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# BINARY MIXTURES OF QUERCETIN WITH SOME PHENOLIC ACIDS IN *TERT*-BUTANOL-WATER SYSTEM: STUDY OF *TERT*-BUTOXYL RADICAL MEDIATED SYNERGISTIC INTERACTION THROUGH KINETIC APPROACH

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## ABSTRACT

An upsurge in the number of studies on flavonoids has been stimulated by the potential health benefits arising from the antioxidant activities of these polyphenolic compounds. Functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions. The rates of oxidation of quercetin (QU) by *t*-BuO$^{\bullet}$ radicals in the presence of phenolic acids *viz.*, chlorogenic acid (CGA), caffeic acid (CA), rosmarinic acid (RA) and gallic acid (GA) have been studied by measuring the absorbance at suitable $\lambda_{max}$ spectrophotometrically. *tert*-butoxyl (*t*-BuO$^{\bullet}$) radicals were generated by the photolysis of *tert*-butyl hydroperoxide (*t*-BuOOH) in *t*-butanol-water (2:1 v/v) to scavenge $^{\bullet}$OH radicals. From competition kinetics, the rate constant of *t*-BuO$^{\bullet}$ radical reaction with QU ($k_{Qu}$) has been determined to be $2.62 \times 10^9$ $dm^3mol^{-1}$ $s^{-1}$. The quantum yields ($\phi_{expt}$) have been calculated from the experimentally determined rates of oxidation of QU/phenolic acids under different experimental conditions. The results indicated that QU showed synergistic/antagonistic interaction with polyphenols. CGA repaired QU to an extent of ~45% whereas QU was found to repair CA, RA and GA to an extent of ~96%, ~81% and ~42% respectively.

**KEYWORDS:** quercetin, phenolic acids, *t*-BuO$^{\bullet}$ radicals, synergistic interaction, repair reactions.

## INTRODUCTION

Plant phenolics are said to be multifunctional antioxidants and act at several levels in the oxidative sequence. They are widely distributed in the plant kingdom and are therefore an integral part of the diet.[1,2] They exhibit a wide range of biological effects including antibacterial, anti-inflammatory, anti-allergic, hepatoprotective, anti-thrombotic, antiviral, anti-carcinogenic and vasodilatory actionsn.[3,4] The multiple potential mechanisms by which the antioxidants act make the diverse group of phenolic compounds, an interesting target in the search of health-beneficial phytochemicals and also extends the shelf life of lipid-rich foods. Recently, attention has been focused on phytochemicals having cancer-preventive properties to understand their modes of antioxidant activity[5] and molecular mechanisms underlying it. Scavenging of Reactive Oxygen Species (ROS) by polyphenols is the generally accepted mechanism of their antioxidant activity. These compounds have been proposed as potential preservatives in food industry to avoid chemical preservatives.[6,7]

The protective effects of the antioxidant constituents of fruits and vegetables have been attributed due to the cooperative interactions among the phytochemicals such as carotenoids, vitamins C and E, flavonoids, etc present together in them.[8] Interaction among antioxidants can be synergistic, antagonistic or merely additive. Strong synergistic activity has also been observed in the mixtures of natural tocopherols and citric acid. α-Tocopherol and β-carotene in combination were found to provide a higher antioxidative capacity in a membrane system than β-carotene or α-tocopherol alone. Due to its low reduction potential, ascorbate is reported to regenerate flavonoids from their aroxyl or semiquinone radicals thus maintaining radical scavenging activities of the phenols.[8] Conversely, flavonoids may exert sparing effects of vitamin E by oxidizing the tocopheroyl radical.[9] Polyphenolic compounds such as quercetin showed additive effects on free radical scavenging activity with ascorbic acid or α-tocopherol.

Flavonoids are a group of natural compounds based upon a fifteen-carbon skeleton consisting of two benzene rings (Fig.1) linked *via* a heterocyclic pyrane ring (C). The antioxidant activity of flavonoids depends upon the arrangement of functional groups about the nuclear structure. The configuration, substitution, and total number of hydroxyl groups substantially influence several mechanisms of antioxidant activity such as radical scavenging and metal ion chelation ability.[10,11]

The B ring hydroxyl configuration is the most significant determinant of scavenging of ROS and RNS because it donates hydrogen and an electron to hydroxyl, peroxyl, and peroxynitrite radicals, stabilizing them and giving rise to a relatively stable flavonoids radical.[12] A number of studies have suggested protective effects of quercetin (QU) against many infectious (bacterial and viral diseases) and degenerative diseases such as cardiovascular diseases, cancers, and other age-related diseases.[13,14] Due to low redox potentials, QU is thermodynamically able to reduce highly oxidizing free radicals such as superoxide, peroxyl, alkoxyl, and hydroxyl radicals by hydrogen atom donation. It has redox properties which allow it to act as reducing agents, hydrogen donators, singlet oxygen quenchers along with metal chelation properties.[15]

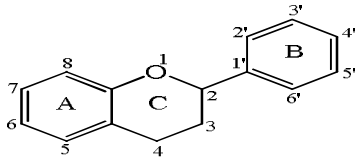



Fig. 1. General structure of flavonoids

There remains a great interest in the possible health promoting effects of antioxidants, but the mechanism (synergy/antagonism) by which these compounds coexist in foods is in need of further study. The regeneration effect of one antioxidant by another antioxidant is a potentially beneficial reaction that needs to be further studied in human and animal models. *tert*-butyl hydroperoxide (*t*-BuOOH), a well-known oxidant, has been used as a model oxygen-centered radical for the present study to investigate mechanisms of its interaction with QU in the presence of polyphenols. Although there are several reports available on such interactions among the antioxidants have been reported, a systematic kinetic approach to understand cooperative mechanisms among the antioxidants is not available. It is in this context that a competitive kinetic study of interaction of QU with antioxidants *viz.*, CGA, CA, RA and GA (Fig. 2) in the presence of *t*-BuO$^{\bullet}$ radicals was carried out to get an insight into the possible synergistic/antagonistic molecular mechanisms which help in selection of co-antioxidants while adding to the food preservatives.

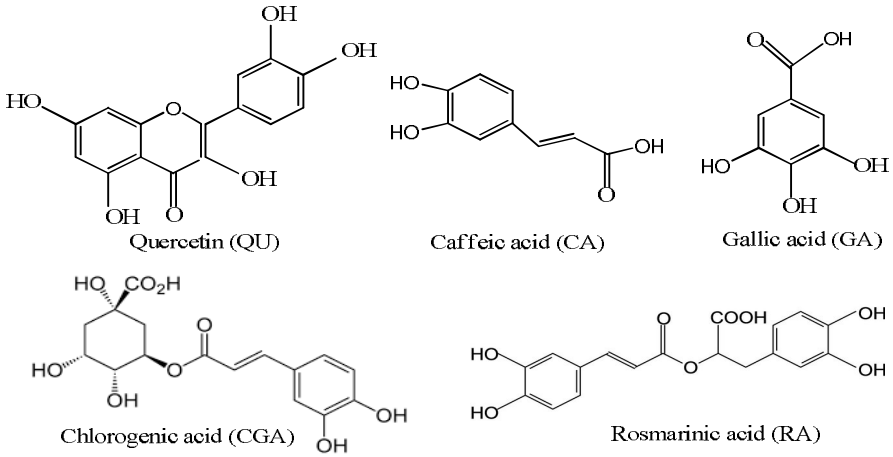



**Fig. 2: Structures of antioxidants.**

Moreover, only a few studies have considered the possible interactions between phenolics, whereas a potent regeneration of an antioxidant by another one can increase or decrease the activity of a mixture of antioxidants. The present investigation was undertaken to confirm structure–activity relationships already pointed out by other measurement methods and to study the synergistic and antagonistic effects occurring between pairs of phenolic antioxidants in a mixture.

## MATERIALS AND METHODS

QU, CGA, CA, RA and GA were purchased from Sigma Aldrich Chemical Co., St. Louis, USA and used as received. All solutions were prepared afresh using double-distilled water. *tert*-Butyl hydroperoxide (*t*-BuOOH) was used as received from Merck-Schuchardt of Germany. *t*-BuOOH was estimated by iodometric method. The irradiations were carried out at room temperature in a quantum yield reactor model QYR-20 supplied by Photophysics, England, attached with 400 W medium pressure mercury lamp. The quartz cuvette containing the sample was irradiated and the irradiations were interrupted at definite intervals of time and the absorbance was noted. The light intensity corresponding to the irradiating wavelength (254 nm) was measured using peroxydisulphate chemical actinometry. On photolysis, *t*-BuOOH was activated at 254 nm to generate $^{\bullet}$OH and *t*-BuO$^{\bullet}$ radicals by homolytic cleavage of –O-O- bond. The $^{\bullet}$OH radicals produced were scavenged by sufficient concentration of *t*-BuOH present in the solvent mixture.[16] The procedure followed here is same as the method described elsewhere.[17] The absorbance measurements were made at suitable $\lambda_{max}$ of

QU (375 nm) and CGA (328 nm) on a Systronics UV-Visible Double-beam spectrophotometer (model 2202).

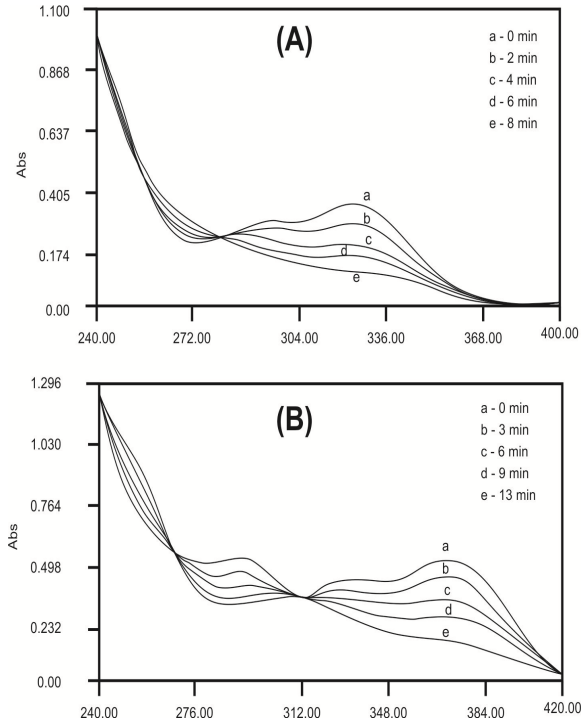



**Fig. 3: (A) Absorption spectra of photooxidation of CGA in the presence of *t*-BuOOH (B) Absorption spectra of photooxidation of CGA in the presence of *t*-BuOOH and QU. [CGA] = 1 × $10^{-5}$ mol $dm^{-3}$, [*t*-BuOOH] = 5 ×$10^{-3}$ mol $dm^{-3}$, [QU] = 1 × $10^{-5}$ mol $dm^{-3}$, Solvent – *t*-BuOH/water (2:1 v/v), Light Intensity = 5.7549 × $10^{15}$ quanta $s^{-1}$, $\lambda_{max}$ = 328 nm, pH ~ 7.5, Room temperature = 298 K**

## RESULTS AND DISCUSSION

The initial rates of photooxidation of QU by *t*-BuOOH in *t*-BuOH-water (2:1 v/v) have been calculated from the plots of absorbance of QU at 375 nm *vs* time. UV-visible absorption spectra of QU in *t*-BuOH-water (2:1 v/v) at different irradiation times in the presence and absence of phenolic acids were recorded (Fig. 3). To understand the nature of interactions between QU with phenolic acids *viz.*, CGA, CA, RA and GA towards oxidation by *t*-BuO$^{\bullet}$ and elucidate the regeneration/repair studies, the reaction mixture containing known concentrations of QU, phenolic acid and *t*-BuOOH in *t*-BuOH-water (2:1 v/v) was irradiated in presence of varying concentrations of phenolic acids. The reactions were followed by measuring the absorbance of QU/ phenolic acid at suitable wavelength (Fig. 4) and the rate data are presented in Tables 1-4. The initial rates and quantum yields of oxidation of QU by *t*-BuO$^{\bullet}$ radicals were found to decrease with increase in concentration of phenolic acids. The rate constant for the reaction of *t*-BuO$^{\bullet}$ with QU has been calculated by the method previously reported and was determined to be 2.62 × $10^{9}$ $dm^{3}mol^{-1}s^{-1}$.

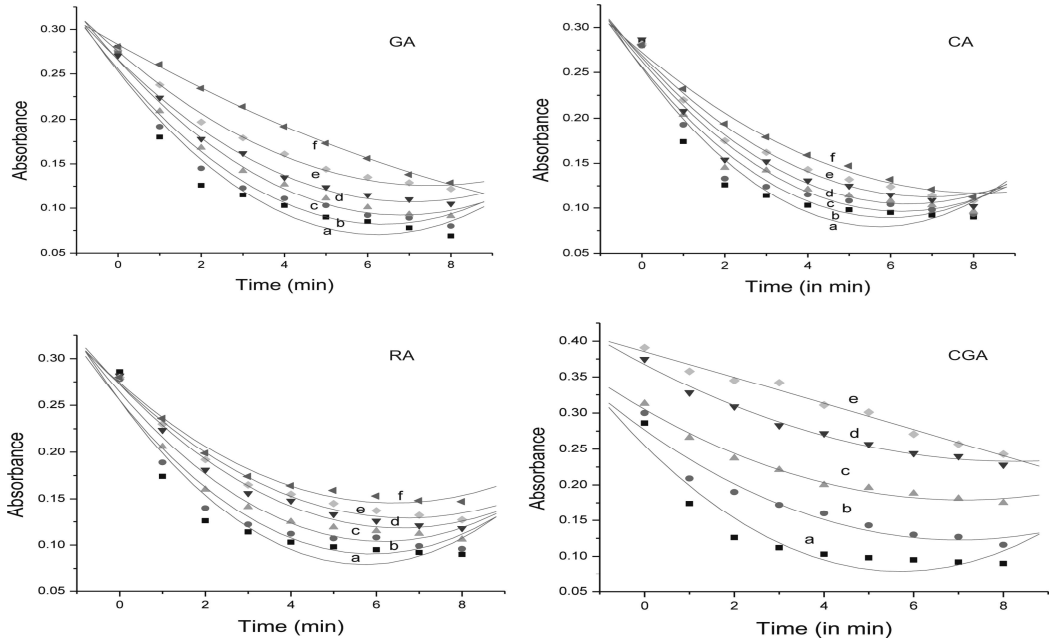
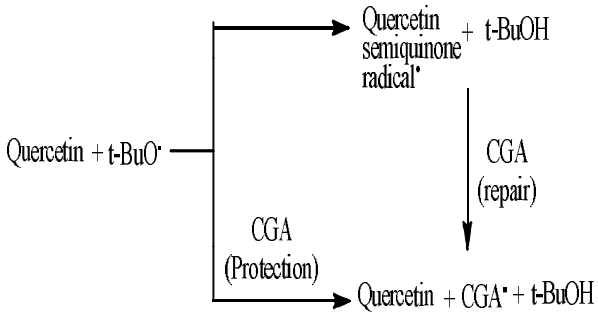



**Fig. 4. Effect of [Phenolic acid] on the oxidation of QU by *t*-BuO$^{\bullet}$ in the presence of GA, CA and RA in *t*-BuOH-water (2:1 v/v) medium. [QU] = 1 × $10^{-5}$ mol $dm^{-3}$, [*t*-BuOOH] = 5 ×$10^{-3}$ mol $dm^{-3}$ at 298 K. [Phenolic acid] = (a) 0.0, (b) 1 × $10^{-6}$ mol $dm^{-3}$, (c) 5 × $10^{-6}$ mol $dm^{-3}$, (d) 8 × $10^{-6}$ mol $dm^{-3}$, (e) 10 × $10^{-6}$ mol $dm^{-3}$, (f) 20 × $10^{-6}$ mol $dm^{-3}$.**

**For CGA, effect of [QU] on the oxidation of CGA by *t*-BuO• in the presence of QU in *t*-BuOH-water (2:1 v/v) medium. Light intensity = $5.7549 \times 10^{15}$ quanta $s^{-1}$, $\lambda_{max}$ = 375 nm, pH ~ 7.5, Temperature = 298 K**

The calculated quantum yield ($\phi_{cal}$) values and experimental quantum yield values ($\phi_{expt}$) at different phenolic acid concentrations (Tables 1-4) show that the $\phi_{cal}$ values are lower than $\phi_{expt}$ values.[17] This indicates that more number of QU molecules is consumed in the system than expected due to H atom donation by QU to phenolic acid radicals. Using the fraction of *t*-BuO• radical scavenged (*p*) by QU and $\phi_{exptl}$ values, $\phi'$ values have been calculated. In the absence of any repair of phenolic acid radicals by QU, the $\phi'$values should all be equal to $\phi^{o}_{expt}$. The observed increase in $\phi'$ with increasing phenolic acid concentration clearly indicated that repair of phenolic acid radicals does occur. The extent of repair of phenolic acid radicals formed due to reaction with *t*-BuO• radicals by QU was calculated for each of the phenolic acid. The results obtained in the present study indicated that phenolic acid radicals *viz.*, CA, RA and GA were efficiently repaired by QU to the extent of ~96%, ~91% and ~42% respectively at ~10μM of [QU] whereas CGA repaired QU radicals to the extent of ~55% at ~10μM of CGA. The protection of QU and repair of QU radicals by CGA as an example is summarized below.




Similar results were reported by Peyrat-Maillard *et al*[18] about the synergistic and antagonistic effects occurring between pairs of antioxidants in a mixture. A synergistic effect was observed between QU and RA whereas an antagonism was pointed out between QU or (+)-catechin and CA. Antagonistic effects were observed in mixtures of CA and QU[18] whereas water soluble antioxidants such as CGA and QU exhibited synergistic effects with α-tocopherol in microemulsions.[19] Pedrielli *et al*[20] studies indicated longer induction periods (synergistic or co-antioxidant effects) between QU and α-TOH in homogeneous solution of peroxidating methyl linoleate in different solvents. Hajimedipoor *et al*[21] have studied synergistic antioxidant effects of phenolic acids and flavonoids using FRAP (Ferric Reducing Antioxidant Power) method and the results showed that combination of GA and CA demonstrated considerable synergistic effects (137.8%) while other combinations were less potent. Meyer *et al*[22] demonstrated that catechin, cyanidin, CA, QU and ellagic acid showed a potential synergistic effect on human LDL oxidation. One of the studies[23] suggested that these phenolic acids are capable not only to donate hydrogen atoms to the radical, but they are also able to donate electrons to regenerate other pro-oxidant phenols. According to Leopoldini *et al*[24], phenolic compounds are capable to transfer electrons to other phenolics or antioxidants, promoting their chemical regeneration. But very few studies are available which focused on the kinetic model to investigate the molecular mechanisms underlying the synergistic/antagonistic interactions among the antioxidants.

The function of an antioxidant is to retard the oxidation of an organic substance, thus increasing the useful life or shelf life of that material. In a combination of antioxidants present in food, exhibit different mechanisms of action and physical properties and inhibition of oxidation occurs in many different phases. It depends on factors *viz.*, the type of oxidation, catalyst, physical state of lipid (bulk, emulsified), pH, temperature and the ability to interact with other components in the food.[25] In general, the less effective antioxidant traps the radicals resulting in protecting more effective antioxidant from the oxidation. QU and α-tocopherol show a synergism in decreasing the oxidation of lard by the mechanism in which α-tocopherol acts as a free radical scavenger while quercetin acts as a metal chelator.[26] To understand the antioxidant interactions, it is essential to examine factors such as the direction of electron transfer of when two or more antioxidants are present, the rate constant of the regeneration, the thermodynamics of radical reactions, one-electron reduction potential (ΔE) and OH bond dissociation enthalpies (BDEs). A synergistic or antagonistic effects occurring between pairs of antioxidants can be explained by regeneration mechanisms, the chemical structure of molecules and formation of stable intermolecular complexes.[27]

**Table 1: Effect of varying [GA] on the rate and quantum yield of photooxidation of QU in *t*-BuOH-water (2:1 v/v) medium. [QU] = $1.0 \times 10^{-5}$ mol dm$^{-3}$, [*t*-BuOOH] = $5.0 \times 10^{-3}$ mol dm$^{-3}$, Light intensity = $5.7549 \times 10^{15}$ quanta s$^{-1}$, $\lambda_{max}$ = 375 nm, pH ~ 7.5, Temperature = 298 K**

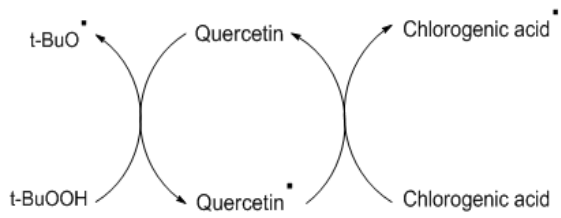
| $10^6 \times$[GA] (mol dm$^{-3}$) | $10^8 \times$ Rate (mol dm$^{-3}$ s$^{-1}$) | $\phi_{expt}$ | $\phi_{cal}$ | *p* | $\phi'$ | % scavenging | % regeneration |
|---|---|---|---|---|---|---|---|
| 0.0 | 4.2632 | 0.01338 | 0.01338 | 1.0000 | 0.01338 | 100.0 | 0.00 |
| 1.0 | 3.9440 | 0.01238 | 0.01221 | 0.9129 | 0.01356 | 98.61 | 1.39 |
| 5.0 | 3.3333 | 0.01046 | 0.00906 | 0.6770 | 0.01546 | 84.46 | 15.54 |
| 8.0 | 3.0798 | 0.00967 | 0.00758 | 0.5670 | 0.01705 | 72.54 | 27.46 |
| 10.0 | 2.9220 | 0.00917 | 0.00685 | 0.5117 | 0.01793 | 65.99 | 34.01 |
| 20.0 | 2.0833 | 0.00654 | 0.00460 | 0.3438 | 0.01902 | 57.81 | 42.19 |

Regeneration of a more effective free radical scavenger (primary antioxidant) by a less effective free radical scavenger (coantioxidant, synergist) occurs mostly when one free radical scavenger has a higher reduction potential than the other.[28] In general, thermodynamics of electron transfer reactions of antioxidants to regenerate other antioxidants can be explained on the basis of their one electron reduction potentials. In fact, reduction potentials are highly dependent on pH, solvent type and ionic strength. Buettner[29] had proposed the prediction of a pecking order or hierarchy by using one-electron reduction potentials. The higher the reduction potential, the higher is the ability to take an electron (or hydrogen atom) from those with a lower reduction potential. For example, α-tocopherol (480 mV) and ascorbic acid (280 mV) have lower the reduction potential than polyunsaturated fatty acid (600 mV) and alkylperoxide (~1000 mV) radicals, so it is thermodynamically feasible for these antioxidants to donate an electron to fatty acid radicals.

**Table 2: Effect of varying [CA] on the rate and quantum yield of photooxidation of QU in *t*-BuOH-water (2:1 v/v) medium. [QU] = $1.0 \times 10^{-5}$ mol dm$^{-3}$, [*t*-BuOOH] = $5.0 \times 10^{-3}$ mol dm$^{-3}$, Light intensity = $5.7549 \times 10^{15}$ quanta s$^{-1}$, $\lambda_{max}$ = 375 nm, pH ~ 7.5, Temperature = 298 K.**

| $10^6 \times$[CA] (mol dm$^{-3}$) | $10^8 \times$ Rate (mol dm$^{-3}$ s$^{-1}$) | $\phi_{expt}$ | $\phi_{cal}$ | *p* | $\phi'$ | % scavenging | % regeneration |
|---|---|---|---|---|---|---|---|
| 0.0 | 4.2632 | 0.01338 | 0.01338 | 1.0000 | 0.01338 | 100.0 | 0.0 |
| 1.0 | 4.0416 | 0.01268 | 0.01188 | 0.8881 | 0.01428 | 93.2 | 6.8 |
| 5.0 | 3.5715 | 0.01121 | 0.00821 | 0.6135 | 0.01827 | 63.4 | 36.5 |
| 8.0 | 3.2520 | 0.01021 | 0.00666 | 0.4981 | 0.02049 | 46.8 | 53.1 |
| 10.0 | 3.0903 | 0.00970 | 0.00592 | 0-4425 | 0.02192 | 36.1 | 63.8 |
| 20.0 | 2.3752 | 0.00745 | 0.00380 | 0.2841 | 0.02624 | 3.83 | 96.2 |

For the antioxidants used in the present work, the order of the one electron reduction potentials (IP) of the antioxidants used are found to be in the order of GA > CGA > CA > QU (Table 5). QU with the least reduction potential among the antioxidants studied can thermodynamically regenerate other phenolic acids. Thus, QU was found to repair/regenerate CA, RA and GA but rather was regenerated by CGA. Since QU is more efficient free radical scavenger compared to other antioxidants, the former regeneration reactions are considered as antagonistic interactions by donating H atom and the latter regeneration reaction is considered as synergistic interaction. The synergistic (co-operative mechanism) reaction between QU and CGA can be represented by the following diagram and in equations (1) and (2).




The synergistic (co-operative) reaction between QU and CGA

In terms of regeneration or interaction among antioxidants, the BDE values also provide useful thermodynamic information for predicting the hierarchy of the reactions, the rate constant and the efficiency of regeneration. As the driving force for hydrogen transfer, the minimum O-H BDE of the phenolic antioxidant is usually correlated to the rate constant of the ability of an antioxidant to inhibit chain propagation[30-32], as well as the efficiency and the rate of regeneration of antioxidants.[33]

$$QU + t\text{-}BuO^{\bullet} \longrightarrow QU^{\bullet} + t\text{-}BuOOH \quad (1)$$

$$CGA + QU^{\bullet} \longrightarrow CGA^{\bullet} + QU \quad (2)$$

**Table 3: Effect of varying [RA] on the rate and quantum yield of photooxidation of QU in *t*-BuOH-water (2:1 v/v) medium. [QU] = $1.0 \times 10^{-5}$ mol $dm^{-3}$, [*t*-BuOOH] = $5.0 \times 10^{-3}$ mol $dm^{-3}$, Light intensity = $5.7549 \times 10^{15}$ quanta $s^{-1}$, $\lambda_{max}$ = 375 nm, pH ~ 7.5, Temperature = 298 K.**

| $10^6 \times$[RA] (mol $dm^{-3}$) | $10^8 \times$ Rate (mol $dm^{-3}$ $s^{-1}$) | $\phi_{expt}$ | $\phi_{cal}$ | *p* | $\phi'$ | % scavenging | % regeneration |
|---|---|---|---|---|---|---|---|
| 0.0 | 4.2632 | 0.01338 | 0.01338 | 1.0000 | 0.01338 | 100.0 | 0.0 |
| 1.0 | 3.8888 | 0.01221 | 0.01181 | 0.8827 | 0.01383 | 96.6 | 3.4 |
| 5.0 | 3.4722 | 0.01090 | 0.00804 | 0.6009 | 0.01814 | 64.4 | 35.6 |
| 8.0 | 3.2465 | 0.01019 | 0.00648 | 0.4848 | 0.02102 | 42.8 | 57.1 |
| 10.0 | 3.0680 | 0.00963 | 0.00575 | 0.4295 | 0.02242 | 32.4 | 67.6 |
| 20.0 | 2.2916 | 0.00719 | 0.00365 | 0.2734 | 0.02631 | 18.3 | 81.7 |

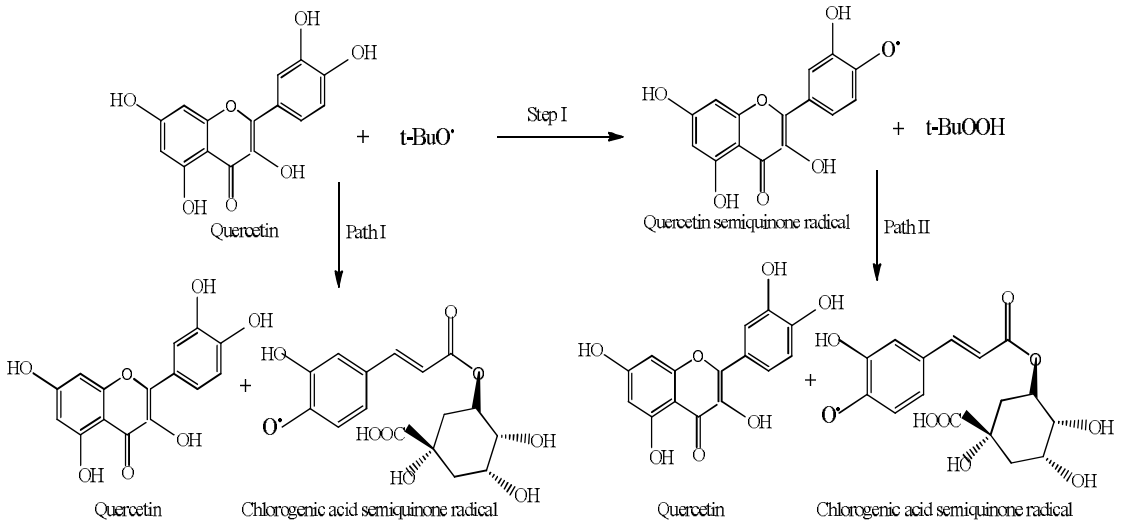
When the difference in BDE of two molecules is reasonably good, then an antioxidant with higher BDE acts as primary antioxidant and an antioxidant with lower BDE acts a synergist (coantioxidants).[34] In our case, the results obtained shows that CGA with higher BDE (73.4 kcal/mol) regenerates QU with lower BDE (71.8 kcal/mol).[35,36] An antioxidant with higher antioxidant power (AOP) is regenerated by an antioxidant with lower AOP is termed as 'synergistic interaction'. QU with higher AOP is regenerated by CGA (at concentration of 10 µM) having lower AOP to an extent of 45 % suggesting synergistic interactions among them.

**Table 4: Effect of varying [QU] on the rate and quantum yield of photooxidation of CGA in *t*-BuOH-water (2:1 v/v) medium. [CGA] = $1.0 \times 10^{-5}$ mol $dm^{-3}$, [*t*-BuOOH] = $5.0 \times 10^{-3}$ mol $dm^{-3}$, Light intensity = $5.7549 \times 10^{15}$ quanta $s^{-1}$, $\lambda_{max}$ = 328 nm, pH ~ 7.5, Temperature = 298 K**

| $10^6 \times$ [QU] (mol $dm^{-3}$) | $10^8 \times$ Rate (mol $dm^{-3}$ $s^{-1}$) | $\phi_{expt}$ | $\phi_{cal}$ | *p* | $\phi'$ | % scavenging | % regeneration |
|---|---|---|---|---|---|---|---|
| 0.0 | 3.0016 | 0.00942 | 0.00942 | 1.0000 | 0.00942 | 100.0 | 0.00 |
| 1.0 | 2.9072 | 0.00913 | 0.00870 | 0.9243 | 0.00981 | 95.1 | 4.84 |
| 5.0 | 2.5063 | 0.00787 | 0.00668 | 0.7095 | 0.01109 | 82.3 | 17.7 |
| 8.0 | 2.1888 | 0.00687 | 0.00569 | 0.6042 | 0.01145 | 79.3 | 20.7 |
| 10.0 | 2.0885 | 0.00656 | 0.00518 | 0.5498 | 0.0119 | 73.4 | 26.6 |
| 20.0 | 1.5037 | 0.00472 | 0.00357 | 0.3791 | 0.0124 | 67.8 | 32.2 |
| 50.0 | 0.8521 | 0.00267 | 0.00185 | 0.1963 | 0.0136 | 55.3 | 44.6 |

However, the redox potentials of CGA (550 mV) and QU (330 mV) are not in conformity with the results in Table 4.[28] These inconsistencies could be due to the fact that besides thermodynamic properties of antioxidants influencing the HAT/ET reactions in homogeneous systems, different physical locations of individual antioxidants may play an important role in exhibiting interactions.[37]

Relatively higher redox potential and BDE of CGA makes it behave as primary antioxidant and QU semiquinone radicals formed by the reaction of QU with *t*-$BuO^{\bullet}$ radicals are regenerated by CGA as shown below.



The order of the decreasing BDE of antioxidants studied are RA < GA < QU ~ CA < CGA (Table 5).[36] CA has almost similar BDE value and higher reduction potential compared to QU. Having lower antioxidant activity compared to QU, CA was found to get regenerated by QU (Table 3). Similarly, the lower BDE and higher IP values of GA and RA would make these phenolic acids show more tendency to loose H atom to *t*-BuO$^{\bullet}$ radicals compared to QU and in turn would get regenerated/repaired by QU present in the medium. Thus, the results obtained in the present study indicate that QU (at the concentration of 10μM) was found to repair CA, RA and GA to an extent of ~96%, ~81% and ~42% respectively.

**Table 5: Bond Dissociation Energies (BDE)* and One.**

| Antioxidant | BDE (kcal/mol) | IP (mV) |
|---|---|---|
| Gallic acid (GA) | 70.2 | 560 |
| Rosmarinic acid (RA) | 69.2 | - |
| Caffeic acid (CA) | 72.1 | 534 |
| Chlorogenic acid (CGA) | 73.4 | 550 |
| Quercetin (QU) | 71.8 | 330 |

**electron Reduction Potential (IP**) of antioxidants**

*adapted from Guitard *et al*., 2016 and the references thereof[36], **adapted from Choe and Min[28], 2009

In addition, stable intermolecular complexes (Fig. 6) could be formed between QU and phenolic acids as suggested in the co-pigmentation mechanism.[38,39] It is suggested that the formation of stable complex between antioxidants due to π-π stacking between the aromatic ring of phenolic acid and the B-ring of flavonol may influence on the overall electron donating capacity.[18,27,39] Also, there is formation of hydrogen bonding between carbonyl and hydroxyl groups of two antioxidant molecules which provides better structural analogy.[22] However, the more stable complex formed between QU and CGA leads to synergistic interactions and the less stable complex formed between QU and other phenolic acids exhibits antagonistic interactions. The nature of interactions of QU with phenolic acids is essential for understanding the effects of this compound in oxidative stress conditions *in vivo*. This supported our contention that QU and phenolic acid radicals might not involve in oxidative stress in our experimental conditions.

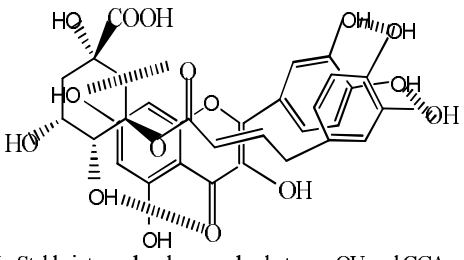



Fig. 6 - Stable intermolecular complex between QU and CGA molecules

## CONCLUSION

For the past two decades, a large amount of inclusive studies have been undertaken to explain the antioxidant efficiencies. Several studies have emphasized that the antioxidant ability of an antioxidant varies linearly with the number of phenolic OH groups present in them. Different structure-activity relationship (SAR) studies have indicated that several factors apart from structural characteristics influence the antioxidant capacity of an antioxidant. Also, innumerable methods have been designed to evaluate antioxidant power of available antioxidants individually and in combinations. Lack of consistency among the data available provides a driving force to explore deeper into the kinetic and other novel approaches to comprehend synergistic/antagonistic antioxidant effects. The molecular mechanisms underlying these interactions need further consideration for optimum dietary recommendations and food preservation strategies by unearthing the desired role of the antioxidants.

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## CONFLICT OF INTEREST

Author declare that there is no conflict of interest associated with this publication.

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