MY does, not improve the effectiveness of flavonoids against aqueous phase radicals.[6]

GE and DA are the two most important isoflavones contained in about equal quantities in soy and are phytoestrogens. Accumulating evidence[48–51] from in vivo studies suggests that isoflavones may have an impact on cardiovascular diseases, cancers, and osteoporosis and that their antioxidant activity might be responsible for their protective effects. GE and DA were found to have comparable *t*-BuO$^\bullet$ radical scavenging capacity in the present study. Similar results were also reported by Foti et al.[52], indicating that DA was just as effective as GE against induced oxidative damage in Jurkat T-cell line and peripheral blood lymphocytes of healthy subjects. This may be due to the structural similarities between DA and GE, the presence of phenolic moiety at 5′ position of the B ring instead of 4′ position as observed in other flavonoids studied.[31,34] Another notable feature that can be observed is that the presence of 5-OH group on the A ring did not affect the antioxidant capacity of GE.

AP is one of the active ingredients found in fruits and vegetables and an outstanding food functional factor and potential therapeutic drug for some diseases.[53] It has been widely investigated for its anti-inflammatory effects, free radical scavenging effects, and growth inhibitory properties in several cancer lines.[54,55] Recent clinical studies demonstrated that the AP intake was associated with a suggestive decrease in woman's risk of ovarian cancer.[56] The least *t*-BuO$^\bullet$ radicals scavenging activity was observed for AP in the present study. Two hydroxyl groups in the B ring are required to obtain a stronger antioxidant potential; a lack of one of them in AP significantly reduced this activity. Similar results were obtained by Majewska et al.,[57] who evaluated the free radical scavenging activity of flavonoids through their ability to quench the synthetic 2,2- diphenyl-1-picrylhydrazyl (DPPH) radical and found that AP had the weakest radical scavenging potential against DPPH.

Flavonoids, in general, undergo photo-oxidation, with *t*-BuO$^\bullet$ radicals producing the corresponding semiquinone radical (phenoxyl radical), which combined with phenoxy or other radicals available in the medium to give either dimer or other stable final products. An analysis of the potential products of oxidation of flavonoids by *t*-BuO$^\bullet$ radicals was carried out using FTIR analysis and LC–MS of the irradiated reaction mixture. In the FTIR spectra of all the flavonoids, two strong peaks were observed: one in the region of

1600–1700 cm^{-1}, indicating the presence of carbonyl group formed due to the oxidation of –OH group to semiquinone or a quinone, and a broad peak in the region of 3000–3500 cm^{-1}, representing H-bonding of the phenolic groups of the polyphenols studied. The FTIR spectrum of the photo-oxidation of QU, as a representative compound, has been given in Figure 4, showing these peaks and clearly indicating the formation of corresponding quinones as one of the possible oxidation products. The LC–MS chromatogram of the oxidized mixture of the flavonoids have indicated the formation of the numerous oxidation products at different retention times (RT), which has made the analysis more complicated, but attempts were made to identify few oxidation products from the complex mixture for QU as given in Figure 5.

The various classes of flavonoids were found to change in the level of oxidation and arrangement of substitution of the C ring, while specific compounds within a class vary in the pattern of substitution of the A and B rings.[1,2] Oxidation studies of quercetin, which is one of the most commonly found flavonoids in plant extracts, is well documented. Few studies have investigated the degradation of quercetin in the dark, when dissolved in an alkaline medium, treated with radical generators, or electrochemically oxidized.[58–60] Zare et al.[61] studied electrochemical behavior of quercetin using cyclic voltammetry, chronoamperometry, rotating disk electrode voltammetry, and quantum mechanical calculations and suggested that the *o*-quinone derived from quercetin participates in Michael addition reaction with quercetin and produces a dimer.

A significant amount of literature[62,63] is available on oxidation of flavonoids by radicals, and a kinetic approach has been used here to investigate the oxidation of flavonoids by *t*-BuO$^{\bullet}$ radicals. In the present study, the phenoxyl (semiquinone) radical of QU was observed in LC–MS at an RT of 2.467–2.533 min by the loss of proton from 4'-OH of the B ring, which further loses another proton from 3'-OH and gives quinone with *m/z* of 300.8 at an RT of 2.567–2.617 min in

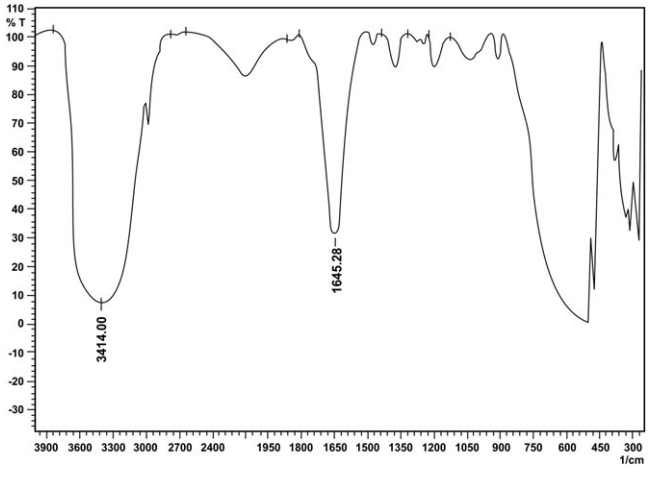

FIGURE 4 FTIR of QU photo-oxidized by *t*-BuO$^{\bullet}$ radicals in *t*-BuOH–water (2:1 v/v) ratio

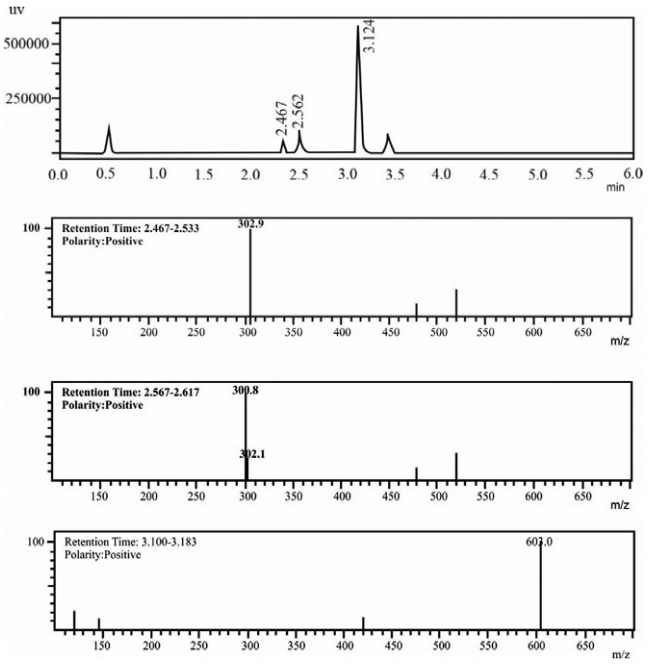

FIGURE 5 LC–MS of QU photo-oxidized by *t*-BuO$^{\bullet}$ radicals in *t*-BuOH–water (2:1 v/v) ratio

the positive ion mode. The increase in the absorbance at 305 nm in the UV spectrum may be attributed to the quinone formed (Figure 3). Previous studies on the oxidation of QU indicated the formation of quinone in which the two –OH groups in ring B are readily oxidized.[64,65] It has recently been reported that polyphenol oxidase (PPO) and mushroom tyrosinase can oxidize QU to four corresponding tautomeric forms of quinones. Timbola et al.[66] studied oxidation of QU by cyclic voltammetry, controlled-potential electrolysis, and UV–Vis spectroscopy. The two-electron, two-proton oxidation process of QU at the first peak directed to the materialization of the conforming ortho-quinone, which is an electrochemically active and unstable species. Xu et al.,[67] in their in situ spectroelectrochemical study of QU oxidation, reported the formation of four possible quinones and their structures. The differential pulse voltammograms of QU showed that the first oxidation peak, with peak potential $E_p = +0.30$ V, corresponds to the loss of two electrons and two protons from ring B, a reversible process giving o-quinone species, which can undergo rearrangements to give other products. About 32% (relative amount) of a dimer of QU was detected at an RT of 3.100–3.183 min with *m/z* of 603 in positive ion mode, for which the possible mechanism is discussed in Figure 6. Chervyakovsky et al.[68] also indicated the formation of dimer as one of the major products in the oligomeric oxidation of QU due to the possible random cyclization of QU leading to dimers and trimers. Es-Safi et al.[69] have studied the enzymatic oxidation of flavonols and indicated the formation of dimers for a few of them through mass spectral analysis and reported that QU allowed the formation of two pseudodimeric compounds at *m/z* 498 and 618. Krishnamachari et al.[58] also indicated the

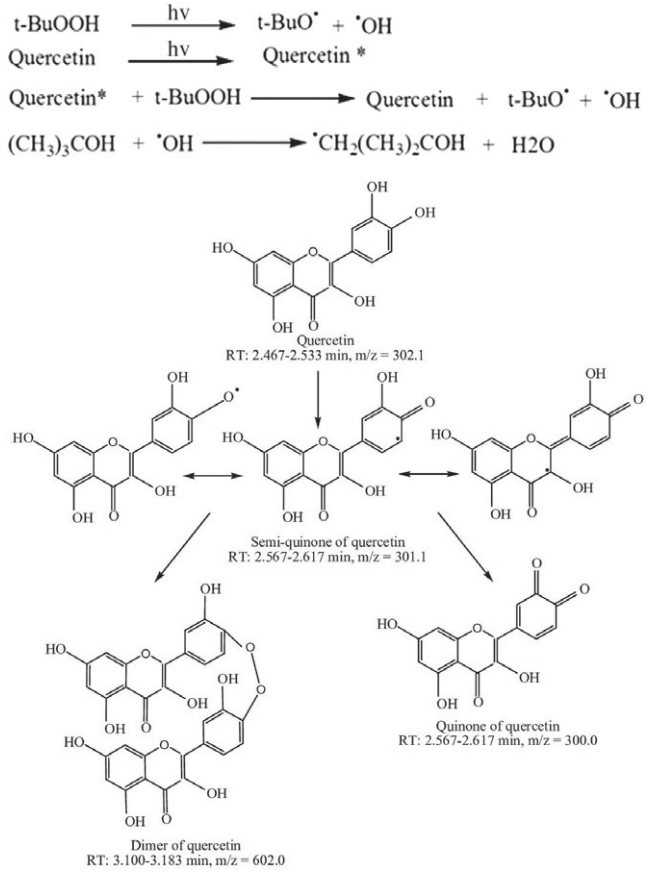

FIGURE 6 Reaction scheme for the oxidation of QU by photochemically generated *t*-BuO• radicals showing one of the possible quinone and dimer as oxidation products

formation of the dimeric product along with other oxidation products in the oxidation of flavonoids by the peroxyl radical generator 2,2′-azobis-isobutyronitrile (AIBN), which were identified by chromatographic techniques and acknowledged by NMR and MS analyses. At RTs of 2.467–2.533 and 2.567–2.617, the two peaks observed in the range of 450–550 *m/z* values indicate the formation of possible dimers (Figure 5).

4 | CONCLUSIONS

Flavonoids are a broad class of secondary metabolites that have been effectively found to exhibit various biological and pharmacological properties. The unwarranted availability of flavonoids in everyday food as well as their multitude of potentially beneficial effects has stimulated a renewed interest in understanding the complex molecular mechanisms underlying these effects. The polyphenolic nature of flavonoids enables them to act as antioxidants by scavenging free radicals by the donation of hydrogen to reactive species. The present study indicated that the order of the reactivity with *t*-BuO• radicals is QU > KA > MY > DA > GE > AP. Hence, QU can be considered one of the powerful antioxidants, while AP has been found to have the least antioxidant nature toward *t*-BuO• radicals. The product analysis showed the formation of phenoxyl radical such as semiquinone for all the flavonoids studied, which may further combine with other species available to form a dimer or other oxidation products. The formation of an intramolecular hydrogen bond between hydroxyl groups of flavonoids increases the stability of the phenoxyl radicals. The fate of the phenoxyl radicals depends on their structure and the presence of species affecting the stability of the free radicals. However, it is tough to clearly establish the pathway for the formation of these comprehensive oxidation products of flavonoids because an antioxidant decreases the oxidative stress at low concentrations. As a consequence, further research should be performed to convincingly address the importance of flavonoids as free radical scavengers. Such studies will also throw light on the understanding of synergistic interactions of these molecules with other phytochemicals forming an integral part of the diet.

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