Chapter

Theoretical Studies on Anti-Oxidant Activity of the Phytochemical, Coumestrol and Its Derivatives

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Abstract

Free radical-induced changes in cellular and organ levels have been studied as a possible underlying cause of various adverse health conditions. Important research efforts have, therefore, been made to discover more powerful and potent antioxidants/free radical scavengers for the treatment of these adverse conditions. The phytoestrogen coumestrol intensively attracted scientific interest due to their efficient pharmacological activities. In this scenario, DFT studies were carried out to test the antiradical activities of coumestrol and its derivatives. The results obtained from FEDAM plots demonstrated that the coumestrol derivatives pointed out were good radical scavengers relative to the parent molecule in the gas phase. The derivatives whose 16thposition substituted with electron-donating groups like -NH_2, -OCH_3 and -CH_3 showed good antioxidant capacity. Three antioxidant mechanisms, including hydrogen atom transfer (HAT), electron transfer followed by proton transfer (SET-PT), and sequential proton loss electron transfer (SPLET), were investigated by measuring thermodynamic parameters.

Keywords: phytochemical, coumestrol, anti-oxidant activity, global descriptive parameters, donor acceptor map, full electron donor acceptor map

1. Introduction

Extreme production of free radicals such as reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulphur species (RSS) with half-lives of just a few nanoseconds is the source of the harmful process called oxidative stress, the effects of which can significantly alter cell structures (e.g. membranes) and destroy bio molecules such as lipids, lipoproteins, proteins, and nuclei [1–3]. Our body has natural defence mechanisms provided by secondary metabolites called antioxidants to neutralise these ROSs. In the respiratory chain, the electron transfer to molecular oxygen takes place and the electron transport chain is located on the mitochondria, suggesting that the ROS is mainly formed in mitochondria [4]. Natural products reflect a diverse community of different kinds of antioxidants that inhibit or postpone the oxidation of essential cell macromolecules by scavenging certain free radicals [5, 6]. Antioxidants are commonly dispersed in different parts of plants, such as fruits, leaves, flowers, etc., and cow milk and honey milk contain a number of antioxidants [7–9].

Various antioxidant techniques have involved either the increase of endogenous antioxidant enzyme defences (e.g., superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase) or the enhancement of non-enzymatic defences (e.g., glutathione, vitamins) by dietary or pharmacological means in order to counteract and neutralise the deleterious effects of ROS/RNS. By scavenging free radicals and decreasing oxidative stress, antioxidants may slow, inhibit or prevent the oxidation of oxidizable substrates. The defence against ROS is, however, impaired or harmed in disease conditions and the oxidant load increases. Under such circumstances, the external supply of antioxidants is sufficient to mitigate the adverse effects of oxidative stress [10]. It is generally recognised that the presence of one or more conjugated -OH groups or -COOH groups, which increases the capacity of such a molecule to quench free radicals, is the most important structural feature that facilitates successful antioxidant activity. Therefore Studies have shown that polyphenols (both natural and synthetic) are promising antioxidants [5, 6].

Coumestrol is a phytoestrogen belongs to the coumestan family of compounds, in plants. Coumestrol exhibit estrogenic and antiestrogenic activity based on oestrogen levels in the body. It has a similar structure to isoflavones and estradiol. It was first isolated from ladino clover in 1956 by E.M. Bickoff. Coumestrol is widely distributed in plants like clover, alfalfa, soya beans, peas, brussels sprouts, spinach, strawberries and a variety of legumes. Coumestrol can easily pass through cell membranes due to its low molecular weight and stable structure. Coumestrol exhibit a neuroprotective effect via cerebral ischemia prevention. Coumestrol exert beneficial effects in cancer, menopause, osteoporosis, atherosclerosis, and cardiovascular disease. In addition to this, coumestrol shows Anti-ageing, Neuroprotective, Anti-adipogenic, Depigmenting activity, Anti-oxidant and Anti-cancer properties. A detailed mechanistic (radical scavenging mechanism) study on coumestrol and its derivatives is needed to describe the antioxidant characteristics in a satisfactory manner, so the current work is an attempt to provide a theoretical exploration of the antioxidant property of the molecules under study.

2. Materials and methods

2.1 Materials

The present study mainly focussed on the anti-oxidant property of coumestrol and its derivatives. The three-dimensional structure of the parent molecule is downloaded from the PubChem database. Using the Gaussview-5.0 graphical user interface, the input structures of coumestrol derivatives were drawn and assigned to the Gaussian 09 software package for computational calculations.

2.2 Computational methodology

2.2.1 Frontier molecular orbital (FMO) analysis

In particular, the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) form the frontier molecular orbitals (FMOs). FMOs are strongly involved in the study of the electrical and chemical properties of substrates. Analysis of frontier molecular orbitals of coumestrol and its derivatives have been carried using density functional theory and their energy gaps were computed. A lower energy gap indicates the reactivity of the molecule. An anti-oxidant ‘s working mechanisms are derived from HOMO, as a weak electron donor represents a lower HOMO, and vice versa. In addition, electron transfer is

involved in hydrogen abstraction, and therefore the study of HOMO-LUMO is important.

2.2.2 Global descriptive parameters

Global descriptive parameters are parameters that give information's about the reactivity of coumestrol derivatives and also give the relation between the reactivity of derivatives and responses to the changes in external conditions. So, by calculating these parameters, we can compare the reactivity of coumestrol with its derivatives. It is an attractive method for understanding the reactive nature of all the products [11]. Global parameters include ionisation potential (I), electron affinity (A), hardness (η), softness (S), electronegativity (χ), chemical potential (μ) and electrophilicity index (Ω) [12]. These parameters depend upon the number of electrons and electron density due to the external changes [13]. Global descriptive parameters can be calculated by two methods; they are according to Koopman's theorem and the Energy vertical method. These methods have particular relevance in the comparison of different molecules. Low ionisation potential, high electron affinity and high electronegativity contribute to high reactivity. So, by analysing the values of these parameters' reactivity can be studied.

According to energy vertical, difference in total electronic energy of the neutral molecule and its corresponding anion and cation were considered. The equations for finding ionisation potential (I) and electron affinity (A) are given below;

$$I = E_{cation} - E_{neutral} \tag{1}$$

$$A = E_{neutral} - E_{anion} \tag{2}$$

According to Koopman's theorem of closed shell compounds;

$$I = -E_{HOMO} \tag{3}$$

$$A = -E_{LUMO} \tag{4}$$

Where E_{HOMO} is the energy of the highest occupied molecular orbital (HOMO) and E_{LUMO} is the energy of the lowest unoccupied molecular orbital (LUMO). The global properties were computed by using the equations given below;

$$\text{Hardness } (\eta) = (I - A)1/2 \tag{5}$$

$$\text{Electronegativity } (\chi) = (I + A)1/2 \tag{6}$$

$$\text{Softness } (s) = 1/(2\eta) \tag{7}$$

$$\text{Chemicalpotential } (\mu) = -\chi \tag{8}$$

$$\text{Electrophilicityindex } (\omega) = \mu^2/2 \tag{9}$$

2.2.3 Donor acceptor map (DAM)

A donator-acceptor map is a useful tool for a qualitative comparison among substances. DAM can be used for classifying molecules in terms of their electron accepting and donating capacity (with respect to coumestrol). Graphical representation of DAM plot is shown in **Figure 1**. DAM also provides information's regarding anti-radical capability of molecules and also gave us a base for antioxidant studies. Single-point calculations (Energy vertical) were used to compute ionisation potential (I) and electron affinity (A). Ionisation potential was calculated as the difference between the energy of the cation and that of the neutral molecule.

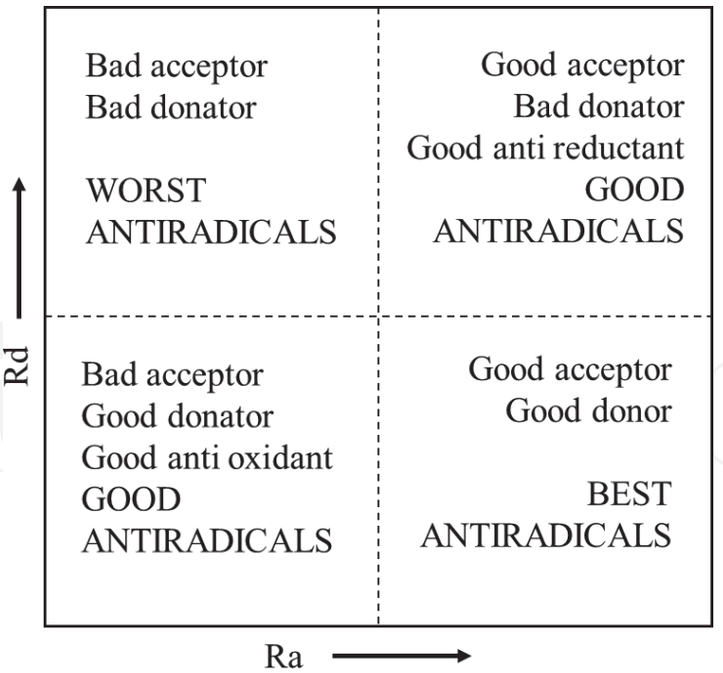

Figure 1.
Graphical representation of DAM.

And electron affinity was calculated as the energy difference between the neutral and the anion, and both were assumed to have ground state nuclear configuration of the neutral molecule.

According to J.J. Gázquez's approximation, the tendency to donate charge, or electron donating power, maybe defined as;

$$\omega- = (3I + A)^2/16(I–A) \tag{10}$$

whereas, the tendency to accept charge, or electron accepting power, maybe defined as;

$$\omega+ = (I + 3A)^2/16(I - A) \tag{11}$$

I and A donate or accept a single electron whereas, ω- and ω + refer to fractional charges. Lower values of electron donating power indicate the greater capacity for donating charge and higher values of electron accepting power indicate the greater capacity for accepting charge. So, it is a simple charge transfer model expressed in terms of chemical potential and hardness. Chemical potential gives more importance for ionisation potential in the context of charge donation and give more importance on electron affinity in the context of charge acceptance.

2.2.4 Full electron donor acceptor map (FEDAM)

FEDAM is a plot of electron donation index (RI) vs. electron acceptance index (RA), which gives information about the radical scavenging activity of different molecules. The ionisation enthalpy (I) and electron affinity (A) were obtained through DFT-B3LYP/6–31 + G(2d,2p) using energy vertical method. The electron donating and accepting indexes of the coumestrol derivatives were calculated with respect to the parent molecule, coumestrol, by using the equations given below;

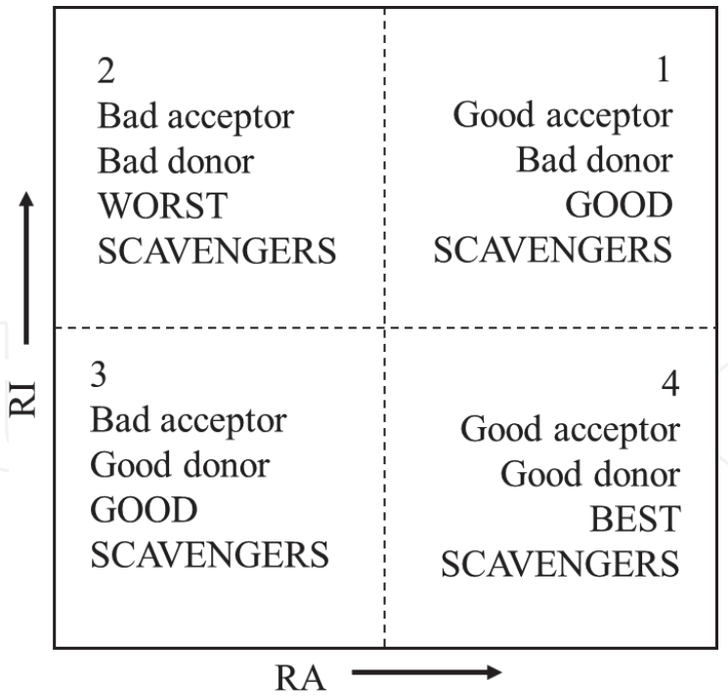

Figure 2.
Graphical representation of FEDAM.

$$RI = I_L/I_{Cou} \tag{12}$$

$$RA = A_L/A_{Cou} \tag{13}$$

Where, L = Ligand (Derivatives).
Cou = Coumestrol.

The graphical representation of FEDAM is shown in **Figure 2**. It is used for evaluating the single electron (SET) transfer processes. Generally, the electron transfer takes place from region-3 (good donor) to region-1 (good acceptor). From this graph, it's vivid that the molecules with low I value and high A value exhibits the best scavenging activity.

2.2.5 Antiradical activity

To clarify the radical scavenging potential of phenolic anti-oxidants, three main mechanisms have been proposed. Consequently, antioxidants can deactivate free radicals according to the following mechanisms [14, 15].

3. HAT (*hydrogen atom transfer*) mechanism

$$ArOH + X^{\cdot} \rightarrow ArO^{\cdot} + XH \tag{14}$$

The phenolic anti-radical interacts directly with a free radical that is neutralised, according to this mechanism, and a radical form of phenolic antiradical develops. The hydrogen atom is transfered (HAT, Eq. (14)) from antioxidant molecules (ArOH) to radicals. Bond dissociation energy(BDE) is a numerical parameter connected to this mechanism. A better anti-radical property is defined by the lower BDE parameter.

$$BDE = H(ArO^{\cdot}) + H(H) - H(ArOH) \tag{15}$$

4. SET (*single electron transfer*) mechanism

It takes place through two steps. Initially, a free radical cation is formed by the transfer of anelectron from a neutral species.

$$ArOH + X^{\cdot} \rightarrow ArOH^{\cdot+} + X^{-} \tag{16}$$

Neumerical parameter associated to this step is AIP.

$$IP = H\left(ArOH^{\dot{+}}\right) + H(e^{-}) - H(ArOH) \tag{17}$$

In the next step, phenolic radical cation decomposes into phenolic radical and proton.

$$ArOH^{\cdot+} \rightarrow ArO^{\cdot} + H^{+} \tag{18}$$

PDE is the neumerical parameter related to this step.

$$PDE = H(ArO^{\cdot}) + H(H^{+}) - H\left(ArOH^{\dot{+}}\right) \tag{19}$$

5. SPLET (*sequential proton loss electron transfer*)

In SPLET mechanism, The phenolic antioxidant dissociates into an anionic form and proton in the first step, and then ions formed in the first reaction react with the free radical.

$$ArOH \rightarrow ArO^{-} + H^{+} \tag{20}$$

$$ArO^{-} + X^{\cdot} + H^{+} \rightarrow ArO^{\cdot} + XH \tag{21}$$

The first step corresponds to the PA and it can be calculated using Eq. (15):

$$PA = H(ArO) + H(H^{+}) - H(ArOH) \tag{22}$$

The numerical parameter for the second step ETE can be calculated by the equation,

$$ETE = H(ArO^{\cdot}) - H(ArO^{-}) \tag{23}$$

6. Results and discussion

6.1 Optimisation of structures

Coumestrol is a polycyclic aromatic compound containing a coumestan moiety, which consists of a benzoxole fused to a chromen-2-one to form 1-Benzoxolo[3, 2-c] chromen-6-one. The lowest energy conformer of coumestrol is obtained through potential energy scanning and is used for further analysis. The derivatives were drawn by substituting 16th position of coumestrol with electron donating groups like -OH, -NH_2, -OCH_3, -CH_3, -Ph, -$CHCR_2$, -OCOR and -NHCOR and electron withdrawing groups like -F, -CL, -BR, -CN, -NO_2, -SO_3H, -CHO, -COR, -COCL, -COOR and -COOH (where 'R' is a methyl group). All the structures were optimised through DFT-B3LYP/6–31 + G(2d,2p). The optimised structure of coumestrol is shown in **Figure 3**.

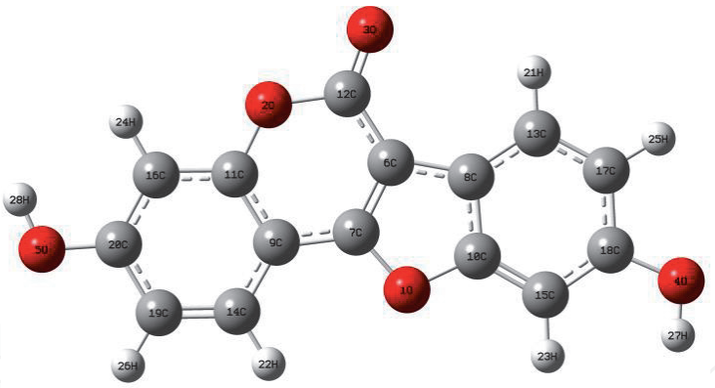

Figure 3.
Optimised lowest energy conformer of coumestrol.

Method	I	A	H	X	S	μ	ω
Energy vertical	7.3311	0.4221	3.4545	3.8766	0.1447	−3.8766	2.1751
Koopman's theorem	5.8556	1.8844	3.9712	3.87	0.1259	−3.97	1.8857

Table 1.
The global descriptive parameters of coumestrol.

6.2 Global descriptive parameters

Global descriptive parameters were calculated for comparing the chemical reactivity of coumestrol derivatives with parent molecule.

The global descriptive parameters of coumestrol are shown in **Table 1**. It can be calculated in two different methods, energy vertical method (single point energy calculations) and Koopman's theorem.

Table 2 indicates the global descriptive values for coumestrol substituted at the C-16th position according to koopman's theorem. Generally, derivatives of coumestrol substituted with an electron withdrawing group showed a common trend; Ionisation potential, electron affinity, electronegativity, softness and electrophilic index increases with increase in electron withdrawing power. Hardness and chemical potential decreases with increase in electron withdrawing power. The trend followed by derivatives of coumestrol substituted with electron donating group is given by; ionisation potential, electron affinity, hardness and electronegativity decreases with increase in electron donating power. And softness, chemical potential and electrophilic index increases with increase in electron donating power. As the electro negativity increases reactivity increases.

Table 3 indicates the global descriptive values for coumestrol substituted at C-16th position calculated by vertical energy method. The derivatives substituted with electron withdrawing groups showed the same trend as in Koopman's, i.e. Ionisation potential, electron affinity, electro negativity, softness and electrophilic index like parameters generally increases with increase in the electron withdrawing power. Hardness and chemical potential decreases with increase in electron withdrawing power. The general trend followed by derivatives of coumestrol substituted with electron donating group was given by; ionisation potential, electron affinity, hardness and electronegativity decreases with increase in electron donating power. And softness, chemical potential and electrophilic index increases with increase in electron donating power. Derivatives substituted with electron donating groups also showed same trend as in Koopman's.

Derivatives	I	A	η	X	S	M	ω
16-F Coumestrol	5.992	2.03	1.981	4.011	0.2524	−4.011	4.0606
16-Cl Coumestrol	5.9903	2.0466	1.9718	4.0184	0.2536	−4.0184	4.095
16-Br Coumestrol	5.9811	2.0387	1.9712	4.0099	0.2536	−4.0099	4.0777
16-CN Coumestrol	6.2015	2.4324	1.8846	4.317	0.2653	−4.317	4.9443
16-NO_2 Coumestrol	6.2243	3.205	1.5096	4.7146	0.3312	−4.7146	7.3617
16-SO_3H Coumestrol	6.1247	2.2855	1.9196	4.2051	0.2605	−4.2051	4.6058
16-CHO Coumestrol	6.0877	2.652	1.7178	4.3698	0.2911	−4.3698	5.5586
16-COR Coumestrol	6.0023	2.4077	1.7973	4.205	0.2782	−4.205	4.9191
16-COCl Coumestrol	6.1607	2.661	1.7498	4.4108	0.2857	−4.4108	5.5592
16-COOR Coumestrol	5.8951	2.0988	1.8981	3.9969	0.2634	−3.9969	4.2082
16-COOH Coumestrol	5.9664	2.2316	1.8674	4.099	0.2678	−4.099	4.4987
16-OH Coumestrol	5.879	1.9369	1.971	3.908	0.2537	−3.908	3.8746
16-NH_2 Coumestrol	5.8148	1.8591	1.9779	3.8369	0.2528	−3.8369	3.7217
16-OCH_3 Coumestrol	5.833	1.871	1.981	3.852	0.2524	−3.852	3.7452
16-CH_3 Coumestrol	5.7974	1.8109	1.9932	3.8042	0.2508	−3.8042	3.6302
16-Ph Coumestrol	5.7849	1.824	1.9804	3.8044	0.2525	−3.8044	3.541
16-$CHCR_2$ Coumestrol	5.7508	1.7879	1.9815	3.7693	0.2523	−3.7693	3.585
16-OCOR Coumestrol	6.07	2.142	1.9645	4.1055	0.2545	−4.1055	4.2899
16-NHCOR Coumestrol	6.0431	2.1162	1.9634	4.0797	0.2546	−4.0797	4.2394

Table 2.
Global descriptive parameters of coumestrol substituted at C-16th position according to Koopman's method.

Analysing the reactivity based on ionisation potential, electronegativity and electron affinity; reactivity increases with increase in electron affinity and electron negativity and decrease in ionisation potential. According to this relation, the derivatives with more reactivity are 16-NO_2 Coumestrol, 16-OH Coumestrol, 16-OCOR Coumestrol, and 16-NHCOR Coumestrol.

6.3 Dam plot

The **Figure 4** shown the DAM plot of coumestrol substituted at C-16th positions. The derivatives like 16-F Coumestrol, 16-Cl Coumestrol, 16-Br Coumestrol, 16-CN Coumestrol, 16-NO_2 Coumestrol, 16-SO_3H Coumestrol, 16-CHO Coumestrol, 16-COR Coumestrol, 16-COCl Coumestrol, 16-COOR Coumestrol, 16-COOH Coumestrol, 16-OH Coumestrol, 16-OCOR Coumestrol, and 16-NHCOR Coumestrol were good anti-reductants with large size and they were good acceptors. Most of the derivatives substituted with electron withdrawing groups were anti-reductants and bad donors. The derivatives substituted with electron donating groups like 16-NH_2, 16-OCH_3 Coumestrol and 16-CH_3 Coumestrol showed good antioxidant capacity through their electron donating power and they were of small size. 16-OH Coumestrol, 16-OCOR Coumestrol, and 16-NHCOR Coumestrol were exceptional derivatives with electron donating substitution and anti-reductant capacity. Therefore, all the derivatives were good anti-radicals.

Derivatives	I	A	η	χ	S	μ	ω
16-F Coumestrol	7.4689	0.5578	3.4556	4.0133	0.1447	−4.0133	2.3306
16-Cl Coumestrol	7.4425	0.6080	3.4173	4.0253	0.1463	−4.0253	2.3707
16-Br Coumestrol	7.4210	0.6148	3.4031	4.0179	0.1469	−4.0179	2.3719
16-CN Coumestrol	7.6594	1.0007	3.3294	4.3300	0.1502	−4.3300	2.8157
16-NO_2 Coumestrol	7.6796	1.5158	3.0819	4.5977	0.1622	−4.5977	3.4295
16-SO_3H Coumestrol	7.5222	3.2371	2.1426	5.3796	0.2334	−5.3796	6.7537
16-CHO Coumestrol	7.5348	1.1064	3.2142	4.3206	0.1556	−4.3206	2.9039
16-COR Coumestrol	7.4306	0.9353	3.2477	4.1830	0.1540	−4.1830	2.6939
16-COCl Coumestrol	7.6040	1.1919	3.2061	4.3980	0.1560	−4.3980	3.0165
16-COOR Coumestrol	7.3310	0.6910	3.3200	4.0110	0.1506	−4.0110	2.4229
16-COOH Coumestrol	7.4149	0.7913	3.3118	4.1031	0.1510	−4.1031	2.5418
16-OH Coumestrol	7.3332	0.4735	3.4298	3.9034	0.1458	−3.9034	2.2211
16-NH_2 Coumestrol	7.2667	0.4105	3.4281	3.8386	0.1459	−3.8386	2.1491
16-OCH_3 Coumestrol	7.2750	0.4329	3.4210	3.8539	0.1462	−3.8539	2.1708
16-CH_3 Coumestrol	7.2491	0.3721	3.4385	3.8106	0.1454	−3.8106	2.1115
16-Ph Coumestrol	7.1791	0.4754	3.3519	3.8272	0.1492	−3.8272	2.1850
16-$CHCR_2$ Coumestrol	7.1559	0.4109	3.3725	3.7834	0.1483	−3.7834	2.1221
16-OCOR Coumestrol	7.5087	0.7162	3.3963	4.1124	0.1472	−4.1124	2.4898
16-NHCOR Coumestrol	7.4808	0.6957	3.3926	4.0882	0.1474	−4.0882	2.4633

Table 3.
Global descriptive parameters of coumestrol substituted at C-16th position according to energy vertical method.

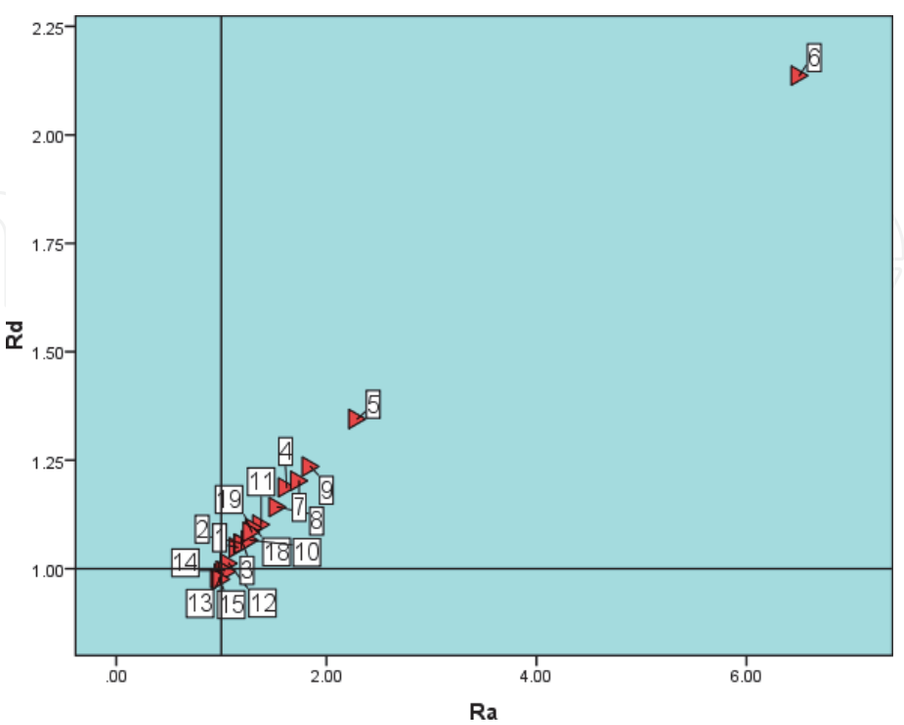

Figure 4.
DAM plot of coumestrol substituted at C-16th position.

6.4 FEDAM plot

All the coumestrol derivatives pointed out were good radical scavengers relative to coumestrol. From the **Figure 4**, it was clear that the derivatives like 16-F Coumestrol, 16-Cl Coumestrol, 16-Br Coumestrol, 16-CN Coumestrol, 16-NO_2 Coumestrol, 16-SO_3H Coumestrol, 16-CHO Coumestrol, 16-COR Coumestrol, 16-COCl Coumestrol, 16-COOR Coumestrol, 16-COOH Coumestrol, 16-OH Coumestrol, 16-OCOR Coumestrol and 16-NHCOR Coumestrolexhibit good accepting capacity and 16-NH_2 Coumestrol, 16-CH_3 Coumestrol, and 16-$CHCR_2$ Coumestrol exhibit good donating capacity of electron. Therefore, all these derivatives were good radical scavengers while 16-PhCoumestrol and 16-OCH_3 Coumestrolwere best radical scavengers with both electron donating and electron accepting capacity. The substitution of electron withdrawing groups on coumestrol imparts electron accepting and donating groups impart electron donating capacity on the derivatives. The size distribution says that good electron acceptors are large in size and good electron donors are small in size (**Figure 5**).

6.5 Anti-oxidant capacity

Numerical parameters corresponding to all the possible anti-oxidant mechanism of coumestrol and selected coumestrol derivatives in gas phase are shown in **Table 4**.

Logically speaking, free energy (ΔG) decides the thermodynamically preferred mechanism. The calculated free-energy equation is $\Delta G = \Delta H - T\Delta S$. In accordance with this equation ΔG is defined by ΔH and ΔS. However the absolute values of the entropic term, TΔS, reach only a few units or tens of kJ/mol, in the case of studied reactions. Free energies are thus predominantly influenced by the enthalpy term ΔH. The mechanisms of HAT, SET-PT and SPLET are primarily regulated by BDEs, IPs and PAs, respectively, and the BDEs, IPs and PAs can therefore specify the thermodynamically preferred reaction pathway involved in the free radical

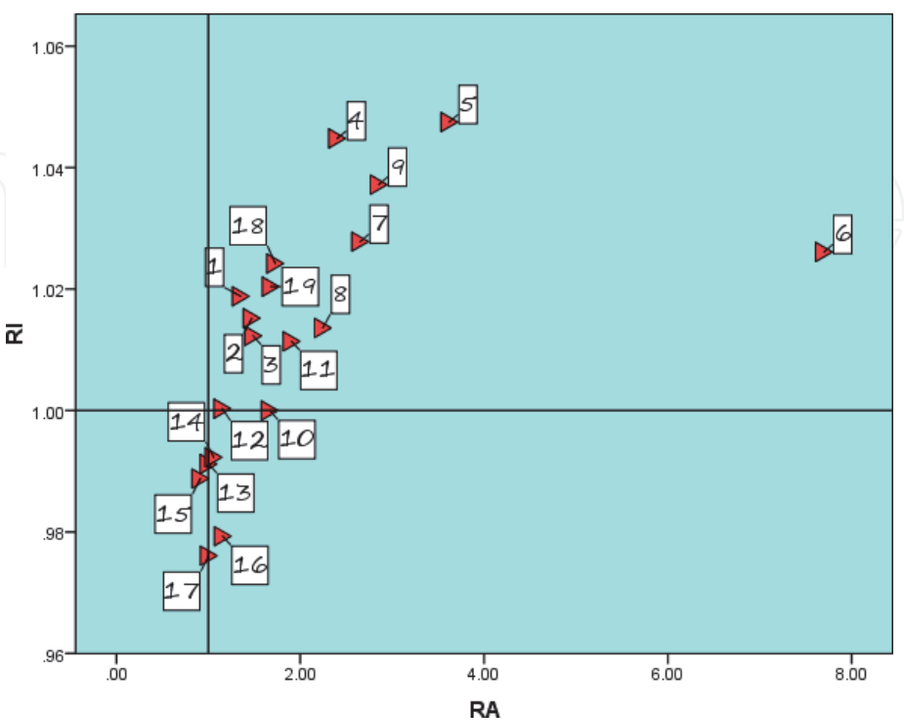

Figure 5.
FEDAM of Coumestrol substituted at C-16th position in gas phase.

Molecule	Bond	BDE	AIP	PDE	PA	ETE
Coumestrol	20-OH	82.9768	166.6985	230.1977	325.5727	71.3235
	18-OH	80.7975		228.0184	332.9728	61.7441
16-NH_2Coumestrol	20-OH	71.3662	125.3958	259.8898	325.0048	60.2808
	18-OH	79.7603		268.2839	333.0714	60.6083
16-OCH_3Coumestrol	20-OH	78.6458	163.4813	229.0839	323.8452	68.7200
	18-OH	79.8399		230.2781	332.7745	60.9848
16-CH_3Coumestrol	20-OH	79.7163	125.1574	268.4784	326.0258	67.6099
	18-OH	79.7063		268.4683	333.6405	59.9852

Table 4.
Numerical parameters corresponding to all the possible antioxidant mechanism of coumestrol and selected coumestrol derivatives in gas phase.

scavenging method. From the table, the measured IPs and PAs of coumestrol and its derivatives in the gas phase have been found to be substantially higher than BDEs and thus, from a thermodynamic point of view, HAT is the most desirable method in the gas phase.

BDE is the numerical parameter that characterises the stability of hydroxyl group and it is related to HAT mechanism. The lower BDE value indicate the lower the stability of the O-H bond, and high antioxidant capacity. Coumestrol contain two different hydroxyl groups which can transfer hydrogen to the free radical present in biological systems. From the table it is observed that, the derivatives substituted with electron donating groups like 16-NH_2, 16-OCH_3 and 16-CH_3 showed good anti-oxidant capacity. In gas phase, the antioxidant power all the selected coumestrol derivatives were higher than that of parent molecule.

For coumestrol, the BDE value observed at 18-OH was less than that of 20-OH which means that 18-OH forms most stable radical. The more stable radical can imply the stronger antioxidant abilities of the compound. Among the three selected coumestrol derivatives, 16-NH_2 showed lowest BDE value implies its higher anti-oxidant potential. In the case of 16-NH_2 Coumestrol, the bond 20-OH showed lowest BDE value compared to that of 18-OH. It may be due to the presence of intra molecular hydrogen bonding between -NH_2 with nearby oxygen radical. 16-OCH_3 coumestrolshowed low BDE value than Coumestrol but higher than 16-NH_2 coumestrol because there is no hydrogen bonding interaction possible between oxygen radical and -OCH_3 group near to it. 16-CH_3Coumestrol, also showed a lower BDE value than parent molecule. -CH_3 group being a weakly electron donating one, only a slight difference in BDE value was observed at 20-OH and 18-OH.

6.6 Frontier molecular orbital analysis

Energy and distribution of frontier orbitals are also significant parameters that correlate with the antioxidant activity of the polyphenols. The calculated frontier orbital distributions and energies in the gas phase for Coumestrol and its derivatives like 16-NH_2Coumestrol, 16-OCH_3Coumestrol, and 16-CH_3Coumestrol are present in **Figure 6**.

The molecule's electron donation potential is linked to the energies of HOMO. Higher HOMO orbital energy molecules have a greater capacity to donate electrons [16, 17]. It can be observed from **Figure 6** that 16-CH3 coumestrol provided the highest HOMO energy (−5.797eV), followed by 16-NH2 coumestrol (−5.815eV), 16-OCH3 coumestrol (−5.833 eV), and coumestrol (−5.855eV). This demonstrates

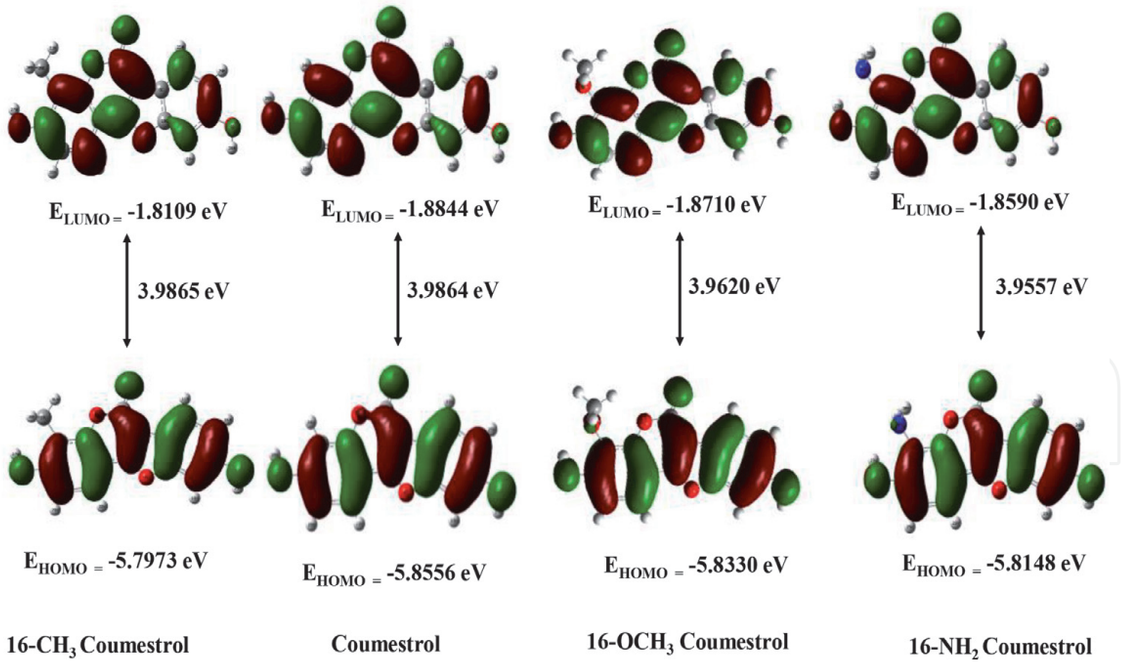

Figure 6.
The energy and distribution of HOMO and LUMO for coumestrol and its selected derivatives.

clearly that all the compounds studied, pocess best electron-donating potential which is in good agreement with IP values. Even though 16-CH3 coumestrol showed high HOMO energy its lectron donating power is less due to the large band gap.

7. Conclusion

The present work explained the antioxidant properties of coumestrol and its derivatives from a theoretical point of view. Since the measured ionisation potential and proton affinities in gas phase are significantly higher than the phenolic O-H group BDEs, we can infer that from a thermodynamic point of view, homolytic O-H bond splitting-off is the most likely process in the gas phase. All the selected derivatives 16-NH_2 Coumestrol, 16-OCH_3 Coumestrol, and 16-CH_3 Coumestrol showed high antiradical activity than parent molecule. Among the selected derivatives, 16-NH2 Coumestrol showed the best antioxidant activity. The calculated molecular properties (electronegativity, ionisation potential, electron affinity, hardness and electrophilicity index) of coumestrol derivatives substituted by electron withdrawing groups at 16^{th} position indicated that, Ionisation potential, electron affinity, electro negativity, softness and electrophilic index increases with increase electron withdrawing power and hardness and chemical potential decreases with increase in electron withdrawing power. In the case of derivatives substituted with electron donating groups, ionisation potential, electron affinity, hardness and electronegativity decrease with increase in electron donating power while the softness, chemical potential and electrophilic index decreases with increase in electron donating power.

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