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METHYLATION EFFECT OF APIGENIN 8-C-GLUCOSIDE TOWARDS ANTIOXIDANT POTENTIAL - A DFT STUDY

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Keywords:

DFT, Apigenin 8-C-glucoside, Methoxyvitexin, Structural activity

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ABSTRACT: The extensive study on O - methylated flavonoids reveals that substitution with methyl group enhances the radical scavenging behavior in certain compounds. Due to potential applications of C-glycosides in metabolic engineering, methylation in these flavonoids needs to be analyzed in both theoretical and experimental levels for their radical scavenging behavior. In this work naturally occurring C- glycosyl flavonoid apigenin 8-C-glucoside (vitexin) is theoretically simulated by substituting the C4′, C5 and C7 hydroxyl positions with methoxy unit and studied with the aid of density functional theory (DFT) for radical scavenging behavior. Structural stability is attained through B3LYP/6-311G(d,p) theory using Gaussian 03 package which provided the stable conformer for the studied compound without imaginary frequency. Structural activity is analyzed with the support of parameters like kinetic energy (temperature independent), HOMO-LUMO, molecular descriptors, Mulliken charge density analysis and compared with vitexin. Combined investigation of the parameters above revealed the superiority of phenyl hydroxyl vitexin over phenyl methoxy vitexin for radical scavenging activity unlike its O methylated flavonoids.

INTRODUCTION: Flavonoids are the secondary metabolites which are well known for their cytoprotective property, anti-inflammatory activity and oxidative stress-reducing action [1]. Exploring the antioxidant mechanism of flavonoid compounds through structural activity relationship (SAR) is gaining interest for nearly two decades. Numerous analyses are done for the investigation of SAR, which includes atoms in molecules (AIM), natural bond orbital analysis (NBO), atomic population analysis and molecular descriptors calculation [2]. Mainly this analysis gives information about the ability of the flavonoids to scavenge the radicals.

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The radical scavenging behavior is governed by the charge transfer mechanism which results in hydrogen atom abstraction, protonation and deprotonation [3]. In the present study, an attempt has been made to evaluate the active site for radical scavenging process through the energy profiles of the phenolic rings present in the flavonoid skeleton of methoxyvitexin, and it is compared with vitexin. Kinetic energy analysis yields information about the response of the atoms in the compound by the substitution of a methoxy group at different positions in terms of instability [4].

RFW Bader *et al*., (1969) proposed that the instability of a molecular system results due to the higher kinetic energy of the system. In vitexin, the rings A and C are tightly bound together thereby exhibiting a higher rate of stability which requires more energy for bond dissociation [5]. Automatically the B-ring captures more attention because this site needs less bond dissociation energy concerning

radical scavenging process and B-ring be the right candidate to prove the instability using kinetic energy analysis.

MATERIALS AND METHODS: Neutral structures of vitexin containing hydroxy and substituted methoxy groups in 4', 5 and 7 positions of respective compounds have been optimized at B3LYP level with the basis set 6-311 G (d,p) using Gaussian 03 package (Gaussian Inc., Wallingford). After the successful completion of the optimization process, the obtained results are taken for computing single point energy (SPE) and harmonic vibrational frequency analysis. The frequency analysis result reveals the absence of negative frequencies and then calculations are performed for HOMO and LUMO energy, Mulliken population analysis, molecular descriptors such as ionization potential (IP), electron affinity (EA), hardness (ω), softness (S), electrophilic index (η) and electronegativity (χ) [6].

Further, plots based on electron density, kinetic energy contours and Lagrangian kinetic energy G(r) are supported by Multiwfn 3.0 data analyzer have been simulated and interpreted [7]. The optimized structures of phenyl hydroxyl vitexin and phenyl methoxy-vitexin4' methoxyvitexin (4' MeOvitexin), 5 methoxyvitexin (5 MeOvitexin), 7 methoxyvitexin (7 MeOvitexin)) along with the numbering schemes are depicted in **Fig. 1**.

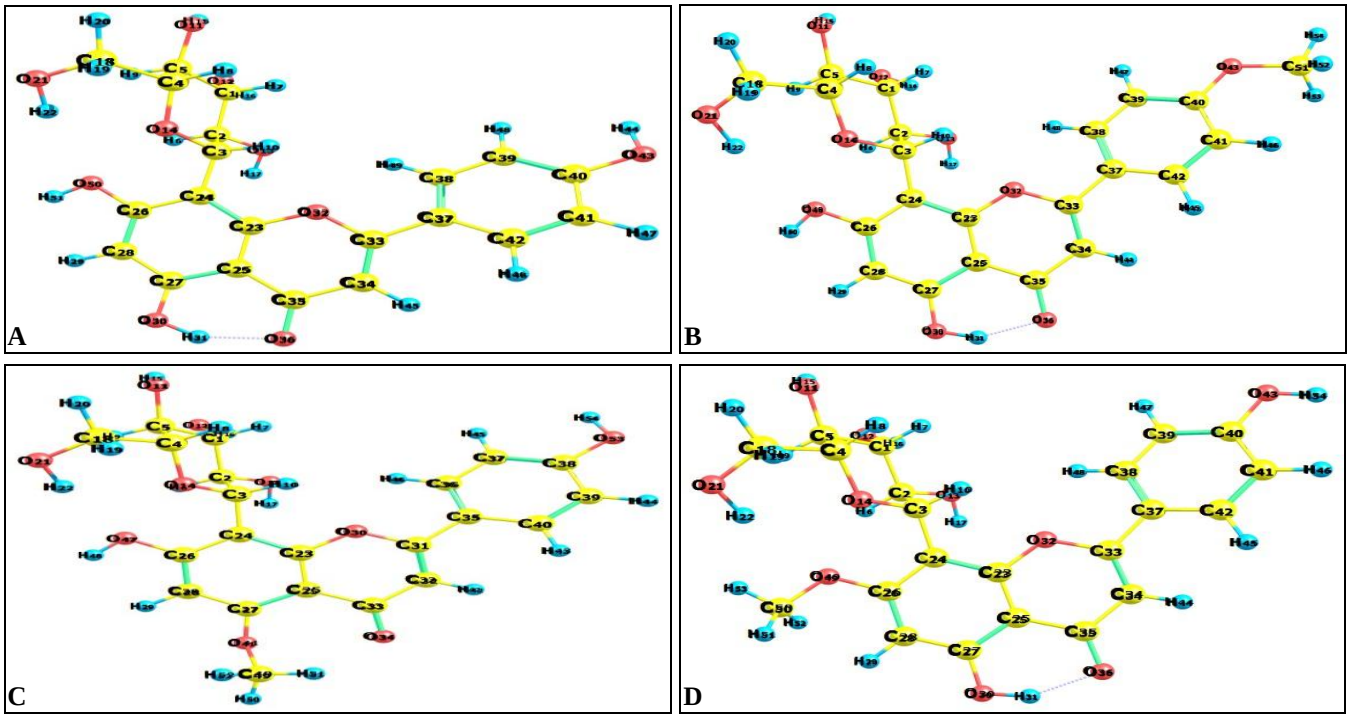

FIG. 1: OPTIMIZED STRUCTURES AND NUMBERING OF (A) VITEXIN, (B) 4' METHOXY VITEXIN (C) 5 METHOXY VITEXIN (D) 7 METHOXY VITEXIN

RESULTS AND DISCUSSION:

Kinetic Energy Distribution: MEP, total charge (electron) density *ρ(x)* and the Lagrangian kinetic energy density distribution G(r) plots for the flavonoid compounded phenyl hydroxyvitaxin and substituted phenyl methoxyvitexin are shown in **Fig. 2** and **3**.

Generally, charge density distribution, molecular binding, and their mechanisms are explained in terms of classical electrostatics. The techniques which are widely used by quantum chemists to observe the above-said parameters are MEP, HOMO, and LUMO contours, Fukui sites prediction [8]. Kinetic energy supports the stability of the molecules by providing information about charge density distribution ρ(r) which serves as a base to examine the stability factor. Kinetic energy distribution can be described with the help of two functions namely Hamiltonian kinetic energy density K(r) and Lagrangian kinetic energy density G(r). K(r) is obtained by integrating the charge density of the system, and *G*(r) is calculated by normalizing K(r) is also known as positive definite kinetic energy density [9].

The equations corresponding to K(r) and G(r) are

$$K(\mathbf{r}) = -\frac{1}{2}\sum_i \eta_i \varphi_i^*(\mathbf{r})\nabla^2\varphi_i(\mathbf{r}) \quad ..1$$

$$G(\mathbf{r}) = \frac{1}{2}\sum_i \eta_i |\nabla\varphi_i(\mathbf{r})|^2 = \frac{1}{2}\sum_i \eta_i \left\{\left(\frac{\partial\varphi_i(\mathbf{r})}{\partial x}\right)^2 + \left(\frac{\partial\varphi_i(\mathbf{r})}{\partial y}\right)^2 + \left(\frac{\partial\varphi_i(\mathbf{r})}{\partial z}\right)^2\right\} \quad ..2$$

K(r) and *G*(r) are directly related by Laplacian of electron density

$$\Delta^2 \rho(r) / 4 = G(r) - K(r) \quad ..3$$

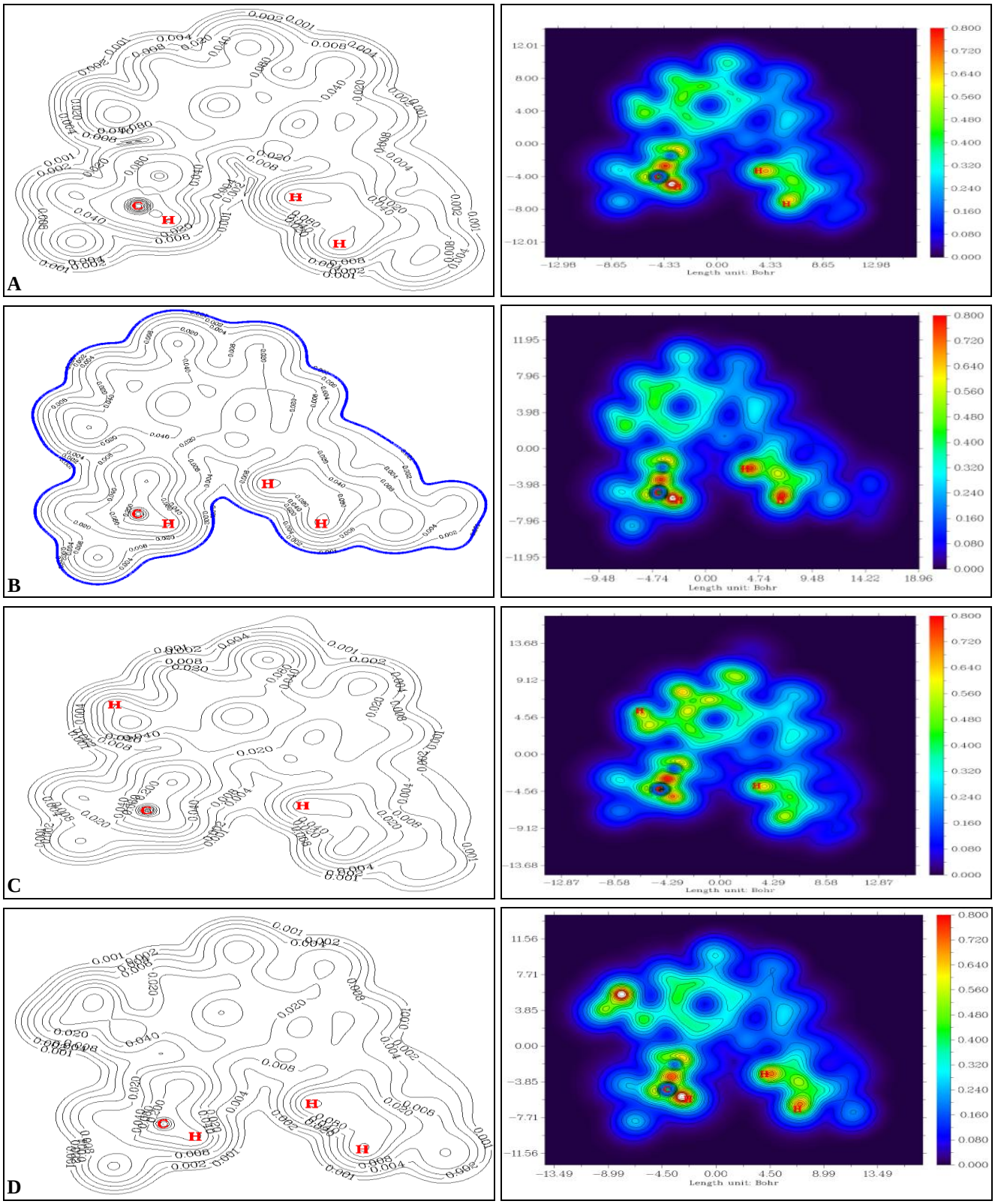

FIG. 2: CHARGE DENSITY CONTOURS MAPS FOR A) VITEXIN B) 4' MEOVITEXIN C) 5 MEOVITEXIN D) 7 MEOVITEXIN

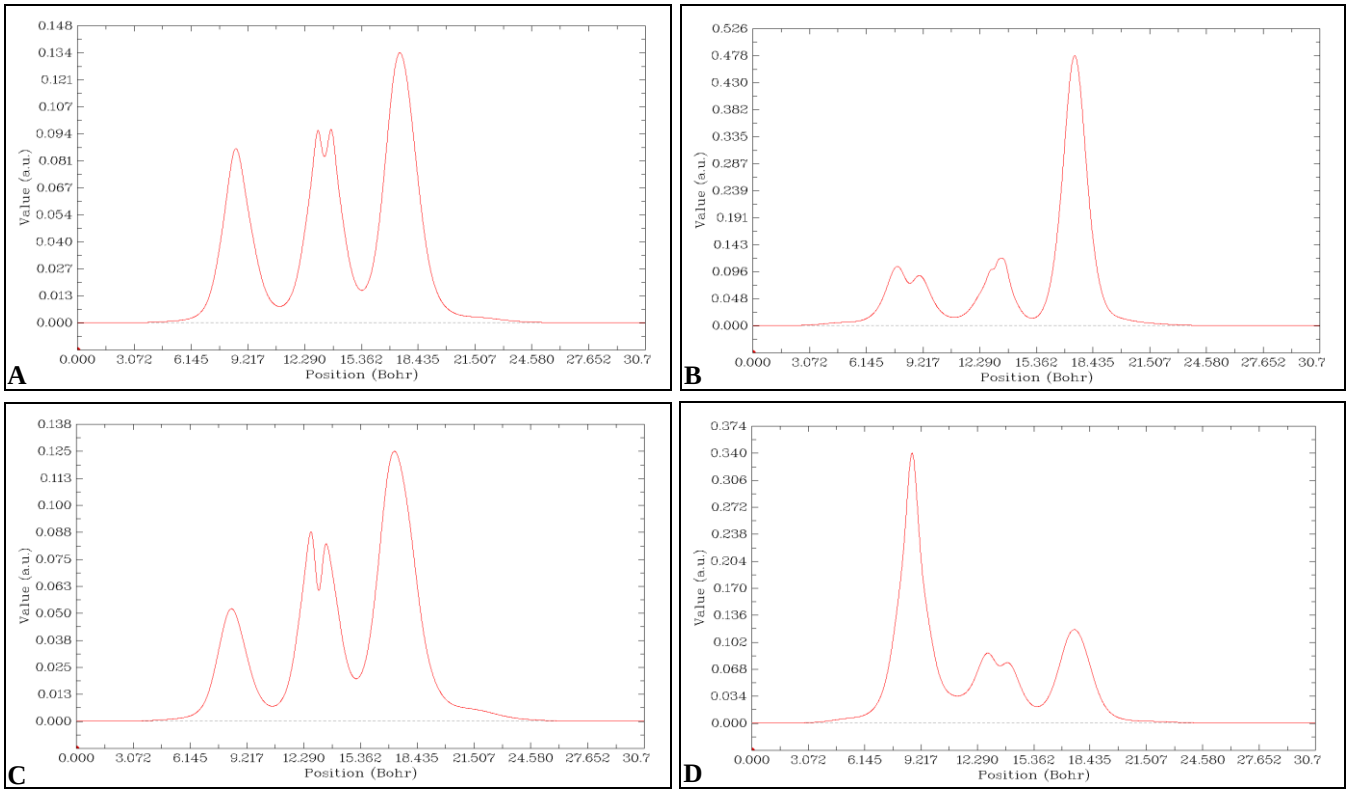

FIG. 3: LAGRANGIAN KINETIC ENERGY DENSITY G(R) FOR (A) VITEXIN (B) 4' MEO VITEXIN (C) 5 MEO VITEXIN AND (D) 7 MEO VITEXIN

It is noted from the electron density plot, that vitexin and 5 methoxyvitexin showing analogous charge density distribution, *i.e.* 4' and 7 positions seem to be electronegative regions and the charge densities are prominent in these regions where C ring is situated along with glucose unit. However, methoxy unit doesn't show any significant development concerning electronegativity when compared with hydroxyl units, and charge density distribution radiates from A ring to C-ring. Regarding antioxidant activity, the flavonoid compounds with more number of –OH groups favors higher reactivity than the compounds with –OCH_3 groups due to the steric effect and increased lipophilicity. But the presence of some hydroxyl units cannot be considered as a deciding factor for antioxidant activity [10]. In the present case, for charge density ρ(r) distribution following order has been observed from **Fig. 2.**

5 MeOvitexin > vitexin > 4' MeOvitexin > 7 MeOvitexin.

This is because of A-ring being more stabilized and consists of a double bonded oxygen atom which shows strong electrostatic attraction over the entire methoxy unit. The reduced charge density in 7 MeOvitexin is due to the strong repulsive force exerted by the hydrogen atoms present in the glucose unit to the methoxy unit since the glucose unit as well as the methoxy unit lies very close to each other.

It is very interesting to note that G(r) follows the same order as that of ρ(r) and the higher degree of the kinetic energy of 5 MeOvitexin is due to the present high density of charge carriers. At the same instant, the same phenomena have been observed in the case of 4'MeOvitexin due to the steric effect, lipophilicity and the kinetic energy of vitexin are strongly influenced by unstable glucose unit. In the case of 4' methoxyvitexin, B ring shows sharp peaks for kinetic energy distribution. Therefore, it is concluded that the presence of methoxy unit results in an unstable structure of C glucoside and increases charge density distribution on the rings. Instability of a ring may be due to a decrease in units of –OH groups. In this context, the B-ring exhibits higher order of reactivity in the presence of –OH groups than –OCH_3 groups.

HOMO and LUMO Energy: The electron occupancy values computed from frontier molecular orbitals supports the reactivity of B-ring

present in both phenyl hydroxyl vitexin and substituted phenyl methoxyvitexin when compared with A and C rings **Table 1**. 5 methoxyvitexin experiences strong steric effect rendered by A ring stays very stable and holds very low occupied and highest unoccupied orbitals whereas higher magnitudes of HOMO of 7 methoxyvitexin leading to poor radical scavenging potential.

TABLE 1: OCCUPIED AND UNOCCUPIED ORBITAL ENERGY VALUES OF VITEXIN, 4' METHOXY VITEXIN, 5 METHOXY VITEXIN AND 7 METHOXY VITEXIN [11]

Orbital Occupancy	*Vitexin	4' Methoxy vitexin	5 Methoxy vitexin	7 Methoxy vitexin
HOMO (eV)	6. 385	6.322	6.372	6.278
LUMO (eV)	2.144	2.092	1.944	2.097

Molecular Descriptors: Molecular descriptors such as IP, EA, χ, η, S and ω are calculated based on the orbital energies and reported in **Table 2**. It reveals that apart from the superiority of phenyl hydroxyl over phenyl methoxy, methoxy functional group is less supportive for the dissociation process and is taken into account during the radical scavenging process.

TABLE 2: MOLECULAR DESCRIPTORS FOR VITEXIN, 4' METHOXY VITEXIN, 5 METHOXY VITEXIN AND 7 METHOXY VITEXIN OPTIMIZED AT THE LEVEL OF THEORY B3LYP/6-311 G(D, P) IN EV [11]

Molecular descriptors	E_o (eV) of *Vitexin	E_o (eV) of Vitexin 4' Methoxy	E_o (eV) of Vitexin 5 Methoxy	E_o (eV) of Vitexin 7 Methoxy
IP	6.38	6.61	6.38	6.27
EA	2.14	2.09	1.89	2.09
ω	2.12	2.25	2.24	2.09
S	0.23	0.22	0.22	0.24
χ	4.26	4.35	4.14	4.18
η	4.29	4.19	3.81	4.19

From **Table 2** it is witnessed from χ that the electron-accepting capability is underprivileged for 5methoxyvitexin when compared with remaining three compounds in the order of 5 MeOvitexin < 4' MeOvitexin < 7 MeOvitexin < vitexin. The energy required to remove a loosely bound electron is high for 4' MeOvitexin, whereas EA shows that vitexin doesn't accept electrons easily concerning the other compounds and hence it is a preferred candidate for antioxidant activity [11]. The tendency of 5 MeOvitexin concerning η is weak, and vitexin exhibits the highest electronegativity. Based on the above analysis the following order of activity has been found: vitexin > 4' MeOvitexin > 7 MeOvitexin > 5 MeOvitexin.

Mulliken Population Analysis: The investigation of charge delocalization and localization has been carried out by Mulliken population analysis technique and the results are depicted in **Table 3**. n- π* transition is witnessed in and around B-ring by the accumulation of atomic charges [12]. In all the studied compounds possession of a higher number of atomic charges are found at B ring rather than A and C rings and this once again proves that the B ring is a better candidate for radical scavenging activity. Another factor which is observed from **Table 3**, in addition to B rings of all the compounds, 7 MeO vitexin is also a good candidate for radical scavenging behavior based on the atomic charges.

TABLE 3: MULLIKEN CHARGES FOR VITEXIN, 4' METHOXY VITEXIN, 5 METHOXY VITEXIN AND 7 METHOXY VITEXIN OPTIMIZED AT THE LEVEL OF THEORY B3LYP/6-311 G(D, P) IN EV [11]

*Vitexin		4' methoxy vitexin		5 methoxy vitexin		7 methoxy vitexin	
Atom	Charge (eV)	Atom	Charge (eV)	Atom	Charge (eV)	Atom	Charge (eV)
1 C	0.040957	1 C	0.039182	1 C	0.039665	1 C	0.037584
2 C	0.057341	2 C	0.058793	2 C	0.056947	2 C	0.056597
3 C	0.148522	3 C	0.148326	3 C	0.150992	3 C	0.152214
4 C	-0.012484	4 C	-0.011992	4 C	-0.011866	4 C	-0.012194
5 C	0.096728	5 C	0.09691	5 C	0.097178	5 C	0.100717
6 H	0.1308	6 H	0.129951	6 H	0.131751	6 H	0.130493
7 H	0.101476	7 H	0.104618	7 H	0.103554	7 H	0.103736
8 H	0.106391	8 H	0.107239	8 H	0.107565	8 H	0.10617
9 H	0.117707	9 H	0.117192	9 H	0.117589	9 H	0.114043

10 H	0.134353	10 H	0.135157	10 H	0.138016	10 H	0.135677
11 O	-0.417404	11 O	-0.417325	11 O	-0.417081	11 O	-0.417832
12 O	-0.421273	12 O	-0.421554	12 O	-0.420792	12 O	-0.422617
13 O	-0.426357	13 O	-0.424617	13 O	-0.423569	13 O	-0.426458
14 O	-0.41171	14 O	-0.412431	14 O	-0.413343	14 O	-0.41319
15 H	0.249185	15 H	0.249107	15 H	0.24934	15 H	0.249056
16 H	0.248369	16 H	0.248803	16 H	0.24861	16 H	0.249138
17 H	0.252934	17 H	0.252814	17 H	0.255072	17 H	0.251713
18 C	0.039755	18 C	0.039576	18 C	0.039373	18 C	0.037742
19 H	0.100914	19 H	0.100335	19 H	0.100651	19 H	0.100792
20 H	0.120764	20 H	0.12068	20 H	0.121033	20 H	0.121741
21 O	-0.40704	21 O	-0.40752	21 O	-0.407241	21 O	-0.407508
22 H	0.240782	22 H	0.240859	22 H	0.240676	22 H	0.241674
23 C	0.254535	23 C	0.254555	23 C	0.239494	23 C	0.255218
24 C	-0.19487	24 C	-0.194838	24 C	-0.188037	24 C	-0.196412
25 C	-0.288785	25 C	-0.288213	25 C	-0.177476	25 C	-0.287038
26 C	0.219333	26 C	0.218037	26 C	0.218166	26 C	0.238216
27 C	0.219182	27 C	0.218914	27 C	0.157507	27 C	0.213725
28 C	-0.08879	28 C	-0.089637	28 C	-0.070068	28 C	-0.101087
29 H	0.099288	29 H	0.098865	29 H	0.094403	29 H	0.115431
30 O	-0.348037	30 O	-0.348734	30 O	-0.348315	30 O	-0.346235
31 H	0.266666	31 H	0.266368	31 C	0.223112	31 H	0.265752
32 O	-0.344061	32 O	-0.342334	32 C	-0.175548	32 O	-0.344926
33 C	0.227935	33 C	0.225679	33 C	0.320353	33 C	0.228058
34 C	-0.15532	34 C	-0.156451	34 O	-0.34374	34 C	-0.154957
35 C	0.365495	35 C	0.365572	35 C	-0.150675	35 C	0.365078
36 O	-0.41604	36 O	-0.417419	36 C	-0.01951	36 O	-0.417843
37 C	-0.155129	37 C	-0.150698	37 C	-0.138785	37 C	-0.152113
38 C	-0.015258	38 C	-0.016002	38 C	0.169122	38 C	-0.013251
39 C	-0.134302	39 C	-0.1008	39 C	-0.094286	39 C	-0.096842
40 C	0.16972	40 C	0.190865	40 C	-0.036922	40 C	0.169899
41 C	-0.092652	41 C	-0.141897	41 O	-0.359669	41 C	-0.129427
42 C	-0.038357	42 C	-0.039995	42 H	0.109018	42 C	-0.042241
43 O	-0.347496	43 O	-0.338747	43 H	0.105954	43 O	-0.347557
44 H	0.253179	44 H	0.110015	44 H	0.112068	44 H	0.109059
45 H	0.111421	45 H	0.104727	45 H	0.096123	45 H	0.10653
46 H	0.10675	46 H	0.11159	46 H	0.121587	46 H	0.097037
47 H	0.113791	47 H	0.110896	47 O	-0.357911	47 H	0.113879
48 H	0.09869	48 H	0.118561	48 H	0.257906	48 H	0.118368
49 H	0.119498	49 O	-0.355257	49 C	-0.107938	49 O	-0.356252
50 O	-0.355923	50 H	0.258432	50 H	0.11694	50 C	-0.142268
51 H	0.258822	51 C	-0.136498	51 H	0.133844	51 H	0.125871
		52 H	0.117066	52 H	0.087175	52 H	0.121139
		53 H	0.116757	53 O	-0.349956	53 H	0.143417
		54 H	0.136518	54 H	0.251947	54 H	0.252482

CONCLUSION: Methylated structure of the naturally occurring flavonoid vitexin has been simulated, analyzed and compared theoretically using B3LYP/6-311G(d,p) level of theory with the vitexin. Kinetic energy distributions studies clarify the instability as well as steric effect created by glucose unit and methoxy unit in both compounds using prominent peaks in the particular position where these functional units are located. This is further supported by HOMO-LUMO energies and molecular descriptors which elucidates the higher order of dissociating ability of hydroxyl group than methoxy group. Mulliken charge density analysis depicts the B-ring activity and contribution offered by methoxy unit for radical scavenging activity.

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CONFLICT OF INTEREST: Nil

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