



## THEORETICAL STUDY OF THE ANTIOXIDANT PROPERTIES OF CURCUMIN: BIS(4-HYDROXY-3-METHOXYPHENYL)-1,6-HEPTADIENE-3,5-DIONE

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### ABSTRACT

*Curcumin* is a yellow pigment of turmeric, a spice manufactured from the root of *Curcuma longa* and is currently the focus of enormous research owing to its antioxidant properties as the mechanism of action of *curcumin* as the antioxidant properties is not very clear. In the present study, the *structures, energetic, one electron oxidation potential and global reactivity descriptors* of *curcumin* in both neutral and charged states have been investigated using density-functional theory. The *keto-enol* tautomerism study shows that *enol* form is the predominant form of the compound. In order to probe the preferred site of reactivity, and hence antioxidant activity, the *Fukui function;  $f_k^-$*  has also been evaluated for *curcumin* in both *keto* and *enol* form of molecules. The trends observed in various global reactivity descriptors along with *Fukui indices* clearly indicates that *curcumin* is a potent antioxidant and the antioxidant activity of *curcumin* is mainly due to phenolic -OH groups in the two phenyl rings present in the molecule. The results obtained are important guidelines towards the detailed understanding of the antioxidant mechanism of *curcumin*.

### INTRODUCTION

Prime ayurvedic therapeutic formulations draw on an impressive array of plants. In the classical ayurveda literature enormous number of plants with therapeutic claims such as *immunomodulators, memory enhancers, neuroprotectives, anti-obesity* and *anti-ageing*, have been described. The vast wealth of information present in ayurveda material-medicina clearly demonstrate the need for carrying out investigations on scientific lines to reinforce the claims of this traditional medicinal system and to put the therapeutic formulations of the system into the modern time frame. There is growing

evidence in literature, which shows that old molecules are finding new applications through a better understanding of traditional knowledge and clinical observations. The ayurvedic medicinal plants, thus, provide a fertile ground for exploration using modern scientific tools. One very active area of research in this regard is the study of antioxidant properties of constituents of prime ayurvedic plants of rich medicinal importance. Excessive production of reactive oxygen species<sup>1-2</sup> (ROS) i.e.  $\text{OH}^\bullet$ ,  $\text{O}_2^\bullet$  and  $\text{ROO}^\bullet$ ,.... etc resulting from the metabolic processes in an organism is involved in the oxidative damage of important biological constituents such as DNA, proteins and lipids and hence causing very serious diseases. Antioxidants<sup>3-4</sup> plays an important role in the inhibition of the oxidative damage of the bio molecules and is present in abundance in many of the fruits<sup>5</sup> and vegetables and more importantly in a large number of ayurvedic medicinal botanicals. Antioxidants are also very useful in prevention of auto-oxidation of biomolecules in food tissues in packaged food when added as food preservatives. Thus, there are two dimensions of application of antioxidants namely the health sector and the food sector. Study of antioxidants in the food sector primarily aimed at better food quality maintenance through prevention of oxidation. On the other hand the Study of antioxidant, in the health sector, is carried out in relation to oxidative stress<sup>6</sup> related disorders. Antioxidants are broadly classified as, according to their mechanism of action, primary or chain breaking antioxidants and secondary or preventive antioxidants.

At present, the plant; *Curcuma longa* (turmeric), is the focus of enormous interest. The interest in the plant is mainly due to the active molecule *curcumin*, present in the rhizome of the plant, being evaluated for its antioxidant properties, however, the mechanism of action of *curcumin* for the antioxidant properties is not very

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clear. With structural formulae bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, the *curcumin* structure contains a variety of functional groups including the  $\beta$ -diketo group, carbon-carbon double bonds and phenyl rings containing varying amounts of hydroxyl and methoxy substituents. Fig. 1 shows two tautomeric forms of *curcumin*. Because of the contradictory results<sup>7-8</sup> reported in literature on the site of activity, moreover, considering the fact that various key factors are being missed in the previous studies, a systematic and thorough investigation is essential for better insight into the site of activity responsible for the antioxidant effects of *curcumin*. In the present study, the structures, energetic, one electron oxidation potential, keto-enol tautomerism and global reactivity descriptors of *curcumin* in both neutral and charged states have been investigated using density-functional theory. In order to probe the preferred site of antioxidant activity, the Fukui function;  $f_k^-$  has also been evaluated for *curcumin* in both *keto* and *enol* form of molecules. The trends observed in various global reactivity descriptors along with Fukui indices clearly indicates that the antioxidant activity of *curcumin* is mainly due to phenolic -OH groups in the two phenyl rings present in the molecule. The results obtained are important guidelines towards the detailed understanding of the antioxidant mechanism of antioxidant activity of *curcumin*.

## METHODOLOGY

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The methodology adopted for determination of physical and chemical properties of the spice under study is being based on the first principal's Quantum Mechanical methods. The methods predict accurate picture of what happens, structurally and dynamically, even at the atomic level and helps in understanding and description of physical, chemical and biological systems, and consequent prediction of their behaviour under a wide variety of conditions.

We work is based on the density functional theory<sup>9</sup> (DFT) approach to estimate the electronic structure of the *curcumin* by employing Beck's three parameters exact exchange functional (B3) combined with non local gradient corrected correlation functional of Lee-Yang-Parr (LYP), denoted as B3LYP and the 6-311G basis sets.

Conceptual density functional theory (DFT) was used to obtain the global chemical reactivity descriptors<sup>10</sup> such as electronegativity ( $\chi$ ), chemical potential ( $\mu$ ), chemical hardness ( $\eta$ ),

chemical softness ( $S$ ), and electrophilicity index ( $\omega$ ).

The ionization potential (IP) and electron affinity (EA) values were calculated using the finite difference approximation, and are given by

$$\begin{aligned} \text{IP} &= E(N-1) - E(N) \\ \text{EA} &= E(N) - E(N+1) \end{aligned}$$

$E(N)$  represents the electronic energy of the  $N$ -electron system.

$$\begin{aligned} \chi &= -\mu = -\left(\frac{\partial E}{\partial N}\right)_{v(\vec{r})} = \frac{(\text{IP} + \text{EA})}{2} \\ \eta &= \frac{1}{2} \left(\frac{\partial^2 E}{\partial N^2}\right)_{v(\vec{r})} = \frac{1}{2} \left(\frac{\partial \mu}{\partial N}\right)_{v(\vec{r})} \\ &= \frac{(\text{IP} - \text{EA})}{2} \\ S &= \frac{1}{2\eta} \end{aligned}$$

The charge transfer parameter determines the fractional number of electrons, transferred from an antioxidant molecule; A to an acceptor system i.e. ROS represented as "D", and is given by

$$\Delta N = \frac{\mu_D - \mu_A}{2(\eta_A + \eta_D)}$$

where  $\mu$  represents the electronic chemical potential and  $\eta$  represents the chemical hardness of the respective chemical species. For electronic charge transfer from the donor to acceptor, the  $\Delta N$  value should be negative.

The condensed Fukui functions<sup>11</sup> obtained from local charges using the finite difference method as follows

$$\begin{aligned} f_k^- &= q_k(N) - q_k(N-1) \\ f_k^+ &= q_k(N+1) - q_k(N) \end{aligned}$$

where  $q_k$  represents the electronic population of atom  $k$  in a molecule and is obtained using NPA. All the calculations were performed using Gaussian 09 computational package.

## RESULT AND DISCUSSION

The fully optimized structures of *Curcumin* in both *keto* (*Cur-K*) and *enol* (*Cur-E*) form, in the gas phase, obtained at the B3LYP level using the 6-311G basis set, are shown in Fig. 2. The optimized geometries of these *curcumin* molecules are being put to use for evaluation of energies and investigation of electronic structures of these molecules. The vertical ionization potential (IP) and electron affinity (EA) for *Cur-K*, *Cur-E* and the ROS, calculated using 6-311G basis set, are given in Table 1. The vertical ionization (or electron affinity)

**Table 1: Calculated IP, EA and various DFT based global reactivity descriptors for curcumin**

Molecules	Medium	IP(eV) Vert.	EA(eV) Vert.	$\chi = -\mu$	$\eta$	S	$\omega$
Curcumin keto form	Gas	7.10	0.96	4.03	3.06	0.16	2.65
	$\epsilon=78.36$	5.88	2.57	4.22	1.65	0.30	5.39
Curcumin enol form	Gas	6.71	1.08	3.89	2.81	0.17	2.69
	$\epsilon=78.36$	5.58	2.73	4.16	1.42	0.35	6.08
$\cdot\text{OH}$	Gas	16.32	1.77	9.05	7.27	0.07	5.65
	$\epsilon=78.36$	12.79	5.21	9.00	3.79	0.13	10.71
$\cdot\text{OOH}$	Gas	12.62	0.54	6.58	6.04	0.08	3.60
	$\epsilon=78.36$	9.48	3.71	6.60	2.88	0.17	7.54
$\text{O}_2^{\cdot-}$	Gas	2.79	-7.52	-2.36	5.15	0.09	0.54
	$\epsilon=78.36$	5.87	1.43	3.65	2.22	0.22	3.00

is the difference in the total energies of the neutral and positively (or negatively) charged molecules where the molecular geometries of these molecules were optimized in the neutral state only. The calculated vertical IP values for *Cur-K* and *Cur-E* molecules in the gas phase are 7.10 eV and 6.71 eV, respectively. The vertical IP values computed for ROS using 6-311G basis set,  $\cdot\text{OH}$  (16.346 eV), and  $\cdot\text{OOH}$  (12.646 eV) are higher than all of those for both the *Cur-K* and *Cur-E*

higher EAs of the neutral ROS compared to those of the antioxidant molecules, clearly demonstrate the antioxidant behavior of *Cur-K* and *Cur-E* molecules. The EAs of ROS (including  $\text{O}_2^{\cdot-}$ ) in aqueous medium are found to be positive, which is a clear indication of the possibility of existence of  $\text{O}_2^{\cdot-}$  in the aqueous medium. The calculations of global reactivity descriptors were performed in order to analyze the reactivities of the studied curcuminoid molecules. The calculated chemical

**Table 2: Calculated electronic energy (in Hartree units) of neutral ( $E_N$ ) and Cationic ( $E_{N-1}$ ) molecular species.**

	Gas phase		Solvent medium	
	$E_N$	$E_{N-1}$	$E_N$	$E_{N-1}$
Curcumin Keto form	-1263.19	-1262.93	-1263.21	-1262.99
Curcumin Enol form	-1263.20	-1262.95	-1263.22	-1263.01

molecules. The electron charge transfer ability is shown by lower IP's of curcuminoids, compared to the ROS. An appreciable decrease in the IP, in the presence of solvent, was observed. As the solvent plays a crucial role in prediction of the reactivities of the ionic species, the study was performed in the aqueous medium for  $\text{O}_2^{\cdot-}$ . Theoretically calculated EAs are particularly useful as the EAs helps in evaluating reactivity descriptors and explaining phenomena such as donor-acceptor interactions between antioxidants and the ROS. The calculated EA values for these molecules are lower than those for the neutral ROS  $\cdot\text{OH}$  and  $\cdot\text{OOH}$  in the same media. The lower IPs of these molecules than those of the neutral ROS and the

hardness ( $\eta$ ) is found to be lower in the solvent medium than in the gas phase. The calculated  $\eta$  values of these molecules are 3.06 and 2.81, for *Cur-K* and *Cur-E*, respectively, in the gas phase and in the solvent medium the corresponding values are 1.65 and 1.42, respectively. The  $\eta$  values of the ROS  $\cdot\text{OH}$ , and  $\cdot\text{OOH}$  are 7.296 and 6.058 eV, respectively which reduce to 3.809 and 2.904 eV in aqueous medium. The presence of the solvent, therefore, increases the reactivities of these molecules and ROS. The common feature of the antioxidant mechanism is the charge transfer between the antioxidant and the ROS. The Mulliken electronegativity ( $\chi$ ) is a measure of the tendency of the molecule to attract electrons. The

**Table 3: Calculated charge transfer descriptors of Keto and Enol forms of curcumin**

	Gas phase			Solvent medium		
	$\dot{\text{O}}\text{H}$	$\dot{\text{O}}\text{OH}$	$\dot{\text{O}}_2^-$	$\dot{\text{O}}\text{H}$	$\dot{\text{O}}\text{OH}$	$\dot{\text{O}}_2^-$
<b>Curcumin Keto form</b>	-8.855	-6.366	-2.110	-8.608	-6.120	-3.085
<b>Curcumin Enol form</b>	-8.856	-6.367	-2.118	-8.620	-6.137	-3.110

computed  $\chi$  values for these antioxidant molecules are lower than those for the neutral ROS. The  $\chi$  of  $\cdot\text{OH}$  is higher than the  $\chi$  of  $\cdot\text{OOH}$ . This suggests that, among the ROS,  $\cdot\text{OH}$  is very reactive in nature<sup>12</sup>. The presence of solvent and the polarity of the solvent are found to have negligible effects on the electronegativities of antioxidant molecules and neutral ROS. The electrophilicity index ( $\omega$ ) also helps to understand the tendency of a molecule to soak up electrons. The high electrophilicities ( $\omega$ ) of  $\cdot\text{OH}$  and  $\cdot\text{OOH}$  are found to be higher than those of all the antioxidant molecules (Table 1), and this clearly indicates the high propensity of the ROS to

A greater magnitude of  $\Delta\text{N}$  for *Cur-E* than that of *Cur-K* (Table 3), indicates that *Cur-E* exhibit greater antioxidant potential than that of *Cur-K*. The one electron oxidation potential values, obtained from the calculated energies, are 0.70 V and 0.52 V in the gas phase and in aqueous medium for the *Cur-K*, while corresponding values for *Cur-E* are 0.79 V and 0.58 V, respectively. These values are nearly consistent with those predicted by experimental studies<sup>14</sup>. This also indicated greater antioxidant potential of *Cur-E* than *Cur-K*.

In order to probe the preferred site of reactivity of these curcuminoids towards the ROS,

**Table 4: Fukui function [ $f_k^-$ ] values at different sites of Keto and Enol forms of curcumin**

Curcumin's Site of Activity [ $f_k^-$ ]	<i>Cur-K</i>		<i>Cur-E</i>	
	Gas	$\epsilon=78.36$	Gas	$\epsilon=78.36$
<b>C<sub>10</sub> Carbon atom</b>	-0.004	-0.002	-0.044	-0.044
<b>O<sub>15</sub> Oxygen atom</b>	-0.065	-0.054	-0.056	-0.060
<b>O<sub>16</sub> Oxygen atom</b>	-0.065	-0.054	-0.056	-0.060

attract electrons. The high  $\omega$  values of  $\cdot\text{OH}$  also determine its highly reactive nature, as mentioned earlier.

*Cur-K* and *Cur-E* are two isomeric forms of curcumin, *Cur-E* has been found to be lower in Gibbs energy at 298 K. For the equilibrium *Cur-K*  $\leftrightarrow$  *Cur-E*, the  $K_{eq}$  calculated values;  $5.94 \times 10^2$  in the gas phase and  $2.62 \times 10^2$  in the aqueous phase show that *Cur-E* is a preferred form of the molecule and is in accordance with the previous studies<sup>13</sup>.

For interaction between the antioxidant molecules and the ROS the charge transfer parameter  $\Delta\text{N}$  which determines the fractional number of electrons, transferred from a system A to system D (ROS), is considered.

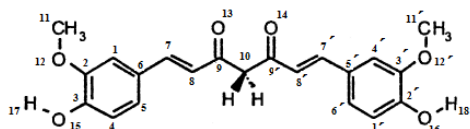
$$\Delta\text{N} = \frac{\mu_{\text{D}} - \mu_{\text{A}}}{2(\eta_{\text{A}} + \eta_{\text{D}})}$$

Where  $\mu_{\text{A}}$ ,  $\mu_{\text{D}}$  and  $\eta_{\text{A}}$ ,  $\eta_{\text{D}}$  are the chemical potential and chemical hardness of the system A (antioxidant species ROS) and D (ROS) respectively.

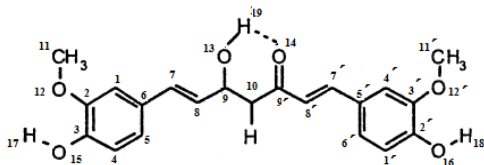
the Fukui function  $f_k^-$  was evaluated at the primary hydroxyl group and also the  $>\text{CH}_2$  group at C<sub>10</sub> site of these antioxidant molecules.  $f_k^-$  indicates the decrease in negative charge from the site k (oxygen atom), upon the removal of one electron from the molecule, the greater the magnitude of  $f_k^-$ , the greater the loss in charge on the oxygen atom and consequently the greater the increase in charge on the ROS. A comparison of the magnitude of  $f_k^-$  values (Table 4) at specified sites in the structure of these molecules shows that the preferred site responsible for the antioxidant activity are the phenolic  $-\text{OH}$  groups present in the aromatic rings of these molecules.

## CONCLUSIONS

We have carried out *first principle's* density functional theory based calculations of the structures, energetics, ionisation potential, electron affinity and various DFT based global chemical reactivity descriptors of interest for keto and enol form of curcumin in both neutral and charged states. The results of our investigations

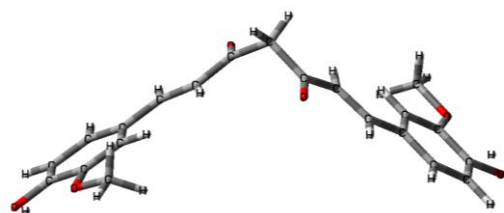
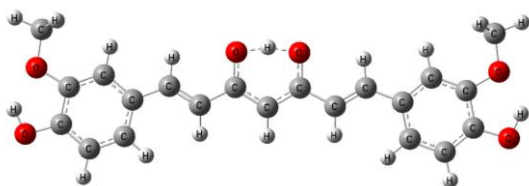


**Curcumin-keto form**



**Curcumin-enol form**

**Fig.1: Keto and Enol forms of curcumin.**



**Fig.2: Optimized Geometries structures of (a) Curcumin-keto form (Cur-K) and (b) Curcuminenol form (Cur-E)**

confirm the rich antioxidant potential of the studied curcuminoids. The trends observed in various reactivity descriptors clearly indicates that the phenolic –OH groups present in the aromatic rings of these molecules are the preferred site responsible for the antioxidant activity. The results support the statement that curcumin is a phenolic chain breaking primary antioxidant.

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