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DFT BASED COMPARATIVE STUDY OF THE ANTIOXIDANT PROPERTIES OF *ROSMARINIC ACID* AND *ALOE EMODIN*

Amit Kumar*, A K Bhagi and Navneet Manav

Department of Chemistry, Dyal Singh College, University of Delhi, Lodi Road New Delhi 110003

ABSTRACT

Naturally occurring polyphenolics are important compounds and with antioxidative pathways can play important role in the inhibition of the oxidative damage of the bio molecules. Rosmarinic acid and Aloe emodin have important roles in this regard. In the present study a quantitative analysis of the antioxidant activity of Rosmarinic acid and Aloe emodin compounds obtained from Rosmery and Aloe vera plants, respectively, has been obtained by the determination of the structures, energetics and global reactivity descriptors in both neutral and charged states using Density Functional Theory. The present study is an important step for complete understanding of the structure property relationship towards establishing the antioxidant mechanism of these compounds.

Keywords : ROS, Antioxidant, Rosmarinic Acid, Aloe Emodin, Density Functional Theory.

INTRODUCTION

Rosmarinic acid[1] a naturally occuring polyphenolic compound is extracted from the rosmery plant (Tulsi), with scientific name *Rosmarinus officinalis L*. Rosmarinic acid is an ester of caffeic acid and 3,4-dihydroxy-phenyl lactic acid. The structural features of the compound shows that it contains two aromatic rings, the ring A and ring B. Both the rings have two hydroxyl groups attached at ortho position. Apart from the two phenolic rings, the compound has other functionalities[2], such as a carbonyl group, a C=C double bond and also a carboxylic acid group. All these functionalities lie between two phenolic rings. The chemical structure of the compound is shown in Fig. 1 (a). Many biological activities[3] have been attributed to the rosmarinic acid such as inhibiting the blood clots antihapatitis and antiinflamatory activities. Rosmarinic acid has also been found to be strong free radical scavenger; a recent study shows that the antioxidant activity is over three times than that of Trolox. Moreover it also acts as inhibitor of Xanthine oxidase producing superoxide anion radicals. Rosmarinic acid has also been shown to reduce Mo (VI) to Mo (V), thereby preventing the production of free radicals caused by the metal[4]. In view of these properties rosmarinic acid can be considered an important naturally occurring compound. The main interest of the scientific researchers lies in its antioxidant activity. Aloe emodin is also an important constituent of Aloe vera. It is an anthraquinone compound present in the leaves of the plant[5]. Several studies have shown the antioxidant, antinocieptive and antiinflamatory activities of the aloe vera species[6]. The chemical structure of Aloe emodin is shown in Fig 1 (b). One of the main objectives of the study is to carry out investigations on scientific lines aimed at understanding antioxidant properties of these active constituents. The use of *Quantum Mechanics* and *Computational Chemistry* offer an innovation pathway in this regard and helps in investigation of *Molecular Geometry, Energy of molecules, transition states, chemical reactivity* and hence *physical* & *biological activities* of substances. The research work in the study is based on *First Principle's*[7] quantum mechanical study of the structure property relationship of naturally occurring *Rosmarinic acid* and *Aloe emodin* compounds aimed at clarifying the active sites and hence mechanism of action of the molecule responsible for the antioxidant activity. A quantitative analysis of the antioxidant activity of *Rosmarinic acid* and *Aloe emodin* compounds, has been obtained by the determination of the *structures*, *energetics* and *global reactivity descriptors* in both neutral and charged states using Density Functional Theory. The present study is an important step for complete understanding of the structure property

Corresponding Author:

Email: akumardsc@gmail.com

Table 1: Structural parameters; *Bond Lenths and Bond Angles* in optimized geometry of *Rosmarinic acid*.

Molecule → / Bond Length ↓	*Rosmarinic Acid*	Bond Angle	*Rosmarinic Acid*
O_1–C_1	1.420	O_1–C_1–C_2	111.483
C_1–C_2	1.437	O_1–C_1–C_6	127.650
C_2–C_3	1.413	C_2–C_1–C_6	120.858
C_2– O_2	1.406	O_2–C_2–C_1	117.982
C_3–C_4	1.406	O_2–C_2–C_3	122.693
C_4–C_5	1.422	C_2–C_3–C_4	119.487
C_5–C_6	1.428	C_3–C_4–C_5	121.718
C_1–C_6	1.401	C_4–C_5–C_6	118.738
C_5–C_7	1.487	C_4–C_5–C_7	122.765
C_{11}–C_{12}	1.578	C_5–C_6–C_1	119.872
C_7–C_8	1.355	C_7–C_8–C_9	120.650
C_8–C_9	1.513	C_8–C_9 –O_9	127.705
C_9– O_9	1.257	O_9–C_9– O_{10}	123.524
C_9– O_{10}	1.443	C_9–O_{10}–C_{11}	109.984
O_{10}–C_{11}	1.490	C_{11}–C_{13}– C'_1	112.734
C_{11}–C_{13}	1.573	C_{11}–C_{12}– O'_{12}	127.875
C_{12}– O'_{12}	1.252	C_{11}–C_{12}– O_{12}	109.335
C_{12}– O_{12}	1.423	C_{13}– C'_1– C'_2	120.153
C'_3– O'_4	1.405	C_{13}– C'_1– C'_6	120.073
C'_4– O'_4	1.421	C'_1– C'_2– C'_3	121.091
C'_4– C'_5	1.435	C'_2– C'_3– C'_4	119.105
C'_5– C'_6	1.409	C'_3– C'_4– C'_5	120.522
C'_5– O'_5	1.411	C'_5– C'_6– C'_1	119.897
O_1–H_1	1.020	C'_3– C'_4– O'_4	127.399
O'_3–H'_3	1.032	O'_4– C'_4– C'_5	112.078
O'_4–H'_4	1.021	O'_5– C'_5– C'_6	122.256
O_2–H_2	1.033	C_3–C_4–C_{14}	–
O_{12}–H_{12}	1.029	C_{14}–C_{13}–C_1	–

relationship towards establishing the antioxidant mechanism of these compounds.

METHODS

We worked within the framework of first principles to estimate the electronic structure of the antioxidant molecules 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. The structures of the antioxidant molecules have been optimized via direct inversion in iterative subspace (DIIS), as implemented in the Gaussian 09.

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

$$\mathbf{IP = E(N-1) - E(N)}$$
$$\mathbf{EA = E(N) - E(N+1)}$$

E(N) is the electronic energy of the N-electron system. The vertical IP (or EA) is calculated as the difference in total energies of the neutral and positively (or negatively) charged molecular species, with the molecular geometry being optimized in the neutral state.

Conceptual density functional theory (DFT)[8] approach has been used to obtain the global chemical descriptors such as electronegativity (χ), chemical potential (μ), chemical hardness (η), chemical softness (*S*) , and electrophilicity index

Table 2: Structural parameters; *Bond Lenths and Bond Angles* in optimized geometry of *Aloe emodin*

Molecule → / Bond Length ↓	*Aloe Emodin*	Bond Angle	*Aloe Emodin*
O_1–C_1	1.430	O_1–C_1–C_2	120.012
C_1–C_2	1.394	C_2–C_3–C_4	119.998
C_2–C_3	1.395	C_6–C_7–C_8	119.768
C_3– C'_3	1.540	C_{11}–C_8–O_8	119.993
C'_3– O'_3	1.430	C_3–C_4–C_{14}	120.000
C_3–C_4	1.394	C_{14}–C_{13}–C_1	119.993
C_4–C_{14}	1.395	C_{13}–C_1–C_2	119.994
C_{13}–C_{14}	1.394	C_9–C_{13}–C_{14}	123.349
C_{13}–C_1	1.395	C_{11}–C_9–C_{13}	115.138
C_{13}–C_9	1.540	C_{13}–C_{14}–C_{10}	120.011
C_9– O_9	1.258	C_{10}–C_{14}–C_4	119.983
C_9–C_{11}	1.615	C_{10}–C_{12}–C_5	120.004
C_{11}–C_{12}	1.395	C_{12}–C_{11}–C_9	121.617
C_{12}–C_{10}	1.540	C_{12}–C_{11}–C_8	120.008
C_{10}– O_{10}	1.258	C_7–C_9–C_{11}	119.994
C_{12}–C_5	1.394	C_{12}–C_5–C_6	120.000
C_5–C_6	1.395	C_5–C_7–C_8	120.004
C_6–C_7	1.394	O_9–C_9–C_{13}	120.226
C_7–C_8	1.395	O_{10}–C_{10}–C_{14}	120.226
C_8– O_8	1.430	O_{10}–C_{10}–C_{12}	119.886
C_8–C_{11}	1.394	O_8–C_8–C_{11}	120.012
O_8–H_8	1.090	O_9–C_9–C_{11}	124.634
O_1–H_1	1.090	C_{13}–C_1 –O_1	119.993
O'_3–H'_3	1.027	O'_3– C'_3– C_3	109.471
		C_2–C_3– C'_3	119.997

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(ω) as well as local chemical descriptors[9]. The global reactivity descriptors describe the reactivity of molecule as a whole. The above specified global reactivity descriptors can be calculated as follows

$$\chi = -\mu = -\left(\frac{\partial E}{\partial N}\right)_{v(\vec{r})} = \frac{(IP + EA)}{2}$$

$$\eta = \frac{1}{2}\left(\frac{\partial^2 E}{\partial N^2}\right)_{v(\vec{r})} = \frac{1}{2}\left(\frac{\partial \mu}{\partial N}\right)_{v(\vec{r})} = \frac{(IP - EA)}{2}$$

$$S = \frac{1}{2\eta}$$

HSAB concept has been employed to assess the reactivity of the antioxidant molecules towards ROS. The global interaction between the Antioxidant (donor) and ROS (acceptor) was obtained by the charge transfer parameter; ΔN. The details of calculation of charge transfer parameter have been discussed in a recent study[10] on antioxidants by us. For electronic charge transfer from the donor to acceptor, the ΔN value should be negative.

RESULTS AND DISCUSSION

The fully optimized structures of the Rosmarinic acid and *Aloe emodin* as well as the ROS in the gas phase and the solvent phase obtained at the B3LYP level using the 6-311G basis set, are shown in Fig. 2. Theoretically calculated bond angles for these molecules are listed in Table 1-2. The electronic structures of a molecule is

Table 3: Calculated IP, EA and various DFT based global reactivity descriptors

Molecules	Medium	IP(eV) Vert.	EA(eV) Vert.	χ = - μ	η	S	Ω
Rosmarinic Acid	**Gas**	7.196	0.572	3.884	3.312	0.151	2.277
	ε=78.36	5.948	2.287	4.118	1.830	0.273	4.632
Aloe emodin	**Gas**	7.991	1.950	4.970	3.020	0.166	4.089
	ε=78.36	6.397	3.624	3.713	1.386	0.361	9.055
$^{\bullet}$OH	**Gas**	16.328	1.771	9.050	7.279	0.069	5.651
	ε=78.36	12.799	5.216	9.008	3.792	0.132	10.711
$^{\bullet}$OOH	**Gas**	12.628	0.547	6.588	6.041	0.083	3.602
	ε=78.36	9.489	3.716	6.603	2.887	0.173	7.543
$O_2^{-\bullet}$	**Gas**	2.795	-7.521	-2.363	5.158	0.097	0.542
	ε=78.36	5.877	1.434	3.656	2.222	0.225	3.007

related to the chemical structure which in turn determine the chemical properties, the optimised geometries of these potential antioxidant molecules has been used for single point energy calculations on these molecules and for understanding the electronic structures and hence properties of these molecules. The vertical ionization potentials and the electron affinities for both the potential antioxidant molecules as well as ROS, calculated using 6-311G basis sets, are given in Table 3. The vertical ionization potential (electron affinity) is calculated as the difference in the total energies of the neutral and positively (or negatively) charged molecules, with molecular geometries of the molecules optimized in the neutral state only.

The calculated vertical IP values for rosmarinic acid in the gas phase are found to be 7.196 and 5.948 eV in the gas and solution phase, respectively. The corresponding vertical IP values of aloe emodin are 7.991 eV and 6.397 eV, respectively. The IP values of ROS, $^{\bullet}$OH, (16.346 eV), and $^{\bullet}$OOH, (12.628 eV) are higher than the corresponding values for the rosmarinic acid aloe emodin molecules. IP and EA values help in determining the nature of electron transfer between the antioxidant and the radical. A higher probability of losing an electron is indicated by a lower value of the IP's of antioxidant molecules. It is clear from the trends observed in IP values that both these molecules are capable of electronic charge transfer to the neutral ROS. The solvent was found to have appreciable effect on the energetics of all these molecules. An appreciable decrease in the IP in the presence of solvent has been observed for both Rosmarinic acid and Aloe emodin, more importantly the order remains similar to that seen in the gas phase, i.e., *Rosmarinic Acid <Aloe emodin*. For neutral ROS i.e. $^{\bullet}$OH and $^{\bullet}$OOH, the average decrease in the vertical IP in aqueous medium is 3.334 and 3.330, respectively. In view of the solvent effect in deciding the reactivities of the ionic species, the study was done in the aqueous medium for superoxide radial anion; $O_2^{-\bullet}$. The EA, along with the IP, has a crucial influence on the electron transfer between the antioxidant and the radical. A higher EA indicates a higher probability of gaining an electron.

Table 4: Calculated charge transfer descriptors of antioxidant molecules

	Gas phase			Solvent medium		
	$^{\bullet}$OH	**$^{\bullet}$OOH**	**$O_2^{-\bullet}$**	**$^{\bullet}$OH**	**$^{\bullet}$OOH**	**$O_2^{-\bullet}$**
Rosmarinic Acid	-8.866	-6.380	-2.133	-8.641	-6.166	-3.147
Aloe emodin	-8.808	-6.313	-2.059	-8.524	-6.016	-2.961

The DFT based methods are increasingly employed by chemists for the theoretical determination of EAs as the method can be applied to a larger range

of atoms and molecules than any other ab initio method with precision. The calculated EA values for these molecules are found to be lower than those for the neutral ROS $^{\bullet}OH$ and $^{\bullet}OOH$ in the same media. The lower IPs of these molecules than those of the neutral ROS and the higher EAs of the neutral ROS compared to those of these molecules give the basis of the antioxidant behaviour of these molecules. The global reactivity descriptors were determined using the accurately calculated vertical ionization potentials and electron affinities, with an objective to arrive at quantitative analysis of the reactivity of the studied molecule. The calculated values of various DFT based global reactivity descriptors, for *Rosmarinic Acid*, *Aloe emodin* and also reactive oxygen species, are given in Table 3. Hardness (η) parameter is directly correlated with the stability of the molecule, whereas, softness (*S*) provides a measure of its reactivity. The calculated chemical hardness (η) value, for both the chemical species found to be lower in the solvent medium than in the gas phase. The calculated η values of these molecules are 3.312 (1.830) for *Rosmarinic acid* and 3.020 (1.386) for *Aloe emodin* in the gas phase (solution phase). The effect of solvent, in terms of difference in hardness, is greater for *Aloe emodin* (1.634) than that for *Rosmarinic Acid* (1.482). The η values of the ROS $^{\bullet}OH$ and $^{\bullet}OOH$ are 7.279 and 6.041, respectively, in the gas phase, and these values change to 3.792 and 2.887 in the aqueous medium. Hence, the presence of the solvent significantly increases the reactivities of these molecules as well as the ROS. The charge transfer forms the basis of determining antioxidant properties. For understanding the charge transfer reaction, the chemical reactivity descriptors such as the Mulliken electronegativity (χ) can be used. On the basis of χ values $^{\bullet}OH$ has been found to be most reactive ROS among the neutral ROS in a recent study[11] by our group, these findings are in consonance with experimental studies[12] on $^{\bullet}OH$. Electrophilicity index (ω), is another very useful reactivity descriptor and quantifies the tendency of a molecule to soak up the electrons. The high ω values of $^{\bullet}OH$ also supports the highly reactive nature of neutral ROS $^{\bullet}OH$. The amount of charge transfer between the antioxidant molecules (*Rosmarinic Acid and Aloe emodin*) and various ROS, calculated using the ΔN formulae, is given in Table 4. The magnitude of the charge transfer parameter for electronic charge transfer to $^{\bullet}OH$ by these potential antioxidants follows the order *Rosmarinic Acid* > *Aloe emodin* in both the gas phase (Table 4) and in aqueous medium. Similar trends were also observed, for variation in magnitude of ΔN parameter for electron transfer to other ROS i.e. $^{\bullet}OOH$ and $O_2^{-\bullet}$ from these

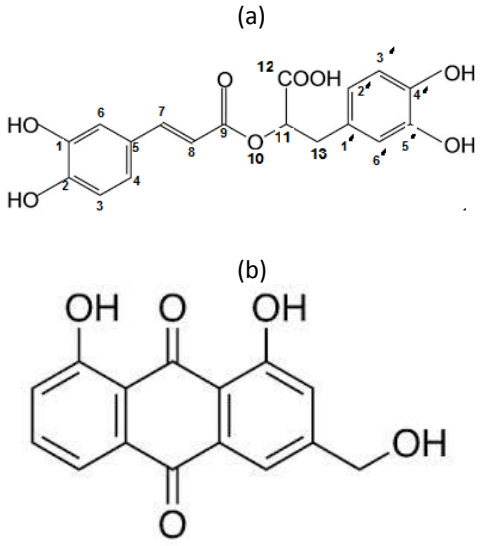

Fig.1: Molecular structures of (a) *Rosmarinic Acid* and (b) Aloe emodin

molecules.

The trends observed in all of these global reactivity descriptors along with charge transfer parameter, unanimously, predict greater antioxidant activity of the *Rosmarinic acid*, as compared to that of *Aloe emodin* studied presently. The extent of hydrogen bonding between two hydroxyl groups at o- position in the phenolic rings of these molecules play important role in stabilisation of the antioxidant radical formed as a result of electronic charge transfer to ROS and thus contributes towards the antioxidant

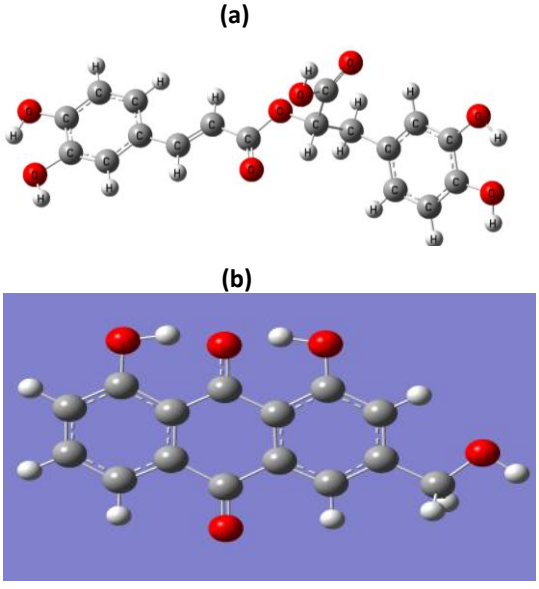

Fig.2: Optimized Geometrical structures of (a) *Rosmarinic Acid* and (d) *Aloe emodin* molecules

activity of Rosmarinic acid and the Aloe emodin.

CONCLUSIONS

We have carried out density functional theory based calculations of the *structures*, *energetics*, *ionisation potential*, *electron affinity* and various DFT based *global chemical reactivity descriptors* of interest for Rosmarinic acid and Aloe emodin molecules in both neutral and charged states. The results of our investigations indicate the rich antioxidant potential of the studied molecules. The trends observed in various reactivity descriptors clearly indicates that the phenolic –OH groups in the structure of these molecules is very important in determining the antioxidant properties of molecules. The present study is an important step for complete understanding of the structure property relationship towards establishing the antioxidant mechanism of these compounds.

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