



## Ab initio and Time Dependent Density Functional Theory Study of the Structural and Spectral Properties of Two Flavones Derived from *Limnophila indica* Species

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Flavonoids constitute a large group of polyphenolic phytochemicals widely distributed in nature. The overwhelming capability of such species to act as an antioxidant by direct free radical scavenging and their capacity of metal chelation have made their properties a subject of continuous research. Herein, we wish to report a theoretical study, both in gas phase and in chloroform medium, carried out on two flavonoids namely 5-hydroxy-7,2'-dimethoxy flavone and 5,2'-dihydroxy-7,8-dimethoxy flavone derived from *Limnophila indica* species. Time dependent density functional theory calculations using the Gaussian 03 suite of programmes gives an excellent opportunity to predict structural and spectroscopic properties of such flavone species. Optimization of the flavonoids by HF, B3LYP, mPW1PW91 methods at 6-31++G(d,p) and 6-311+G(2d,p) levels confirmed their twisted structure. The UV-VIS spectra of both the flavones were calculated by time dependant density function theory under the polarizable continuum model and the results are in good agreement with the experimentally observed value. The theoretically calculated infrared absorption bands, <sup>1</sup>H and <sup>13</sup>C NMR spectra of these flavones agree well with the reported experimental data.

**Key Words:** 5-Hydroxy-7,2'-dimethoxyflavone, 5,2'-Dihydroxy-7,8-dimethoxyflavone, Time dependent density functional theory, Conformation, UV-VIS, IR, NMR.

### INTRODUCTION

Flavonoids are a group of low molecular weight polyphenolic compounds found in most plants, concentrated in seeds, barks, fruit skins and flowers<sup>1</sup>. Principal sources of dietary flavonoids are buck wheat, apple, onion and beverages prepared from plant extracts like wine, fruit juice, beer and tea<sup>2</sup>. Not only are these compounds important for normal plant growth they play a potential important role in maintaining human health. They stimulate or inhibit a wide variety of enzyme systems and have a potential application as antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, antithrombotic agents and have vasodilatory action<sup>3-6</sup>. The pharmacological activities are overwhelmingly related to the antioxidant activity of the flavonoids arising through their ability to scavenge free radicals<sup>7-9</sup>. In spite of the immense biological importance of the flavonoids and their increasing use in medicine, little information about their molecular structure is available in literature.

The density functional theory (DFT) is currently being applied for carrying out a variety of quantum chemical calculations ranging from geometry optimization of large clusters to the study of reaction rates<sup>10</sup>. For theoretical prediction of

spectroscopic properties (transition energy, oscillator strength *etc.*), the time dependent density functional theory (TDDFT) has been developed<sup>10,11</sup> and is now being widely used<sup>12,13</sup>. The application of such theoretical studies on predicting various properties of flavone moiety has just began and lots of work are still to be done to gain a better knowledge on such an important natural antioxidant.

The objective of the present work is to predict a stable conformation of the two flavones derived from *Limnophila indica* species namely 5-hydroxy-7,2'-dimethoxyflavone (Fig. 2a) and 5,2'-dihydroxy-7,8-dimethoxyflavone (Fig. 2b) in vacuum and chloroform by using different TDDFT methods. The infrared absorption frequencies, UV-VIS absorption maxima and NMR chemical shifts of these two flavones are also predicted in the two medium by applying the computational method of TDDFT and compared with available experimental data<sup>14-17</sup>. Such theoretical studies and the results thus arrived at are expected to have some predictive value for spectroscopic and biochemical investigations of similar compounds.

### COMPUTATIONAL METHOD

All computations were performed on a Pentium computer with Gaussian 03 Revision D.01 suite of programmes<sup>18</sup>. DFT

calculations were done by using the Becke's three parameter hybrid<sup>19</sup> exchange potential with the correlation function of Lee, Yang and Parr<sup>20</sup> (B3LYP). Another level of DFT introduced by Adamo and Barone<sup>21</sup> and abbreviated as mPW1PW91, which utilizes a modification of the gradient corrected Perdew-Wang exchange function, PW91, combined with the non-local Perdew-Wang correlation function<sup>22,23</sup> was also tried. The basis set 6-31++G(d,p) and 6-311+G(2d,p) were used. Optimization of the ground state geometry of the molecules at HF, B3LYP and mPW1PW91 levels was carried out in vacuum and also in chloroform solution by the polarizable continuum model<sup>24</sup>. Using the ground state optimized geometry, the vibrational frequencies, <sup>1</sup>H and <sup>13</sup>C NMR chemical shifts and electronic transition energies were calculated. Similarly by using the TDDFT method under the polarizable continuum model formalism the electronic transition energies in vacuum, CHCl<sub>3</sub> were calculated. In the polarizable continuum model, the solute is placed in the solvent reaction field cavity created by a series of overlapping spheres initially devised by Tomasi *et al.*<sup>24</sup> and implemented by Barone *et al.*<sup>25-27</sup> also by Tomasi and co-workers<sup>28, 29</sup>.

## RESULTS AND DISCUSSION

**Optimized geometry:** The structural details for the two flavones namely 5-hydroxy-7,2'-dimethoxyflavone (flavone 1, Fig. 2a) and 5,2'-dihydroxy-7,8-dimethoxyflavone (flavone 2, Fig. 2b) are calculated at different levels of theory namely HF, B3LYP, mPW1PW91 and their optimized geometries computed at DFT/B3LYP/6-31++G(d,p) level *in vacuo* is shown in Fig. 3c-d, respectively. As expected both the flavones are twisted, at ring B (Fig. 1, view of flavone with the chemical numbering used for flavonoids) of the flavone moiety subtends an angle with the rest of the flavone molecule. It can be seen that the phenyl rings A and B (Figs. 1, 2a, 2b) are quite planar with an average C-C bond lengths of 1.39 Å calculated at every levels of theory (HF, B3LYP, mPW1PW91) and both in vacuum and chloroform medium. These lengths are close to the normal aromatic values.

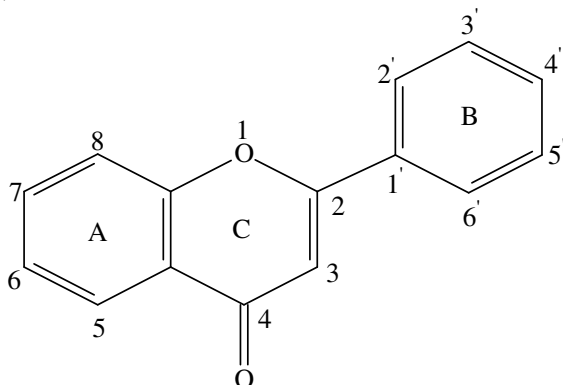
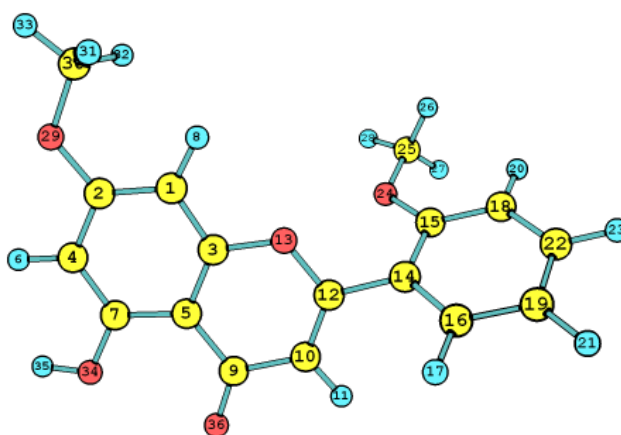


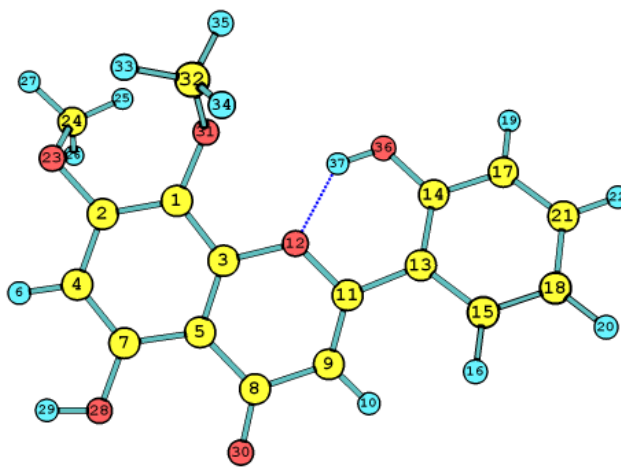
Fig. 1 View of a general flavone unit with the chemical numbering used for flavonoids

In flavone 1 the hydroxyl group and the methoxyl group in ring A lies in the same plane of the chromone ring. The methoxyl group in the phenyl ring (ring B) also lies in the same plane of the benzene ring. In flavone 2 also the hydroxyl

group in ring A is coplanar with the chromone moiety. The hydroxyl group in ring A subtends an angle of 10°, whereas the 8-methoxy group of the same ring subtends an angle of 80° and 7-methoxy group subtends an angle of 65° with the chromone ring. The  $\gamma$ -pyrone ring (ring C, Figs. 1-3) is quasi planar and shows an average dihedral angle of 179° (C1-C3-O13-C12) for flavone 1 and 177° (C7-C5-C8-C9) for flavone 2. This variation of dihedral angles on going from flavone 1 to flavone 2 may be attributed to the developed intramolecular H-bonding (O12...H37-O36) in flavone 2. Judging from the average bond lengths in the  $\gamma$ -pyrone ring for flavone 1 and flavone 2, it can be concluded that there exists very little conjugation within the bonds in this intermediate ring (ring C).



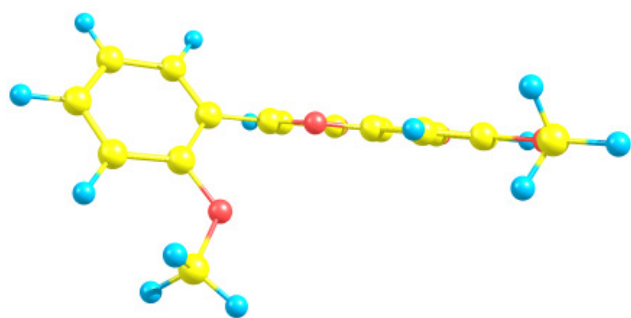
(a) 5-Hydroxy-7,2'-dimethoxyflavone



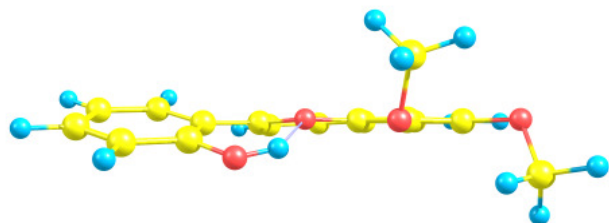
(b) 5,2'-Dihydroxy-7,8-dimethoxyflavone

Fig. 2. Optimized geometry with atom labels calculated at DFT/B3LYP/6-31++G(d,p) level

For flavone 1, the dihedral angles, subtended between the phenyl ring (ring B) and the chromone part calculated at different levels of theory (HF, B3LYP, mPW1PW91) clearly shows a deviation from planarity. The angle is about 126° in vacuum and 125° in chloroform medium. It seems that the slight change in dihedral angle, on changing the medium to chloroform, may be due to the existence of an electronic repulsive interaction between the lone pair electrons of the oxygen atoms on methoxy group in ring B (at position 2') and



(c) 5-Hydroxy-7,2'-dimethoxyflavone



(d) 5,2'-Dihydroxy-7,8-dimethoxyflavone

Fig. 3. Conformational preference of the phenyl ring with respect to the  $\gamma$ -pyrone ring in  $\text{CHCl}_3$  medium as viewed along the inter-ring bond calculated at DFT/B3LYP/6-31++G(d,p) level

the lone pairs residing on the chlorine atoms of the chloroform solvent. Conformational preference of the phenyl ring with respect to the  $\gamma$ -pyrone ring of 5-hydroxy-7,2'-dimethoxyflavone (flavone 1) as viewed along the inter-ring bond and calculated at DFT/B3LYP/6-31++G(d,p) level in  $\text{CHCl}_3$  medium is shown in Fig. 3a.

For flavone 2 the dihedral angle subtended between the phenyl ring (ring B) and the chromone part is about  $159^\circ$  (in vacuum) and  $160^\circ$  (in chloroform) suggesting that the hydrogen bonding which is already prevalent in vacuum medium further tightens on introduction of a polar aprotic solvent like chloroform thus forcing the phenyl ring (ring C) to twist further. Conformational preference of the phenyl ring with respect to the  $\gamma$ -pyrone ring of 5,2'-dihydroxy-7,8-dimethoxyflavone (flavone 2) as viewed along the inter-ring bond and calculated at DFT/B3LYP/6-31++G(d,p) level in chloroform medium is shown in Fig. 3b.

Flavone 1 and 2 are polar, which is suggested by their appreciable dipole moment as computed in both vacuum ( $\mu = 7.6$  D for flavone 1 and  $\mu = 4.4$  D for flavone 2) and chloroform ( $\mu = 8.6$  D for flavone 1 and  $\mu = 5.5$  D for flavone 2) medium. The dipole moment of flavone 1 is higher than flavone 2. Each flavone has a higher dipole moment in chloroform medium than in vacuum as chloroform being a polar aprotic solvent stabilizes the more polar structure.

**UV-VIS spectra:** The electronic transitions were calculated by the TDDFT method in vacuum and in chloroform at HF, B3LYP and mPW1PW91 levels of theory with basis set 6-31++G(d,p), using 30 singlet states in each case. The polarizable continuum model formalism was used for calculation in solution phase and the respective optimized ground state geometries in the above mentioned solvents were used as input. Absorption bands having reasonable oscillator strength are shown in Table-1 for Flavone-1 and Table-2 for flavone-2. Some more absorption bands with strong oscillator strength were observed below 150 nm, but such bands fall deep within the vacuum UV region and were discarded as they might not be observed experimentally.

It is known that flavones exhibit two major absorption bands near-UV region<sup>30</sup>. The two bands, one at 290 nm and the other at 250 nm represent the B-ring and A-ring absorption properties, respectively. Our theoretical experiment also reveals that, except HF method, the methods namely B3LYP and mPW1PW91 has yielded results in close proximity with what is predicted for flavonoid compound. Even these values are in good agreement with the experimental values<sup>14-17</sup>. A representative UV-VIS spectrum of 5,2'-dihydroxy-7,8-dimethoxyflavone (flavone 2) in  $\text{CHCl}_3$  medium calculated at TDDFT/B3LYP/6-31++G(d,p) level is given in Fig. 4.

**Infrared spectra:** With the optimized geometries obtained at HF, B3LYP and mPW1PW91 levels in vacuum and  $\text{CHCl}_3$ , the vibrational frequencies were calculated with 6-31++G(d,p) basis set and compared with the reported experimental results of the IR absorption bands of the flavones. The theoretical results obtained are in good agreement with the experimentally observed values.

The absorption around the region of  $600\text{-}300\text{ cm}^{-1}$  is due to various bending vibrations present in the molecule, the absorption within the region between  $1300\text{-}1000\text{ cm}^{-1}$  is due

TABLE-1  
TRANSITION ENERGIES (eV), WAVE LENGTHS OF ABSORPTION MAXIMA (nm) AND OSCILLATOR STRENGTHS (f) OF ELECTRONIC TRANSITIONS OF 5-HYDROXY-7,2'- DIMETHOXYFLAVONE (FLAVONE 1)

Basis sets	Vacuum			$\text{CHCl}_3$		
	eV	nm	f	eV	nm	f
HF/6-31++G(d,p)	5.1349	241.46	0.0021	5.3760	230.63	0.0143
	4.1488	298.85	0.1197	4.3520	299.79	0.3125
	4.3408	285.62	0.1011	4.5746	280.97	0.1636
	4.4701	277.36	0.0442	4.6497	258.00	0.2279
	4.7844	259.14	0.0554	4.8124	257.63	0.1216
B3LYP/6-31++G(d,p)	4.9278	251.60	0.0909	5.3017	253.44	0.0774
	5.0918	243.50	0.2141	5.5182	248.18	0.1187
	4.3135	287.43	0.1569	4.1684	297.44	0.2598
	4.5097	274.93	0.0949	4.3295	286.37	0.0378
	4.6442	266.96	0.0437	4.5890	270.17	0.0996
MPW1PW91/6-31++G(d,p)	4.9563	250.15	0.0750	4.9742	249.26	0.1830
	5.1043	242.90	0.1457	4.9953	248.20	0.2193

TABLE-2  
TRANSITION ENERGIES (eV), WAVE LENGTHS OF ABSORPTION MAXIMA (nm) AND OSCILLATOR STRENGTHS (f) OF ELECTRONIC TRANSITIONS OF 5,2'-DIHYDROXY-7,8-DIMETHOXYFLAVONE (FLAVONE 2)

Basis sets	Vacuum			CHCl <sub>3</sub>		
	eV	nm	f	eV	nm	f
HF/6-31++G(d,p)	5.0453	245.74	0.0200	5.0961	243.29	0.6027
	5.2196	237.54	0.3969	5.0961	234.39	0.0224
B3LYP/6-31++G(d,p)	4.3937	282.19	0.0315	4.3251	286.66	0.4580
	4.4912	276.06	0.1741	4.3520	284.89	0.3125
	4.7475	261.15	0.2971	4.5746	271.03	0.1636
MPW1PM91/6-31++G(d,p)				4.6497	266.65	0.2279
	4.1222	300.77	0.0237	3.9974	310.16	0.1041
	4.6690	265.55	0.1728	4.5382	273.20	0.3304
	4.8056	258.00	0.0591	4.7669	260.10	0.2737
	4.9617	249.88	0.3092	4.8720	254.49	0.1647

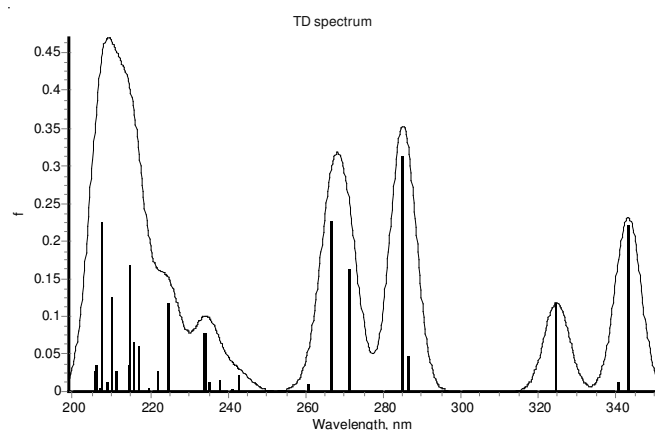


Fig. 4: UV-VIS spectrum of 5,2'-dihydroxy-7,8-dimethoxyflavone in CHCl<sub>3</sub> medium calculated at TDDFT/B3LYP/6-31++G(d,p) level (flavone 2)

to different O-C stretching and bending vibrations of the ether linkage present in the molecule, the absorption region around 1720-1650 cm<sup>-1</sup> is due to the C=O stretching vibrations, the absorption region around 3200-3100 cm<sup>-1</sup> is due to C-H stretching vibrations and the absorption region in between 3800-3500 cm<sup>-1</sup> is due to O-H stretching vibrations.

Tables 3 and 4 lists the important vibrations observed in flavone 1 and flavone 2, respectively in vacuum and CHCl<sub>3</sub> medium. One calculated IR spectrum of flavone 1 is shown in Fig. 5.

**<sup>1</sup>H and <sup>13</sup>C NMR spectra:** For calculation of NMR chemical shifts the structure was optimized at B3LYP/6-311G(2d,p) level in CHCl<sub>3</sub> medium by the polarizable continuum model formalism<sup>20-24</sup> and the optimized geometry was subjected to NMR calculations by the gauge-independent atomic orbital method. For flavone 1 and flavone 2, the <sup>1</sup>H NMR spectrums

TABLE-3  
VIBRATIONAL FREQUENCY DATA OF 5-HYDROXY-7,2'-DIMETHOXYFLAVONE (FLAVONE 1)

Basis sets	Frequency (cm <sup>-1</sup> )	
	Vacuum	CHCl <sub>3</sub>
HF/6-31++G(d,p)	4183, 3259, 3193, 1937, 1855, 1802, 1754, 1660, 1601, 1536, 1492, 1403, 1389, 1370, 1350, 1303, 1212, 1152, 909, 423	3931, 3269, 3197, 1866, 1839, 1792, 1785, 1741, 1654, 1533, 1488, 1397, 1385, 1370, 1346, 1339, 1299, 1228, 1210, 1143, 910, 435
B3LYP/6-31++G(d,p)	3810, 3020, 1717, 1680, 1655, 1612, 1506, 1471, 1410, 1366, 1292, 1283, 1245, 1225, 1189, 1121, 1106, 766	3549, 3030, 3024, 1673, 1666, 1643, 1638, 1601, 1522, 1500, 1466, 1410, 1363, 1307, 1287, 1282, 1264, 1245, 1223, 1186, 1123, 1106, 765
MPW1PM91/6-31++G(d,p)	3878, 3045, 1759, 1710, 1686, 1546, 1514, 1495, 1445, 1394, 1324, 1296, 1274, 1266, 1242, 1202, 1126, 772, 421	3619, 3048, 1711, 1700, 1674, 1669, 1632, 1540, 1508, 1489, 1475, 1443, 1392, 1320, 1315, 1295, 1274, 1264, 1240, 1201, 1126, 771, 439
Experimental	3416, 2934, 1656, 1609, 1497, 1454, 1329, 1238, 1159	

TABLE-4  
VIBRATIONAL FREQUENCY DATA OF 5,2'-DIHYDROXY-7,8-DIMETHOXYFLAVONE (FLAVONE 2)

Basis sets	Frequency (cm <sup>-1</sup> )	
	Vacuum	CHCl <sub>3</sub>
HF/6-31++G(d,p)	4137, 3294, 3186, 1942, 1840, 1800, 1776, 1580, 1495, 1372, 1217, 1210, 1154, 856, 393	4138, 3931, 3293, 3202, 1876, 1825, 1802, 1790, 1765, 1679, 1616, 1574, 1494, 1454, 1383, 1371, 1283, 1213, 1148, 854, 399
B3LYP/6-31++G(d,p)	3812, 3715, 3219, 3029, 3036, 1717, 1671, 1662, 1638, 1616, 1536, 1503, 1444, 1367, 1330, 1252, 1227, 1119, 1105, 611, 402	3698, 3547, 3041, 1671, 1655, 1629, 1609, 1604, 1530, 1496, 1438, 1366, 1353, 1328, 1263, 1249, 1225, 1139, 1117, 1106, 1037, 581, 414
MPW1PM91/6-31++G(d,p)	3879, 3764, 3064, 3061, 1761, 1703, 1671, 1649, 1566, 1535, 1522, 1503, 1470, 1395, 1377, 1300, 1269, 1248, 1154, 1141, 1118, 1075, 618, 413	3747, 3617, 3066, 3061, 1713, 1694, 1684, 1661, 1638, 1559, 1530, 1514, 1498, 1466, 1395, 1377, 1350, 1296, 1285, 1267, 1245, 1215, 1155, 1139, 1118, 1031, 1007, 778, 589
Experimental	3434, 2934, 1649, 1611, 1575, 1509, 1447	

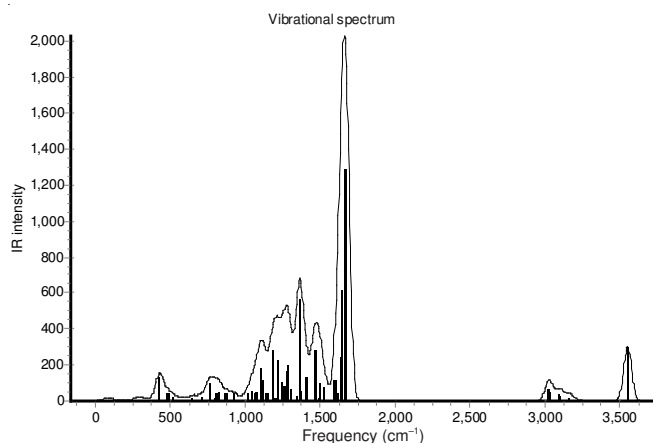


Fig. 5. Theoretically predicted infrared spectrum of 5-hydroxy-7,2'-dimethoxyflavone in  $\text{CHCl}_3$  medium calculated at TDDFT/B3LYP/6-311+G(d,p) level (flavone 1)

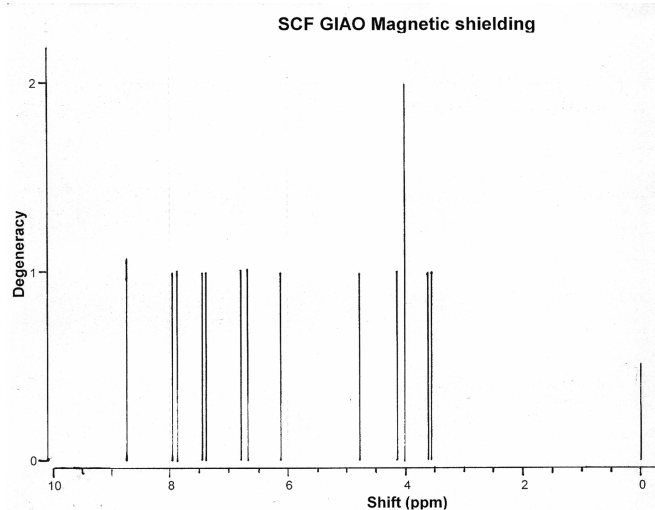


Fig. 8. Theoretically predicted  $^1\text{H}$  NMR spectrum of 5,2'-dihydroxy-7,8-dimethoxyflavone in  $\text{CHCl}_3$  medium calculated at TDDFT/B3LYP/6-311+G(2d,p) level (flavone 2)

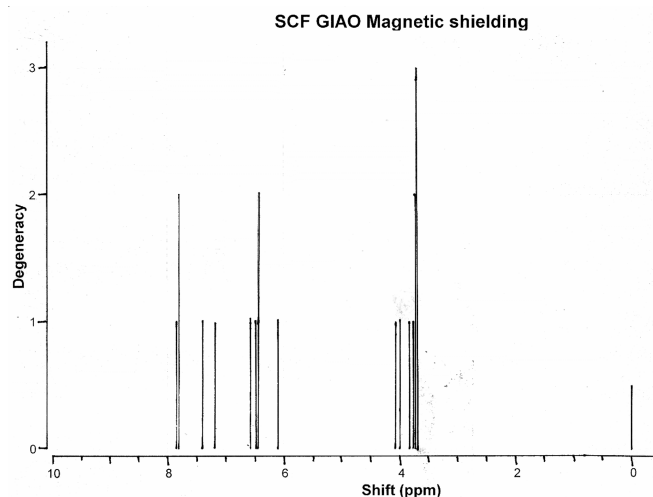


Fig. 6. Theoretically predicted  $^1\text{H}$  NMR spectrum of 5-hydroxy-7,2'-dimethoxyflavone in  $\text{CHCl}_3$  medium calculated at TDDFT/B3LYP/6-311+G(2d,p) level (flavone 1)

are represented in Figs. 6 and 8 and the  $^{13}\text{C}$  NMR spectrum is represented in Figs. 7 and 9, respectively.

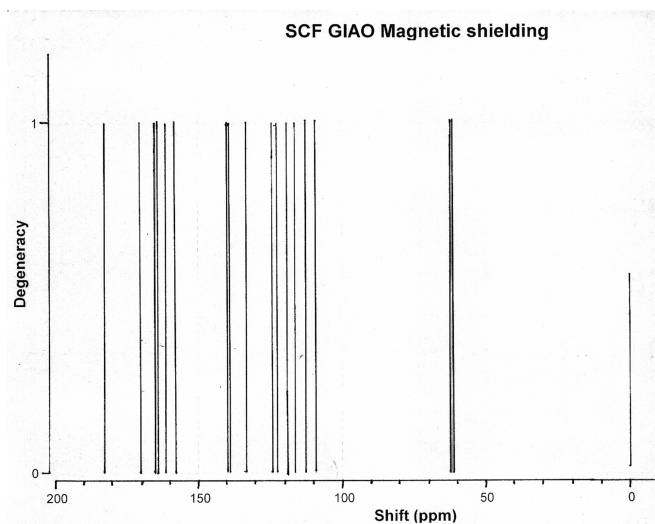


Fig. 9. Theoretically predicted  $^{13}\text{C}$  NMR spectrum of 5,2'-dihydroxy-7,8-dimethoxyflavone in  $\text{CHCl}_3$  medium calculated at TDDFT/B3LYP/6-311+G(2d,p) level (flavone 2)

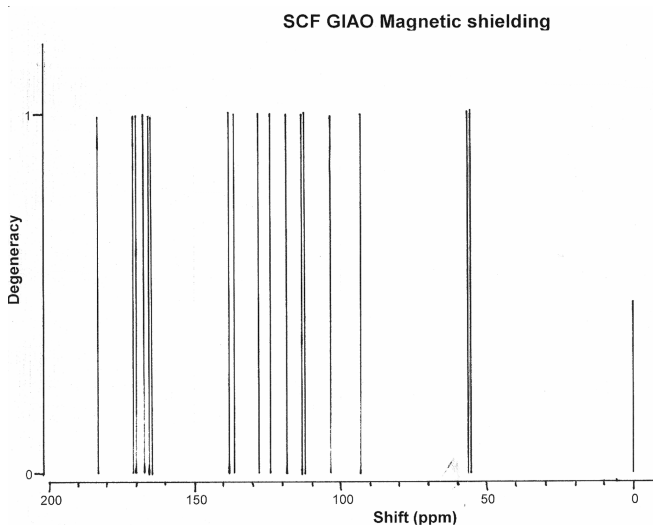


Fig. 7. Theoretically predicted  $^{13}\text{C}$  NMR spectrum of 5-hydroxy-7,2'-dimethoxyflavone in  $\text{CHCl}_3$  medium calculated at TDDFT/B3LYP/6-311+G(2d,p) level (flavone 1)

The calculated  $^{13}\text{C}$  NMR spectrum shows 17 distinct peaks corresponding to 17 different carbon environment present in both the flavone moiety; the theoretically obtained chemical shifts are in accordance with the electronic charge distribution obtained by natural population analysis.

The calculated chemical shifts for  $^1\text{H}$  NMR of flavone 1 are clustered around the region (6.45-6.57)  $\delta$  for ring A, around (7.17-7.84)  $\delta$  for ring B, around (3.71-3.99)  $\delta$  for C-7 OMe protons, around (3.73-4.07)  $\delta$  for C-6' OMe protons and at 6.48  $\delta$  for the C-3 proton. For flavone 2, the  $^1\text{H}$  NMR signal is at 6.68  $\delta$  for proton at C-6 in ring A, around (7.38-7.95)  $\delta$  for ring B, around (3.55-4.77)  $\delta$  for C-7 OMe protons, around (3.60-4.01)  $\delta$  for C-8 OMe protons and at 6.80 for the C-3 proton. The reported experimental values<sup>14-17</sup> are in good agreement with the theoretical findings.

## Conclusion

Flavones are natural antioxidants and by virtue of their metal chelating ability, they can act as metal scavengers. In order to know and investigate these properties it is essential to know their three dimensional structures. This project aims at acquiring a better idea of the structural and spectroscopic properties (namely UV-VIS, IR, NMR) of two flavones 5-hydroxy-7,2'-dimethoxyflavone and 5,2'-dihydroxy-7,8-dimethoxyflavone. Present TDDFT study shows that both the flavones are twisted and polar in nature in both vacuum and chloroform. In both the flavones, ring B subtends an angle with the rest of the flavone molecule. Results also indicate the presence of intra molecular H-bonding in flavone 2. The spectroscopic properties predicted by present study are also in good agreement with the experimentally observed values.

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