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Reactions of hydroxyl radical with bergenin, a natural poly phenol studied by pulse radiolysis

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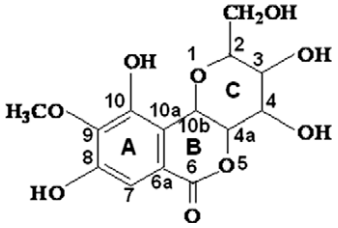
ABSTRACT

Reactions of pulse radiolytically generated hydroxyl (·OH) radicals and one-electron specific oxidants, Br_2^- radicals with bergenin, a polyphenolic tannin derivative, were studied and the transients detected by absorption spectrometry. The transient absorption spectrum produced during the reaction of ·OH radicals with bergenin was broad, and pH dependent. Different modes of reactions of ·OH radicals with bergenin, viz., addition to the aromatic ring adduct and hydrogen abstraction was established by time resolved (5–400 μs) transient absorption studies and also by the reaction of Br_2^- radicals. Comparing the transient spectra with ·OH radicals and Br_2^- radicals at pH 4.5 and 8.5, the absorption maximum of the phenoxyl radical was found to be at 440 nm at pH 4.5 and 480 nm at pH 8.5. Phenoxyl radicals are produced during ·OH radical reaction through the formation of ·OH radical adduct followed by water/OH^- elimination. While the phenoxyl radicals of bergenin are oxidizing in nature, the hydroxyl radical adducts and the radicals produced from hydrogen abstraction are of reducing nature. The yield of the oxidizing radicals produced from the ·OH radical reaction with bergenin was determined to be 26.2% by secondary electron transfer reaction from TMPD. On the other hand the yield of reducing radicals produced from the ·OH radical reaction with bergenin was determined to be 74.1% by secondary electron transfer reaction to MV^{2+}. ·OH radical reactions with bergenin under oxygenated conditions and reaction with trichloro methyl peroxyl radicals with bergenin produced a new transient absorbing at 400 nm, which is attributed to peroxyl type of radicals. The one-electron reduction potential for the formation of phenoxyl radical from bergenin was determined to be 0.938 V versus NHE at pH 7, by electron transfer equilibrium between bergenin and chlorpromazine. The above results confirmed that reaction of ·OH radicals with bergenin, mainly produced radical adducts and one-electron oxidation accounts to only a minor process. The radical adducts may be converted to peroxyl radicals in presence of oxygen. Based on these results it can be concluded that although bergenin is a polyphenol, it may not act as a potent antioxidant, but may be act as pro-oxidant.

1. Introduction

Bergenin is a colourless crystalline polyphenol isolated from medicinal plants like *Bergenial crassifolia, Corylopsis spicata, Caesalpinia digyna, Mallotus japonicus, Sacoglottis gabonensis* etc.[1–4] It is hydrolyzable tannin and an isocoumarin derivative with three hydroxyl (OH) groups and two phenolic OH groups.[5] Bergenin containing extracts have long been used as a folk medicine in several parts of Asia. *Molloti Cortex* extract, containing 11–18% bergenin, was used for the treatment and therapy of gastrointestinal diseases,[1,3] and detectable amounts of bergenin were observed in the urine of the treated animals. The molecular formula and the chemical structure of bergenin (referred to Scheme 1) was confirmed by several spectroscopic methods and also by its synthesis.[6–8] In some fungal systems, bergenin undergoes biotransformation to a dimer.[8]

Bergenin exhibits antihepatotoxic, antiulcerogenic, anti-HIV, antiarrhythmic, neuroprotective, anti-inflammatory and immunomodulatory properties.[2,9–14] Bergenin isolated from *S. gabonensis* showed protective action against oxidative stress induced by 2,4-dinitro phenyl hydrazine (DNPH) and ethanol.[4] In most of these



Scheme 1.

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biological activities, bergenin either acts as a free radical scavenger or a redox regulatory agent. Recently we have studied the antioxidant and free radical scavenging activity of bergenin by following its effect on γ-radiation induced liposomal lipid peroxidation, protein carbonylation and DNA (pBR322) damage.[15] Excessive production of oxidizing free radicals has been implicated in radiation injury and oxidative stress. It is therefore necessary to evaluate the mechanisms of oxidation reactions of bergenin during its reaction with these free radicals. Such studies are also useful in evaluating the antioxidant and other biological abilities of bergenin and bergenin enriched plant extracts.

Among the reactive free radicals, hydroxyl (·OH) radicals are considered as the most powerful oxidizing agents. Hydroxyl radicals are produced during Fenton reaction and are the major agents produced during exposure to radiation. Thus to understand the mechanism of oxidation reactions and also to evaluate the role of reactions of ·OH radicals with bergenin, in this paper, detailed pulse radiolysis studies of bergenin in aqueous solutions have been carried out and the resultant transients detected by absorption spectroscopy. Pulse radiolysis offers a unique tool, which can be used to study the reactions of short-lived free radical reactions during their life times.

2. Results and discussion

2.1. Reaction of ·OH radical and specific one-electron oxidant at different pH

Bergenin in the ground state absorbs from 200 to 330 nm region in the pH range from 1 to 10. Since the transients obtained on reaction of bergenin with ·OH and other oxidizing radicals, absorb at wavelengths above 300 nm, no correction for ground state absorption is made. Figure 1 shows the absorption spectrum in the wavelength region 300–600 nm of the transient, recorded with in 5 μs, after pulse radiolysis of N_2O saturated aqueous solution of 0.1 mM bergenin at pH 4.5, 7, 8.5. Depending on the pH of the solution, the transient spectra showed a clear shift in the absorption maximum. At pH 4.5, the spectrum showed absorption maximum at 440 nm, with a shoulder at 350 nm. At neutral pH, the transient spectrum is very broad with three apparent peaks at 350 nm, 440 nm and 500 nm. At pH 8.5, the 440 nm absorbing transient reduced to a large extent and the absorption band at 500 nm became more intense. The rate constant for the reaction of ·OH radical with the solute was measured by following the formation kinetics at 440 nm and the bimolecular rate constant at different pH is reported in Table 1. The decay kinetics of the transient absorbing at 440 nm also showed significant pH dependency. At pH 1, the transient decayed in less than 20 μs, but the decay became slower with increasing pH up to pH 7 and further increase in pH to alkaline region, it showed negligible decay in the maximum detectable time scale of 10 ms.

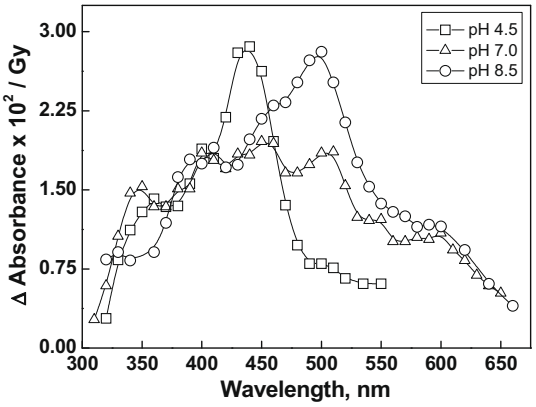


Figure 1. Transient absorption spectra obtained on pulse radiolysis of N_2O saturated aqueous solution of 0.1 mM bergenin at pH 4.5, 7.0 and 8.5 (dose = 14 Gy).

·OH radical is known to react with organic compounds by different reaction channels like one-electron oxidation, addition to double bonds of the aromatic ring and H atom abstraction.[16] In case of phenolic compounds, formation of phenoxyl radical through one-electron oxidation is thermodynamically favourable process.[17–20] However, radical addition and H-atom abstraction are also important modes of reactions and being electrophilic in nature ·OH radical easily adds to aromatic double bonds forming radical adduct.[17–20] Depending on the pH and the nature of substituent, all the above ·OH radical reactions can occur simultaneously in the same time scale or in the different time scale. The site of ·OH radical addition to the aromatic ring, depends on the electron donating and with drawing properties of the substituent, and in most of the cases distribution of isomeric radical adducts are obtained. In the present molecule, the aromatic ring of bergenin possesses phenolic OH with methoxyl group at *ortho*-substituent (ring A) and also a non-aromatic part (ring C). Hydrogen abstraction can take place at ring C. The most preferred position for ·OH radical addition is at 7 position at ring A, which is *ortho* (8 position at ring A) and *para* (10 position at ring A) to the phenolic OH group and *meta* to the methoxyl group in bergenin. There is a certain probability for ·OH radical to add to other positions in the aromatic ring. It is very difficult to identify the individual isomers from the spectral behaviour, because the isomers differ only slightly with respect to their physical and chemical properties. The ·OH radical adducts can undergo acid/base catalyzed elimination of water to yield more resonance stabilized phenoxyl radical.[18,19,21] It is reported that acid catalyzed water elimination reaction of OH adduct of phenol is slower than the base catalyzed reaction.[19]

To resolve the contribution of phenoxyl radicals to the above reactions of ·OH radical with bergenin, one-electron specific oxidant, $Br_2^{\cdot-}$ radical was used. This being a specific one-electron oxidant with $E^0 = 1.66$ V versus NHE,[22] in general reacts with substrates like phenols by outer sphere electron transfer reaction, forming phenoxyl radical. The transient spectra obtained from $Br_2^{\cdot-}$ radical reaction with bergenin showed absorption maximum around 440 nm at both pH 4.5 and 8.5 (Fig. 2).

The bimolecular rate constant for the reaction of $Br_2^{\cdot-}$ radical with bergenin at different pH was determined by measuring the decay kinetics of $Br_2^{\cdot-}$ radical at 365 nm, in presence of varying concentration of bergenin (0.5–2 mM). The rate constant values given in Table 1 indicate that bergenin shows very low reactivity at low pH, but with increase in pH, as it gets deprotonated, the bimolecular rate constant for the reaction increased. Comparing the transient spectra produced during the reaction of ·OH radical and $Br_2^{\cdot-}$ radical with bergenin at pH 4.5 and 8.5, it can be seen that the two spectra show similarity around 440 nm region (Fig. 2), however the absorbance of the transient from ·OH radical reaction is higher than that from $Br_2^{\cdot-}$ radical reaction at pH 4.5. This indicates two things either bergenin concentration is not enough to

Table 1
Bimolecular rate constant values for different reactions

Reaction	pH	Wavelength (nm)	Bimolecular rate constant (M^{-1} s^{-1})
Bergenin + ·OH	4.5	440	$4.29 \pm 0.31 \times 10^9$
Bergenin + ·OH	7.0	440	$3.33 \pm 0.23 \times 10^9$
Bergenin + ·OH	8.5	440	$1.00 \pm 0.06 \times 10^{10}$
Bergenin + $Br_2^{\cdot-}$	4.5	365	$1.96 \pm 0.02 \times 10^7$
Bergenin + $Br_2^{\cdot-}$	7.0	365	$3.36 \pm 0.02 \times 10^7$
Bergenin + $Br_2^{\cdot-}$	8.5	365	$1.09 \pm 0.15 \times 10^8$
Bergenin + $CCl_3O_2^{\cdot}$	7.0	400	$4.16 \pm 0.37 \times 10^6$

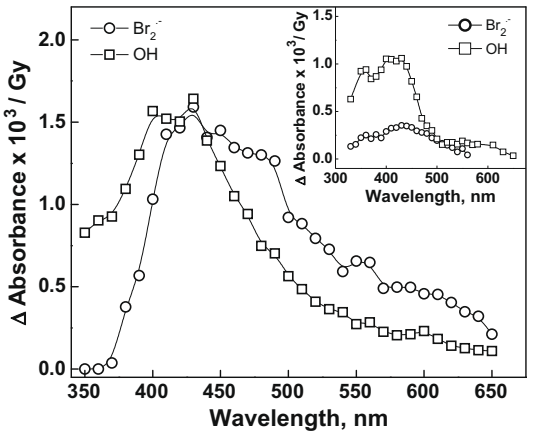


Figure 2. Transient absorption spectra obtained on pulse radiolysis of N_2O saturated aqueous solution of 1 mM bergenin and in presence of 0.1 M KBr at pH 8.5 after 400 μs of pulse (dose = 50 Gy). Inset shows the transient absorption spectra at pH 4.5 under similar condition.

scavenge all the $Br_2^{\bullet-}$ radicals or the radical adduct produced from ·OH radical reaction absorb at the same wavelength region. However by comparing the reactivity parameters at different pHs it appears that the reaction with $Br_2^{\bullet-}$ radicals is nearly complete at 1 mM, at pH >9. Therefore these results suggest that during the reaction of ·OH radicals with bergenin, along with the phenoxyl radicals other radical products, which may be due to addition or H atom abstraction reaction, too absorb in the same overlapping wavelength region. The observed transient absorption spectra from ·OH radical reaction at different pH are therefore ascribed to the mixture of oxidized product and other radical products.

To understand the pH dependent spectral changes of the transients of bergenin produced during the reaction of ·OH radical with bergenin, we have recorded the transient spectra with higher concentration of bergenin (1 mM) and higher dose (50 Gy/pulse) were recorded at 5, 20, 40 and 400 μs after pulse. At pH 8.5 the transient spectra up to 20 μs, did not show any change with increasing bergenin concentration from 0.1 mM to 1 mM. Also no dose effect was seen on this. However, with increasing time, the 500 nm absorbing transient decreased gradually and at 400 μs after the pulse absorption maximum shifted to 440 nm (Fig. 3). Inset of Figure 3 shows the spectra of the transient formed from the reaction of 1 mM bergenin with $Br_2^{\bullet-}$ radical at 40 and 400 μs after the pulse at pH 8.5. Figure 4 shows time-dependent absorption changes at 550 nm (trace a) and also at 440 nm (trace b). The nature of the absorption–time plot at 440 nm was found to be identical with that was obtained on reaction of 1 mM bergenin with $Br_2^{\bullet-}$ radical (trace c). However, the absorbance in trace b is of higher magnitude than that in trace c, confirming that $Br_2^{\bullet-}$ radical is not able to completely oxidize bergenin at this concentration. With increase in pH from 8.5 to 10.5, the decay of the transient at 500 nm became faster and the k_{obs} showed linear dependency (inset of Fig. 4) on the OH^- concentration, further confirming that this process is base catalyzed. This was also supported by the fact that similar studies on the spectra of the transients formed from the reaction of bergenin with $Br_2^{\bullet-}$ radical, did not show any change in the spectra at 40 and 400 μs after the pulse.

At pH 4.5 the transient spectra for ·OH radical and $Br_2^{\bullet-}$ radical with bergenin were more or less similar with absorption maximum at 440 nm (spectra not shown). All these experimental evidences confirmed that the ·OH radical adduct of bergenin absorbing at ~500 nm wavelength region in the alkaline pH region undergoes water elimination and ~440 nm absorption band correspond to the phenoxyl radical and H-atom reaction product of bergenin. The over all reaction of ·OH radical with bergenin is presented in Scheme 2.

2.2. Yield of oxidizing and reducing radicals produced on reaction of ·OH radicals with bergenin

As mentioned earlier ·OH radicals react with bergenin by electrophilic addition to the aromatic ring forming radical adducts, and H-atom abstraction mostly from the C ring through abstraction of hydrogen from β-carbon of the CH_2OH group. Both these reactions lead to production of carbon centred radicals, which are generally reducing in nature.[23,24] The phenoxyl radicals are mostly oxidizing in nature.[25–29] Thus the overall reaction of ·OH radical with bergenin produces a mixture of oxidizing and reducing radicals. It is possible to estimate the relative percentage of oxidizing and reducing radicals. Their relative yield can be estimated by studying the secondary electron transfer reactions with *N,N,N′N′*-tetramethyl-*p*-phenylenediamine (TMPD)[22,30] and methyl viologen (MV^{2+}).[22,31,32] TMPD can undergo easy oxidation to produce $TMPD^{\bullet+}$ (E^0 = 0.265 V vs NHE) which can be detected by monitoring the absorbance at 565 nm and with extinction coefficient of

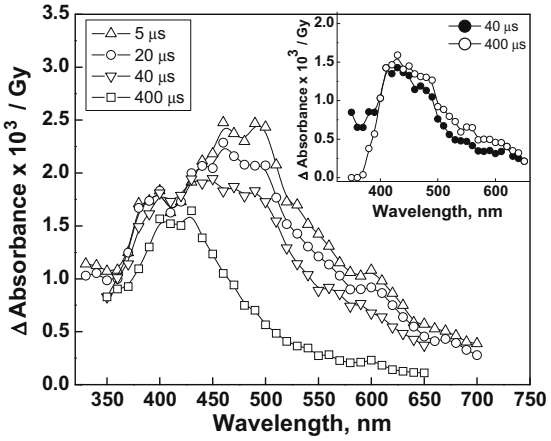


Figure 3. Transient absorption spectra obtained on pulse radiolysis of N_2O saturated aqueous solution of 1 mM bergenin at pH 8.5 after 5, 20, 40, 400 μs of pulse (dose = 50 Gy). Inset: Transient absorption spectra obtained on pulse radiolysis of N_2O saturated aqueous solution of 1 mM bergenin and in presence of 0.1 M KBr at pH 8.5 after 40 and 400 μs of pulse (dose = 50 Gy).

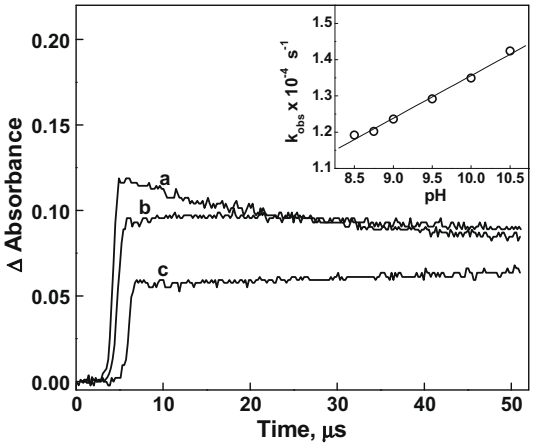
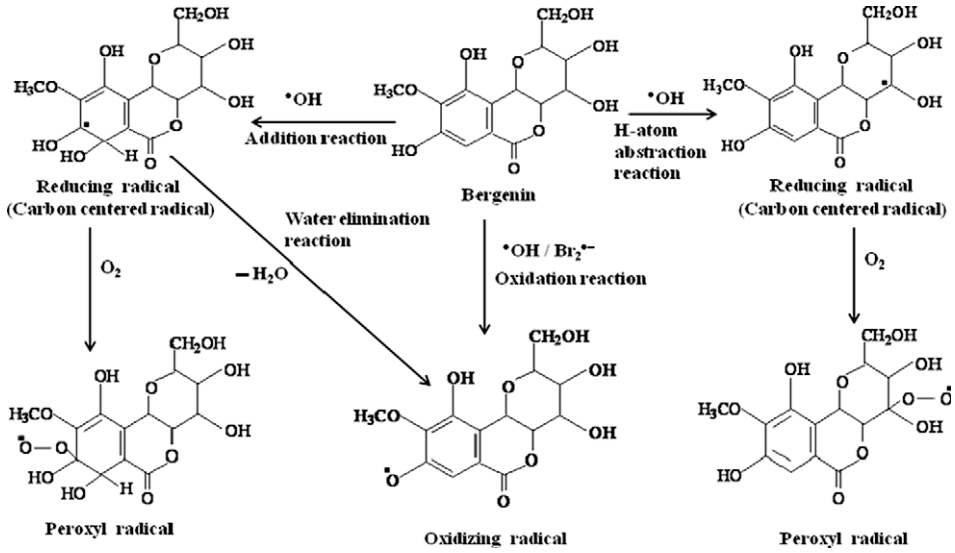
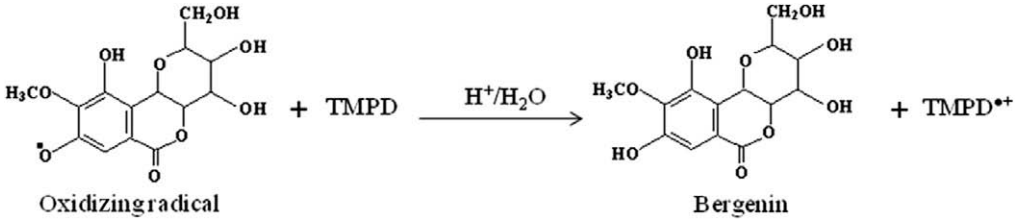


Figure 4. Absorbance versus time trace obtained on pulse radiolysis of N_2O saturated aqueous solution of 1 mM bergenin at 500 nm (trace a), at 440 nm (trace b) and in presence of 0.1 M KBr (trace c) at pH 8.5 (dose = 50 Gy). Inset shows the plot of decay rate constant of N_2O saturated aqueous solution of 1 mM bergenin at 500 nm with varying pH.



Scheme 2.

12,000 M^{-1} cm^{-1}. Similarly, MV^{2+} undergoes easy reduction producing $MV^{\bullet+}$, ($E^0 = -0.443$ V vs NHE) which has an absorption at 605 nm with extinction coefficient of 12,800 M^{-1} cm^{-1}.



(1)

Pulse radiolysis of N_2O saturated aqueous solution of bergenin (2.45 mM, pH 7) containing different concentration of TMPD, showed formation of $TMPD^{\bullet+}$ (λ_{max} = 565 nm) whose absorbance increased with increase in concentration of TMPD (25–200 μM) and reached a saturation value at concentration of 200 μM (inset of Fig. 5). From this saturation value of absorbance and using the extinction coefficient of TMPD at 565 nm, the oxidized product of bergenin reacting with TMPD by electron transfer reaction were determined to be 26.2% and the bimolecular rate constant (k) for this reaction (1) was 5.08×10^8 M^{-1} s^{-1}. Linear plot showing the change in observed rate constant for the formation $TMPD^{\bullet+}$ radical cation at pH 7 in different concentrations of TMPD in N_2O saturated aqueous solution of bergenin is given in Figure 5.

The yield of the reducing radical was determined by following the absorbance of $MV^{\bullet+}$ radical. For these studies N_2O saturated aqueous solution of bergenin (1.8 mM) at pH 7 was radiolyzed in presence of increasing concentration of MV^{2+} and the change in

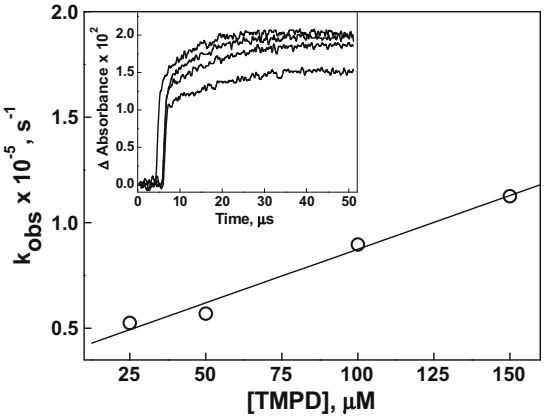


Figure 5. Linear plot showing the change in observed rate constant for the formation of $[TMPD]^{\bullet+}$ at 565 nm at pH 7 with change in concentration of TMPD produced on pulse radiolysis of N_2O saturated aqueous solution containing 2.45 mM of bergenin. Inset shows the formation of $[TMPD]^{\bullet+}$ at 565 nm in the presence of 50, 100, 150, 200 μM TMPD.

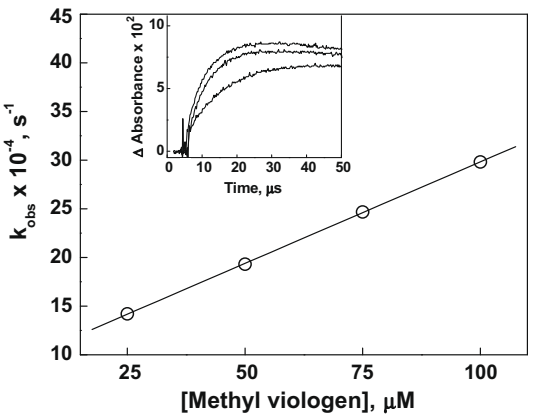
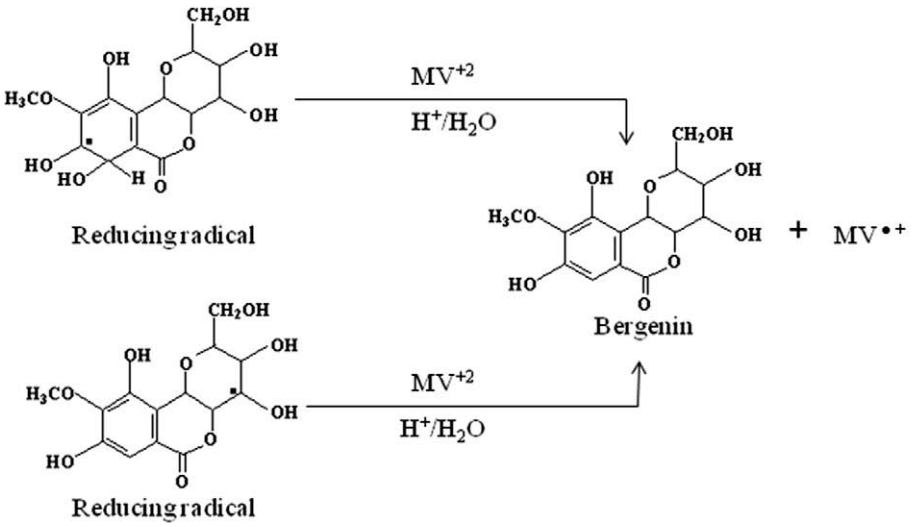


Figure 6. Linear plot showing the change in observed rate constant for the formation of $[MV]^{\bullet+}$ at 605 nm at pH 7 with change in concentration of MV^{2+} produced on pulse radiolysis of N_2O saturated aqueous solution containing 1.8 mM of bergenin. Inset shows the formation of $[MV]^{\bullet+}$ at 605 nm in the presence of 50, 75, 100 μM MV^{2+}.

rate of formation of the $MV^{\cdot+}$ at 605 nm was monitored and plotted as a function of MV^{2+} concentration as shown in Figure 6. Inset of Figure 6 confirms that absorbance of $MV^{\cdot+}$ increases with increase in concentration of MV^{2+}. From the slope of the linear plot, the bimolecular rate constant for reaction (2) was found to be $2 \times 10^9\ M^{-1}\ s^{-1}$. The yield of $MV^{\cdot+}$ increased with increase in concentration of MV^{2+} and reached a saturation value at a concentration of 100 μM of MV^{2+}. From this saturation value of absorbance and extinction coefficient of $MV^{\cdot+}$ at 605 nm, the percentage yield of reducing radical was determined to be 74.1%.

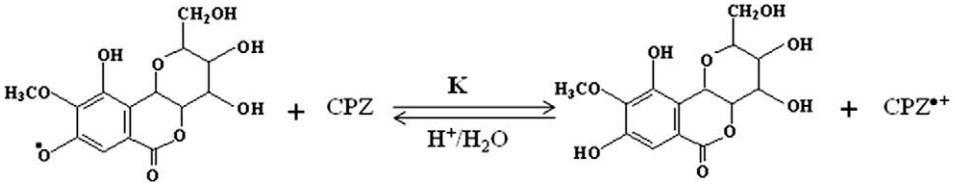


(2)

The above two results indicate that ·OH radical reaction with bergenin, produces reducing radicals in much larger yields than oxidizing radicals. Higher yield of reducing radicals compared to oxidizing radical may be due to the fact that oxidation is possible only on the phenolic OH group while reducing type radicals are produced both from hydrogen abstraction from C ring and ·OH radical addition to the phenyl ring of bergenin.

2.3. One-electron reduction potential of bergenin at pH 7

The one-electron reduction potential of bergenin was determined by monitoring reversible electron transfer involving the phenoxyl radical of bergenin and chlorpromazine radical cation ($CPZ^{\cdot+}$), following the formation of radical cation of $CPZ^{\cdot+}$ at 530 nm at different concentration of bergenin and CPZ. Under these conditions, the following equilibrium (reaction 3) was established:



(3)

At the equilibrium, the observed first order rate constant (k_{obs}) for the formation of $CPZ^{\cdot+}$ is related to the concentration of CPZ and bergenin according to Eq. 4

$$k_{obs} = k_f[CPZ] + k_b[Bergenin] \quad (4)$$

where k_f and k_b are forward and backward rate constants. Rearranging this equation we get[28,29]

$$\frac{k_{obs}}{[Bergenin]} = \frac{k_f[CPZ]}{[Bergenin]} + k_b \quad (5)$$

The k_{obs} was determined by monitoring the formation of $CPZ^{\cdot+}$ at 530 nm at different concentrations of bergenin (0.5–2 mM) and CPZ (100 μM) in presence of 0.1 M of sodium azide. The plot of k_{obs}/[Bergenin] as a function of [CPZ]/[Bergenin] as shown in Figure 7 gave a straight line with slope/intercept value of 19.79 corresponding to the equilibrium constant K (k_f/k_b). The K is also related to the equilibrium absorbance of the transients and its ground state concentration as

$$\frac{1}{A_{eq}} = \frac{1}{K\varepsilon l[R]}\left(\frac{[Bergenin]}{[CPZ]}\right) + \frac{1}{\varepsilon l[R]} \quad (6)$$

where A_{eq} is the absorbance at 530 nm for the equilibrium condition (Eq. 3), ε the molar absorptivity at 530 nm, the path length (l = 1 cm) and [R] total radical concentration.[28,29] The validity of this equation is based on the assumption that, as [Bergenin] or [CPZ] was varied the total radiolytic yield (i.e., [R] = [Bergenin·] + [$CPZ^{\cdot+}$]) remained constant under the fixed dose condition. The plot (inset of Fig. 7) of $1/A_{eq}$ as a function of [Bergenin]/[CPZ] gave a straight line with intercept/slope (K) of 22.35. An average value of K = 21.07 was obtained from the above two methods. According to Nernst's equation, using the average K value as 21.07, the difference in the electrode potential of the two couples (ΔE), was determined to be 0.078 V. Using the standard reduction potential of $CPZ^{\cdot+}$/CPZ as 0.86 V versus NHE,[22] the reduction potential of the couple Bergenin·, H^+/Bergenin was estimated as 0.938 V versus NHE at pH 7. This value is much higher than that of many known antioxidants like vitamin E, trolox etc.[33–35]

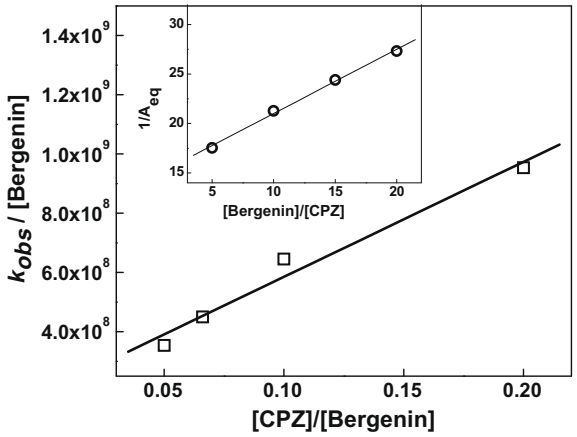


Figure 7. Plot of k_{obs}/[Bergenin] versus [CPZ]/[Bergenin]obtained on pulse radiolysis of N_2O saturated aqueous solution of 0.1 M N_3^- and different concentration of CPZ and bergenin. Inset plot of $1/A_{eq}$ versus [Bergenin]/[CPZ] obtained on pulse radiolysis of N_2O saturated aqueous solution of 0.1 M N_3^- and different concentration of CPZ and bergenin.

2.4. Effect of oxygen on the transient behaviour

To further confirm that the reaction of bergenin with ·OH radical proceeds, as given in Scheme 2, effect of oxygen on the ·OH radical reaction was studied. While phenoxyl radicals generally do not react with oxygen, the carbon-centred radicals produced from the addition and hydrogen abstraction are mostly reactive towards oxygen.[19,29,36] For this, pulse radiolysis of 0.1 mM bergenin at pH 7 was carried out in N_2O–oxygen purged system. The transient spectrum as given in the inset (a) of Figure 8, under these conditions looked significantly different (with λ_{max} = 400 nm), from that obtained in N_2O saturated system. Similar absorbing transient was observed on reaction of bergenin with model peroxyl radicals, $CCl_3O_2^{\cdot}$ radicals at pH 7. Figure 8 shows the transient optical absorption spectrum obtained on pulse radiolysis of aerated aqueous solution of bergenin with $CCl_3O_2^{\cdot}$ radical, which looks similar to that given in the inset (a) of Figure 8. By monitoring the k_{obs} (inset (b) of Fig. 8) for the formation of the transient at 400 nm, as a function of bergenin concentration from 0.5 to 2 mM, the bimolecular rate constant for the reaction of $CCl_3O_2^{\cdot}$ radical with bergenin was evaluated and listed in Table 1. The transient formation under these oxygenated systems may correspond to formation of peroxyl type radicals, as given in Scheme 2.

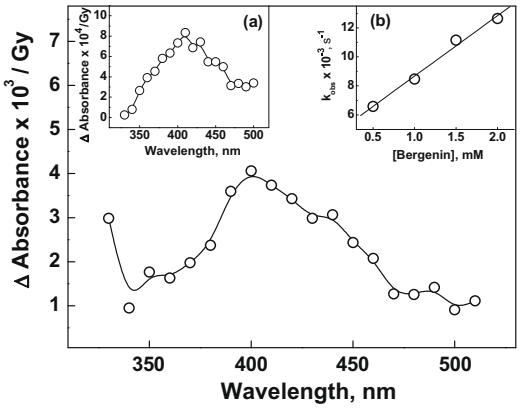


Figure 8. Transient absorption spectra obtained on reaction of trichloro methyl peroxyl radical ($CCl_3O_2^{\cdot}$) reaction with 1 mM bergenin (dose 14 Gy). Inset (a) shows the transient absorption spectra on reaction of ·OH radical with bergenin at pH 7 in aerated condition and inset (b) shows the linear plot obtained by the formation kinetics at 400 nm which shows that observed rate of formation (k_{obs}) increases with increase in concentration of bergenin.

3. Conclusions

Bergenin, a natural polyphenol, reacts with ·OH radical by multiple reaction pathways leading to formation of different products. Phenoxyl radicals of bergenin are formed by one-electron oxidation and addition–elimination reaction. Bergenin radical products are formed by ·OH radical addition and H-atom abstraction. Formation of phenoxyl radicals was confirmed by comparing the transient spectra and decay kinetics with that produced from $Br_2^{\cdot-}$ radical reaction. Although in most of the natural polyphenolic compounds, ·OH radical reaction produces, phenoxyl radical as a major reaction product, in the present case reducing radical adducts are the major transients formed. The reducing radicals are formed by addition of ·OH radical to the phenyl ring and also by H-atom abstraction from the C ring in bergenin. The one-electron reduction potential of Bergenin·, H^+/Bergenin was 0.938 V versus NHE at pH 7. The radical adducts react with oxygen which may result in formation of peroxyl type radicals. The results confirm that like other natural polyphenols, bergenin is able to scavenge oxidant free radicals very effectively, but the resultant radicals are reactive and they act as a source of peroxyl radical. Therefore bergenin may not act as an antioxidant in protecting the biomolecules from free radical induced oxidative damage. However such compounds, may act as pro-oxidants and such pro-oxidants may find application as anti-tumour agents.

4. Experimental

Bergenin, sodium azide, potassium bromide, methyl viologen (MV^{2+}), chlorpromazine (CPZ), were purchased from Sigma/Aldrich Chemicals, USA. *N,N,N′N′*-tetramethyl-*p*-phenylenediamine (TMPD) was from Lancaster, UK. All other reagents used were of highest purity available. Nanopure water from Millipore system was used for preparing the solutions and freshly prepared solutions were used for each experiment.

The pulse radiolysis experiments were carried out with high energy electron pulses (7 Mev, 500 ns) obtained from Linear electron accelerator.[37] The transients were detected by absorption spectrometry. The absorbed dose was measured by using aerated thiocyanate dosimeter by monitoring the $(SCN)_2^{\cdot-}$ species at 475 nm with $G\varepsilon$ value of 2.59×10^{-4} m^2 J^{-1}.[38] Here G denotes the radiation chemical yield in mol/J and ε, the molar absorption coefficient in m^2/mol. Typical dose/pulse used for these studies were varied from 14 Gy and 50 Gy.

The rate constant values were obtained from that kinetic analysis for which very good correlation was obtained between the experimental and calculated results. The bimolecular rate constant was determined from the linear regression plot of k_{obs} versus solute concentration for at least three experiments and the variation was within ±10%.

Radiolysis of water produces e_{aq}^-, H· and ·OH radicals in addition to the molecular products H_2, H_2O_2 and H_3O^+.[16,39–41] The reaction of ·OH radicals was studied in N_2O saturated system, where all the e_{aq}^- is quantitatively converted into ·OH. G-value for ·OH radicals at neutral pH conditions is 0.6 μmol J^{-1}. Specific one-electron oxidants, $N_3^{\cdot}$ and $Br_2^{\cdot-}$ radicals were produced by radiolysis of N_2O saturated aqueous solutions containing 0.1 M NaN_3 and KBr and G-values of $N_3^{\cdot}$ and $Br_2^{\cdot-}$ radicals at pH 7 is 0.69 μmol J^{-1}.[42,43] Trichloromethyl peroxyl radicals ($CCl_3O_2^{\cdot}$) were generated by radiolysis of aerated aqueous solution of bergenin containing 48% of

2-propanol and 4% of CCl_4 which has the *G*-value of 0.64 μmol J^{-1}.[44,45]

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