


Higher Reactive Potential of Bioactive Isoflavonoid Genistein over Daidzein Established through Density Functional Studies: Endorsing the Phytoestrogenic Role

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Abstract: Genistein [5,7-dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one] is a naturally occurring isoflavone commonly found in soy products and other legumes. Daidzein (4',7-dihydroxyisoflavone) is another isoflavone phytoestrogen, classified as a non-steroidal estrogen. Its main sources include leguminous plants like soybean and mung bean. In the current study, Density functional theory (DFT) has been applied to compute and compare the structural and electronic properties of Genistein and Daidzein, including bond length, Mulliken atomic charges, electrostatic potential surfaces, vibrational frequencies, dipole moment, and IR spectra using B3LYP/6-311G, 6-311G(d), and 6-311G(d,p) basis sets. Redistribution of charges in genistein was reported to be over a larger range, while the Bond length values of C2-O15, C6-O13, and C26-O29 were found to be higher as compared to O-H bond lengths. This led us to conclude that the Genistein molecule displays enhanced reactivity due to the presence of 3 hydroxyl groups and a carbonyl group (C8=O17). In Daidzein, C6-O24 and C21-O28 bond lengths were observed to be higher than O-H bond lengths. Daidzein exhibits its activity due to 2 hydroxyl groups and a carbonyl group (C9=O26). A quantum-mechanical study of the electronic structure, ground-state properties, and electrostatic potential surface further suggests more reactive sites in genistein than in daidzein. Overall, these findings suggest that genistein is chemically more reactive, which may contribute to its relatively higher biological activity than daidzein.

Keywords: Density Functional Studies; electronic structure; electrostatic potential; Mulliken atomic charges; Genistein, Daidzein.

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1. Introduction

Organic compounds such as isoflavones have many health benefits, including the prevention of many diseases. Genistein [5,7-dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one] [1] present in Tofu, Fava, and Soyabean is an isoflavone phytoestrogen which harbours antineoplastic activity along with other properties including antioxidant, antiangiogenic, anthelmintic, and anticancer effects [2,3]. Earlier studies have shown that genistein is very effective in the treatment of common liver fluke, pork trematode, and poultry cestode, has selective estrogen receptor modulator properties, and is also thought to be an alternative to classical hormonal therapy, which helps prevent cardiovascular disease in postmenopausal women.

Isoflavones, which are widely consumed in Asian countries, have been proposed for their role in cancer prevention and antitumor potential as established through *in vitro* and *in vivo* studies [4,5]. Moreover, genistein also demonstrates chemopreventive and therapeutic potential by regulating pathways associated with oxidative stress, inflammatory signaling, angiogenesis, programmed cell death, and uncontrolled cell proliferation [6-8]. The estimated daily intake of isoflavones among adults in Asian populations, particularly in Japan and China, ranges between 25 and 50 mg, which is many-fold higher than that of women in Western countries, and this difference may be correlated with the lower incidence of certain types of cancers in Asian populations. [9].

Similarly, another phytoestrogen, daidzein (4',7-dihydroxyisoflavone), derived from legumes such as soybean and mung bean, is a naturally occurring non-steroidal estrogen belonging to the isoflavone family [10]. Traditional Chinese medicine Gegen contains daidzein as its major bioactive component [11], which has been reported to be useful for conditions such as fever, acute dysentery, diarrhea, diabetes, cardiac disorders, liver damage, and other ailments [12]. The chemical structure of daidzein bears a striking similarity to that of mammalian estrogens, exerting dual functionality by either replacing or interfering with estrogen and the estrogen receptor (ER) complex. As a result, daidzein has protective effects against breast cancer, diabetes, cardiovascular disorders, osteoporosis, etc., especially the diseases linked to the regulation of estrogen [13]. Moreover, daidzein has anti-tumorigenic, anti-inflammatory properties, as well as inhibiting oxidative damage and protecting the skin and nerves [14].

As we know, breast cancer among all cancer types has been one of the most common malignant tumours in women, seriously threatening public health globally. Higher intake of phytoestrogens by the Asian population is attributed to a lower incidence of breast cancer cases in comparison to the Western women's population [15]. This points to the fact that phytoestrogen use could be a vital approach for breast cancer prevention and therapy through mechanisms such as estrogen receptor modulation and inhibition of angiogenesis [16]. Hence, the use of Daidzein and Genistein for their anti-cancer role has attracted significant attention recently owing to the presence of phytoestrogen constituents in them. Equol, another metabolite of daidzein, was shown to display stronger estrogenic activity than other isoflavones. It is therefore interesting to compare the preventive and therapeutic potential of these phytoestrogens, including Genistein and Daidzein. In fact, daidzein was reportedly quite distinct from other isoflavones, with promising potential to treat osteoporosis and anti-diabetic activity, as established through *in vitro* and *in vivo* studies [17,18]. In terms of mechanism of action, daidzein, a natural antioxidant, was reported to inhibit lipid oxidation by directly scavenging free radicals, altering membrane fluidity, and thereby reducing radical migration

[19]. Daidzein has also been reported to enhance the activity of key antioxidant enzymes, such as catalase, glutathione peroxidase (GPx), and superoxide dismutase (SOD) [20,21]. Daidzein was also reportedly effective in neuroprotection and functional recovery after stroke [22]. Despite having substantial studies on the anticancer potential of such phytoestrogens, cancer remains one of the serious diseases worldwide. Still, it is of paramount importance to examine the salient features and their comparative account in terms of the reactive potential of Genistein and Daidzein, which has been successfully explored in the current study.

2. Materials and Methods

The molecular structures were drawn by using G-03W and GAUSS VIEW 4.1 VERSION (4) of the ab-initio quantum mechanical program [23]. The molecular geometries of the compounds were optimized in the gas phase using density functional theory at the B3LYP level with the 6-311G(d,p) basis set. Figures 1a and b are the optimized structures of Genistein and Daidzein using the 6-311G(d,p) basis set at the B3 LYP level. The optimized structures of 3. The compounds have been used to calculate the molecular Bond lengths, Mulliken atomic charges, electrostatic potential surfaces, vibrational frequencies (scaled 0.9614), IR spectra, HOMO-LUMO energy gaps, dipole moments, and polarizability tensor components.

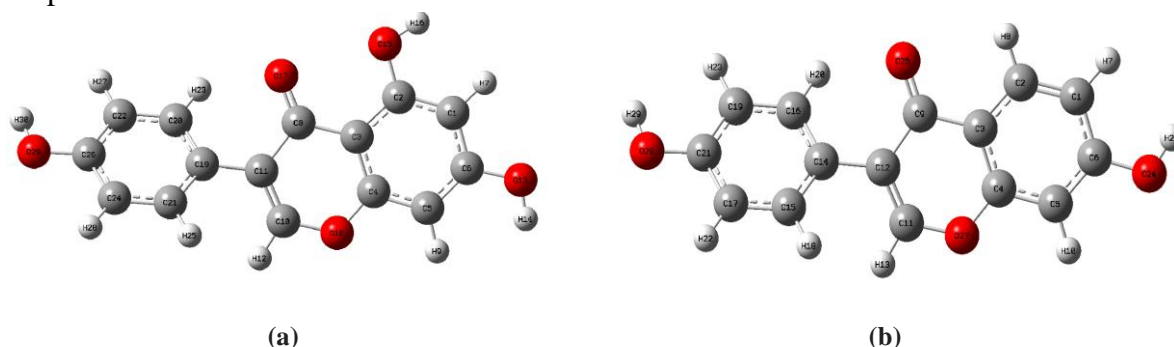


Figure 1. Optimised Structure of (a) Genistein; (b) Daidzein with DFT(B3LYP) 6-311G(d,p) basis sets.

3. Results and Discussion

3.1. Bond length.

Density functional theory (DFT) at the B3LYP level with 6-311G(d,p) basis set has been employed to study the bond lengths of Genistein and Daidzein. The comparative values of the bond lengths of Genistein and Daidzein in the units of 'Å' have been shown in Figures 2a and 2b. Upon studying the bond lengths of Genistein and Daidzein, it was observed that the bond length values of C2-O12, C6-O13, and C26-O29 are 1.35Å, 1.3588Å, and 1.3657Å, respectively, in genistein, whereas in daidzein, the bond length values of C6-O24 and C21-O28 are 1.3594Å and 1.3653Å, respectively. These bond length values are higher than the O-H bond length values in both molecules. So, it can be concluded that the reactivity is due to the presence of a hydroxyl group(-OH) in both molecules. Genistein is a more bioactive compound due to its 3 hydroxyl groups, compared to daidzein, which has 2 hydroxyl groups. The bond length of C9=O26 is 1.225Å in daidzein, and in Genistein is C8=O17 it is 1.221Å. These bond lengths are higher than those to other C-H bonds in both molecules. Hence, there is a possibility of nucleophilic or electrophilic attack on these positions. The phenolic -OH groups, together with

the conjugated C=O group and extended π -system, contribute to hydrogen bonding, redox behavior, and potential biological activity of both genistein and daidzein

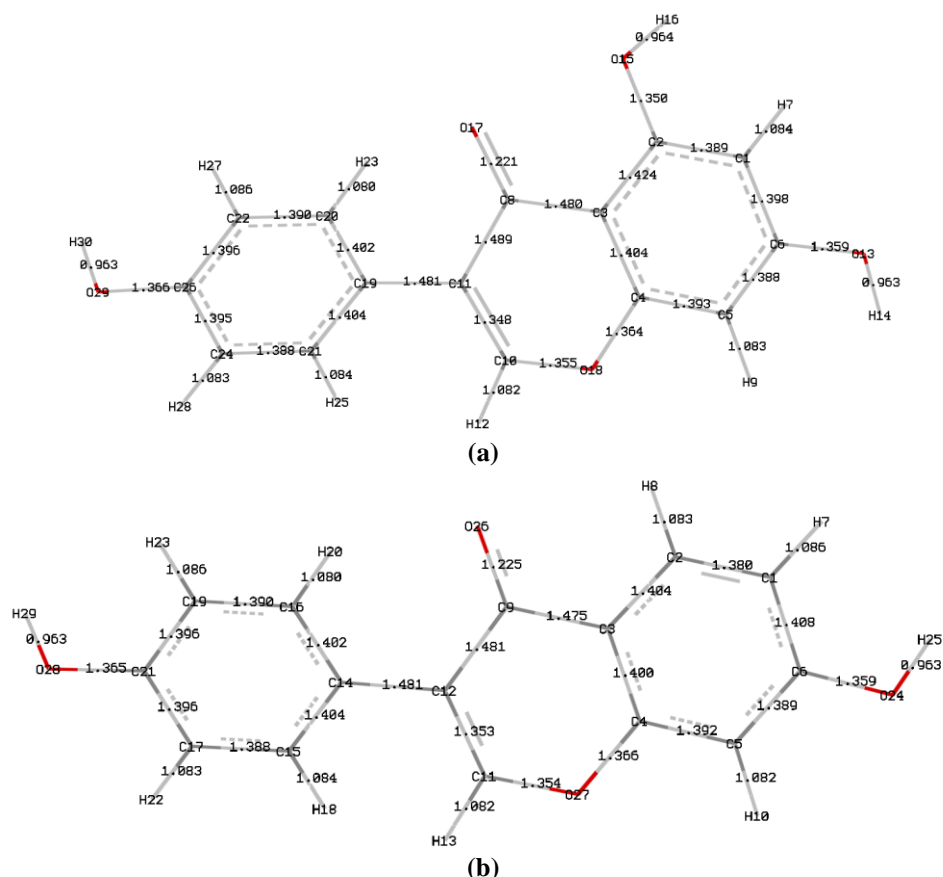


Figure 2. Bond length (Å) values of (a) Genistein; (b) Daidzein computed with DFT(B3LYP) at 6-311G(d,p) level.

3.2. Mulliken population analysis.

The values of the net atomic charges at various atomic sites in the units of ‘e’ have been shown in Figures 3a and 3b. These have been obtained using Mulliken Population Analysis within density functional theory (DFT) at the B3LYP level with the 6-311G(d,p) basis set. The positive and negative values of the net charges at various atomic sites in a molecule are indicative of the fact that the total charges on the orbitals after the molecule is formed are less than or greater than the free atomic charges. This indicates electron-deficient and electron-rich regions of the molecule, reflecting charge redistribution upon bond formation compared to the free atoms.

The partial charge on carbonyl C8, O17 is 0.359180 e, -0.312961 e, respectively, in genistein. Whereas the partial charge on carbonyl C9, O26 is 0.345634 e, and -0.518016 e respectively in daidzein. These results indicate that the carbonyl groups in both molecules are highly polar, with daidzein exhibiting somewhat greater charge separation ($\Delta q \approx 0.86$ e) than Genistein ($\Delta q \approx 0.67$ e). While Mulliken charges are basis-set dependent and should be interpreted qualitatively, the broader charge delocalization observed in genistein, together with its additional hydroxyl group (three versus two in daidzein), suggests that genistein possesses more potential sites for hydrogen bonding and polar interactions. These features, supported by literature reports of higher antioxidant and anticancer activity, imply that genistein is generally more reactive and bioactive than daidzein.

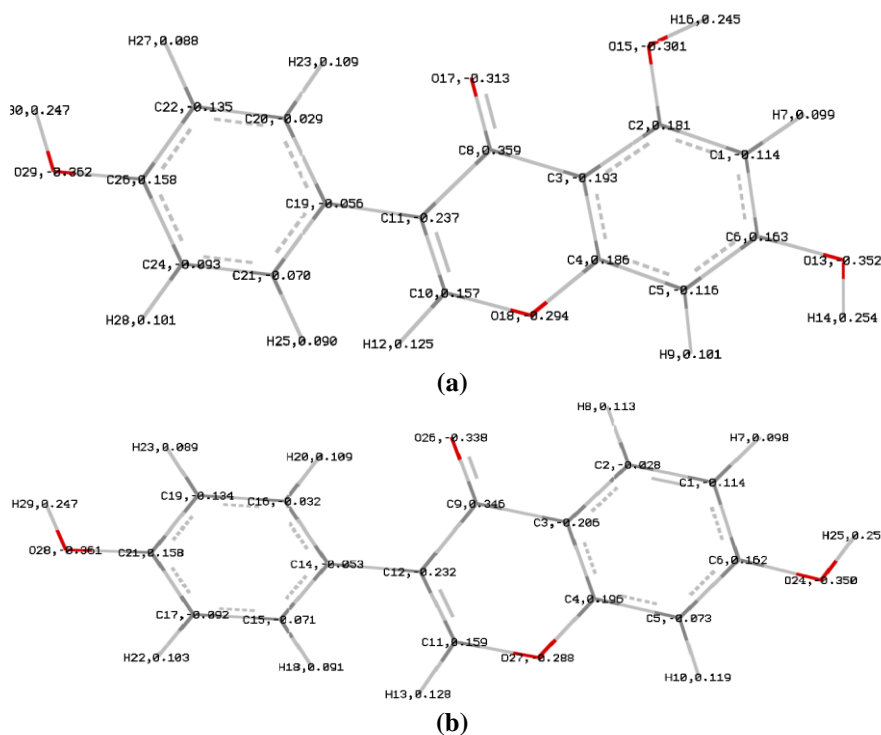


Figure 3. Mulliken atomic charges(e) values of **(a)** Genistein; **(b)** Daidzein computed with DFT(B3LYP) at 6-311G(d,p) level.

3.3. IR spectra.

IR spectra of these molecules calculated at DFT(B3LYP)/6-311G(d,p) level have been shown in Figures 4a and 4b. Computed frequencies, modes of vibrations, and total energy distribution (TED) values calculated at DFT(B3LYP)/6-311(d, p) are shown in Tables 1 and 2. A typical infrared spectrum is usually divided into two regions.

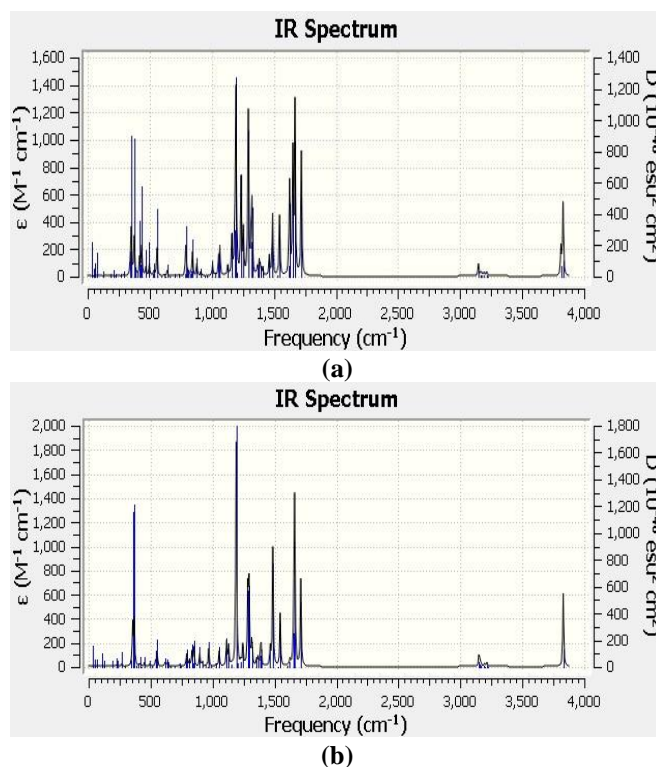


Figure 4. IR spectra of **(a)** Genistein; **(b)** Daidzein computed with DFT(B3LYP) at 6-311G(d,p) level.

The left half, below 1000 cm⁻¹ to 400 cm⁻¹, is the fingerprint region, and the right half, above 1000 cm⁻¹ to 4000 cm⁻¹, is the functional group region. The right half, above 2000 cm⁻¹, although it contains relatively few peaks, provides some diagnostic information in this region of the spectrum. A very broad peak in this region between 3100 cm⁻¹ and 3600 cm⁻¹ indicates the presence of exchangeable protons, typically from alcohol, amine, amide, or carboxylic acid groups [24,25].

Table 1. Frequencies/TED % values for the Stretching mode of vibration of genistein calculated by VEDA at DFT /6-311G(d,p) level.

Mode	Atom no.	Atoms	Frequencies (cm ⁻¹), TED%
s 1	13, 14	O, H	f3829, 100
s 2	15, 16	O, H	f3813, 100
s 3	29, 30	O, H	f3831, 100
s 4	1, 7	C, H	f3175, 99
s 5	5, 9	C, H	f3189, 99
s 6	10, 12	C, H	f3216, 99
s 7	20, 23	C, H	f3226, 99
s 8	21, 25	C, H	f3168, 89
s 9	22, 27	C, H	f3148, 99
s 10	24, 28	C, H	f3194, 89
s 11	17, 8	O, C	f1722, 80
s 12	10, 11	C, C	f1653, 30
s 13	4, 5	C, C	f1391, 34
s 14	1, 6	C, C	f1626, 25
s 15	20, 22	C, C	f1464, 33
s 16	3, 4	C, C	f1065, 31
s 17	29, 26	O, C	f1294, 57
s 18	1, 2	C, C	f1490, 23
s 19	22, 26	C, C	f1621, 23
s 20	20, 22	C, C	f1655, 60
s 21	1, 2	C, C	f1670, 37
s 22	20, 22	C, C	f836, 47
s 23	5, 6	C, C	f1411, 49
s 24	15, 2	O, C	f594, 58
s 25	1, 6	C, C	f1006, 50
s 26	1, 6	C, C	f1254, 27
s 27	19, 20	C, C	f1317, 21
s 28	8, 3	C, C	f1199, 12
s 29	11, 19	C, C	f224, 18

Table 2. Frequencies/TED% values for the stretching mode of vibration of daidzein calculated by VEDA at DFT/6-311G(d,p) level.

Mode	Assignment	Atom no.	Atoms	Frequencies (cm ⁻¹),TED%
s 1	STRE	24, 25	O, H	f3830, 90
s 2	STRE	28, 29	O, H	f3830, 90
s 3	STRE	1, 7	C, H	f3157, 97
s 4	STRE	2, 8	C, H	f3202,97
s 5	STRE	5, 10	C, H	f3213, 97
s 6	STRE	11, 13	C, H	f3215, 97
s 7	STRE	15, 18	C, H	f3168, 89
s 8	STRE	16, 20	C, H	f3223, 98
s 9	STRE	17, 22	C, H	f3194, 90
s 10	STRE	19, 23	C, H	f3149, 98
s 11	STRE	26, 9	O, C	f1712, 76
s 12	STRE	11, 12	C, C	f1651, 52
s 13	STRE	2, 3	C, C	f1243, 22
s 14	STRE	14, 16	C, C	f1319, 38
s 15	STRE	1, 2	C, C	f1662, 52
s 16	STRE	3, 4	C, C	f1607, 41
s 17	STRE	15, 17	C, C	f1295, 62
s 18	STRE	14, 16	C, C	f1465, 17
s 19	STRE	15, 17	C, C	f1624, 44
s 20	STRE	1, 2	C, C	f1486, 35
s 21	STRE	3, 4	C, C	f743, 36
s 22	STRE	14, 16	C, C	f1656, 50

Mode	Assignment	Atom no.	Atoms	Frequencies (cm ⁻¹),TED%
s 23	STRE	27, 11	O, C	f1054, 13
s 24	STRE	24, 6	O, C	f1284, 20
s 25	STRE	1, 2	C, C	f1392, 56
s 26	STRE	15, 17	C, C	f839, 47
s 27	STRE	9, 3	C, C	f1226, 14
s 28	STRE	12, 4	C, C	f227, 23

From the vibrational frequency analysis of genistein, it was found that the O15-H16, O13-H14, and O29-H30 stretching vibrations occur at 3812.54 cm⁻¹, 3829 cm⁻¹, and 3830.57 cm⁻¹, respectively. These stretching vibrations correspond to phenolic-OH groups. Carbonyl group (C=O) stretching vibration was observed at 1721.55 cm⁻¹. The carbonyl peak of the ketone group has shown absorption at longer wavelengths. The reason behind this could be the conjugation with the C=C bond and the delocalization of the electrons in the C=O group, thereby reducing the C=O character and causing absorption at a higher wavelength. Furthermore, C2-O15, C4-O18, and C6-O13 stretchings were observed at 1410.72 cm⁻¹. These results are very much closer to the earlier experimental reporting [26].

Vibrational analysis of daidzein shows that O24-H25 and O28-H29 stretching vibrations are taking place at 3830.90 cm⁻¹, while O26=C9, O27-C11, and O24-C6 stretchings are taking place at 1712,1054,1284 cm⁻¹. In the case of genistein, the hydroxyl and carbonyl stretching frequencies are lower than those of daidzein, indicating stronger hydrogen-bonding interactions and greater electron delocalization. This analysis led us to conclude that genistein is more bioactive than daidzein.

3.4. Molecular electrostatic potential surfaces.

From molecular electrostatic potential (MEP) surfaces, the relative reactive sites within a molecule can be predicted for possible nucleophilic and electrophilic attacks. The MEP surface analysis of both compounds was performed using DFT calculations on the optimized structures with the B3LYP/6-311G(d,p) basis set. The electrostatic potential surface maps of the said compounds are shown in Figures 5a and 5b. The colour code of the compound lies in the range of $-4.618e^{-3}$ (red) to $+4.618e^{-3}$ (blue).

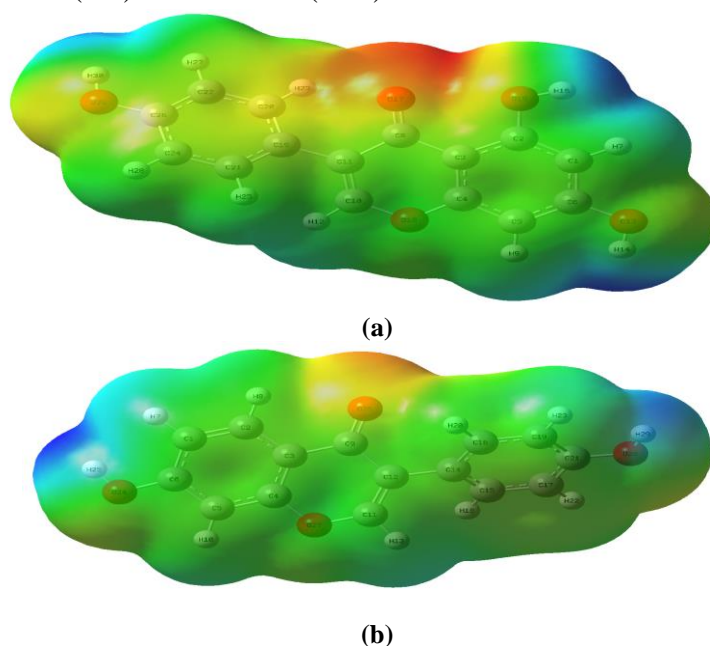


Figure 5. Electrostatic potential surfaces of (a) Genistein; (b) Daidzein computed with DFT(B3LYP)/6-311G(d,p) level.

The electrostatic potential increase has been reported to be in the order blue>green>yellow>orange>red. Blue colour represents the positive regions, which are prone to nucleophilic attack, and red colour represents the negative regions that are more prone to electrophilic attack [27,28]. Red colour in the MEP structure points towards an electron-rich region, and blue colour points towards the electron-deficient region. The polarization effect can be clearly seen in the compounds. Analysis of the molecular electrostatic potential revealed that electron-rich (negative) regions are localized on oxygen atoms, while electron-deficient (positive) regions are predominantly associated with hydrogen atoms. As shown in the ESP surfaces, genistein has three possible nucleophilic attack sites and one electrophilic site, whereas daidzein has two nucleophilic and one electrophilic site. On comparing the intensity of red colour on ESP surfaces, it can be concluded that there is a strong likelihood of electrophilic attack on genistein compared to daidzein. Thus, MEP analysis supports the conclusion that genistein exhibits higher chemical reactivity than daidzein, primarily due to the presence of an additional hydroxyl group and stronger electron density localization at reactive sites.

3.5. Analysis of HOMO and LUMO.

Molecular orbital theory provides an important application in quantum studies of molecules, investigating their electronic level structure, optical, and electrical properties [29]. By using the frontier orbitals, HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) energy values, the ability to receive and donate electrons can be defined. These molecular orbitals also provide information about biological mechanisms, quantum chemistry, optical properties, and photochemical reactions [30-33]. The computed HOMO and LUMO energies of Genistein and Daidzein were obtained at the B3LYP/6-311G(d,p) level of theory, as shown in Table 3. Energy gap computation by using HOMO-LUMO of Genistein and Daidzein has been done to check the stability and reactivity of the molecules. Energy level gaps support the active properties of these molecules. HOMO acts as an electron donor, and LUMO is an electron acceptor. The red colour of HOMO and LUMO represents the negative charge, and the green colour indicates positive charge for the said molecule. In the case of Genistein, HOMO is confined at the locations hydroxyl (O-H), carbonyl (C=O), and C-O bonds, while the LUMO is concentrated on C=O, C-O-C, O-H, and C-H regions. The energy level diagram with frontier orbitals of genistein and daidzein is shown in Figure 6(a,b). The computed energies of corresponding levels are given in Table 3.

Table 3. Energy Values of HOMO and LUMO of **Genistein** and Daidzein computed using DFT (B3LYP) at 6-311G(d,p) level.

Parameter	Genistein (eV)	Daidzein (eV)
HOMO (for the ground level)	-5.790042 eV	-5.886642 eV
LUMO (for the first excited level)	-1.337712 eV	-1.633500 eV
HOMO -LUMO Energy Gap	4.452330 eV	4.253142 eV

In the case of genistein, the energy for HOMO is calculated as - 5.790042 eV, and LUMO is -1.337712 eV. The energy gap between HOMO and LUMO is computed as 4.452330 eV. In the case of Daidzein, HOMO is more confined at O-H, C-C, C-H, and C=O bond locations, whereas LUMO is more confined at C-H, C-C, C=O, and at C-O bond locations. The energy for HOMO is calculated as -5.886642 eV, and LUMO is -1.633500 eV. The energy gap between HOMO and LUMO is computed as 4.253142 eV. The energy gap of both molecules confirms their bioactivity as related to the intramolecular energy transfer. Comparatively,

daidzein possesses a relatively smaller HOMO–LUMO gap compared to genistein, indicating increased chemical reactivity and reduced kinetic stability, and thereby could be the reason for its increased bioactivity. Localization of orbitals on reactive functional groups also facilitates the ability of genistein to form stronger interactions in biological systems.

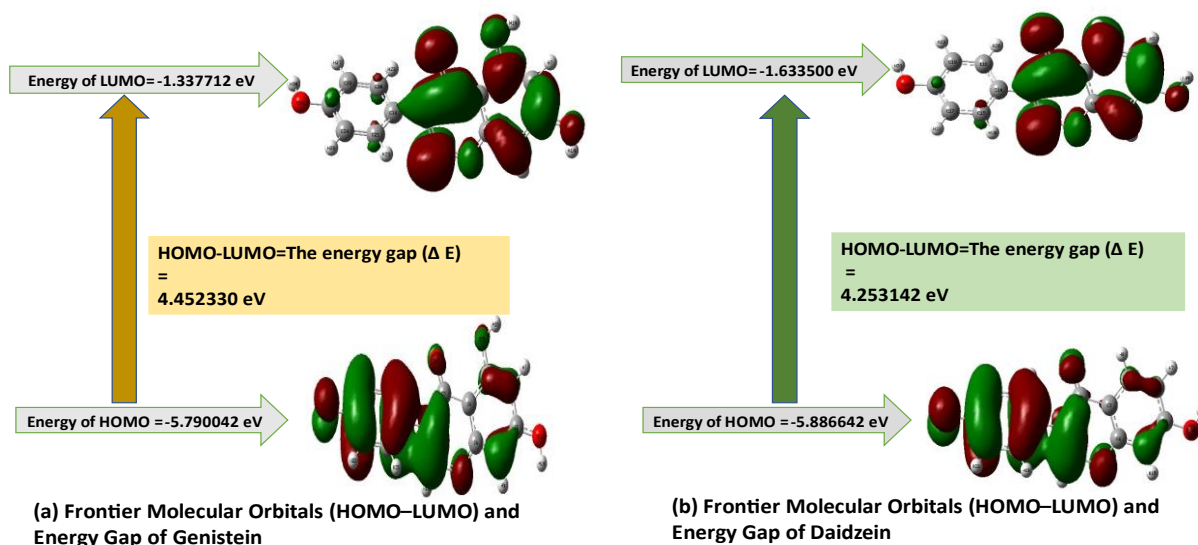


Figure 6. (a) Frontier Molecular Orbitals (HOMO–LUMO) and Energy Gap of Genistein, (b) Frontier Molecular Orbitals (HOMO–LUMO) and Energy Gap of Daidzein.

3.6. Analysis of polarizability tensor components and dipole moments.

Polarizability indicates how an electron cloud gets distorted in the presence of an electric field. If the electron cloud is easily distorted, then it affects the reactivity of the molecules, intermolecular interactions, and overall bioactivity. In this study, The static electronic Dipole polarizability tensor components and dipole moments of Genistein and Daidzein are computed using Hartree–Fock (HF) and density functional theory (DFT) with different basis sets such as with HF/6-311G,6-311G(d),6-311G(d,p) and DFT(B3LYP)/6-311G,6-311G(d),6-311G(d,p).

The computed polarizability tensor components and acquired dipole moments of both molecules are listed in Tables 4-6. It was observed that B3LYP/DFT calculations yielded larger polarizability values than HF calculations. On the other hand, HF results show higher dipole moments than DFT(B3LYP)/6-311G(d,p) for both molecules. Daidzein has a relatively smaller dipole moment compared to genistein. Dipole moment of Genistein by DFT(B3LYP)/ 6-311G(d,p) was found to be 3.4238 Debye, whereas with the same method, the Dipole moment of daidzein was reported to be 2.206 Debye. These observations led us to conclude that genistein has higher polarity and is likely to be biologically more reactive than daidzein. Comparison of the Dipole moments of both molecules is shown in Figure 7.

Table 4. Dipole polarizability tensor components of **Genistein** computed with HF/6-311G,6-311G(d),6-311G(d,p) and DFT(B3LYP)/6-311G,6-311G(d),6-311G(d,p).

Dipole polarizability tensor components of genistein							
Method and basis sets	α_{xx}	α_{yy}	α_{zz}	α_{xy}	α_{xz}	α_{yz}	α_{av}
HF/6-311G	237.65	-16.112	157.422	-2.37	-9.526	82.361	74.90417
HF/6-311G(d)	241.803	-13.981	156.928	-2.109	-9.671	87.243	76.70217
HF/6-311G(d,p)	237.728	-14.354	153.588	-2.386	-10.409	77.125	73.54867
DFT/6-311 G	237.641	-16.118	157.405	-2.365	-9.529	82.381	74.9025
DFT/6-311 G(d)	299.349	-13.251	174.808	-1.107	-9.176	84.601	89.204
DFT/6-311 G(d,p)	300.467	-13.221	175.901	-1.126	-9.083	85.742	89.78

Table 5. Dipole polarizability tensor components of Daidzein computed with HF/6-311G,6-311G(d),6-311G(d,p) and DFT(B3LYP)/6-311G,6-311G(d),6-311G(d,p).

Dipole polarizability tensor components of daidzein							
Method and basis sets	α_{xx}	α_{yy}	α_{zz}	α_{xy}	α_{xz}	α_{yz}	α_{av}
HF/6-311G	240.434	-9.566	149.962	-1.707	-6.356	78.656	75.23717
HF/6-311G(d)	244.36	-7.66	149.855	-1.326	-6.672	83.742	77.04983
HF/6-311G(d,p)	245.22	-7.636	150.938	-1.374	-6.584	84.972	77.58933
DFT/6-311 G	299.316	-6.934	164.107	-0.243	-5.923	76.097	87.73667
DFT/6-311 G(d)	301.34	-5.456	164.524	-0.006	-6.132	80.676	89.15767
DFT/6-311 G(d,p)	302.453	-5.431	165.691	-0.042	-6.035	81.675	89.7185

Table 6. Dipole Moments (Debye)of Genistein and Daidzein computed using HF and DFT(B3LYP) methods with 6-311G, 6-311G(d), and 6-311G(d,p) basis sets.

Basis Set	HF/6-311G	HF/6-311G(d)	HF/6-311G(d,p)	DFT/6-311G	DFT/6-311G(d)	DFT/6-311G(d,p)
Genistein	4.1726	3.8343	3.7668	4.1729	3.4875	3.4238
Daidzein	2.5738	2.3958	2.3489	2.4255	2.2489	2.206

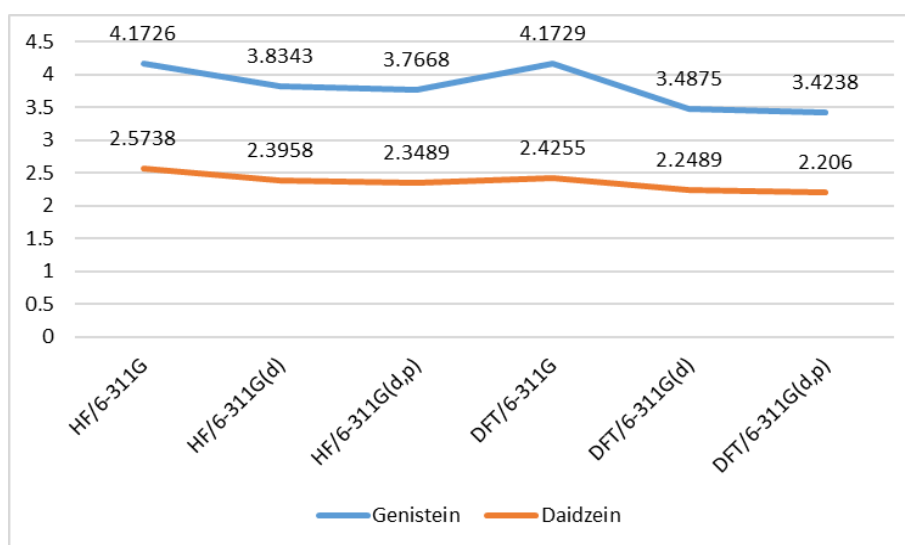


Figure 7. Comparative account of dipole moments of Genistein and Daidzein computed with HF/6-311G, 6-311G(d), 6-311G(d,p) and DFT(B3LYP)/6-311G, 6-311G(d), 6-311G(d,p).

4. Conclusions

Genistein and daidzein are phytoestrogenic isoflavones with strong anticancer, antioxidant, anti-inflammatory, and therapeutic properties. In the current study, Density functional theory (DFT) has been applied to Genistein and Daidzein in order to compute and compare bond lengths, Mulliken atomic charges, electrostatic potential surfaces, vibrational frequencies, IR spectra, Molecular electrostatic potential surfaces, HOMO and LUMO energy gaps, and dipole moments with different basis sets using DFT(B3LYP)/6-311G,6-311G(d),6-311G(d,p) geometries. Redistribution of charges on genistein was reported to be over a larger range, while the bond length values of C2-O15, C6-O13, and C26-O29 were found to be higher as compared to O-H bond lengths. This led us to conclude that the genistein molecule displays significant reactivity due to its 3 hydroxyl groups and the C8=O17 carbonyl group. In daidzein, C6-O24 and C21-O28 bond lengths were observed to be higher than O-H bond lengths. Daidzein exhibits its activity due to 2 hydroxyl groups and the presence of the C9=O26 carbonyl group. Molecular Electrostatic Potential (MEP) surface analysis revealed that genistein has three nucleophilic and one electrophilic site, whereas daidzein has two

nucleophilic and one electrophilic site. Frontier Molecular Orbital (FMO) studies showed that daidzein possesses a narrower HOMO–LUMO gap (4.253 eV) than genistein (4.452 eV). The narrower gap indicates increased electronic transitions, higher chemical reactivity, and lower kinetic stability in daidzein, which could be behind its more robust bioactivity. Additionally, the localization of HOMO over hydroxyl and carbonyl groups in genistein enhances its biological interactions, supporting its observed bioactivity. Genistein had a greater dipole moment (3.42 Debye) than daidzein (2.21 Debye), signifying greater polarity and greater scope for intermolecular interactions within biological systems. Collectively, the results from MEP, HOMO–LUMO, and dipole polarizability indicate that genistein is more chemically reactive and bioactive than daidzein, largely because it has one additional hydroxyl group, a narrower HOMO–LUMO gap, greater electron density localization, and a larger dipole moment.

Author Contributions

Conceptualization, A.S. and A.K.S.; methodology, A.S., AB.S., V.S., S.K.U., and A.K.S.; software, VA.S., J.S., AA.S., and O.P.S.; validation, AB.S. and V.S.; formal analysis, A.S., AB.S., V.S., VA.S., J.S., and AA.S.; investigation, A.S.; data curation, O.P.S. and AJ.S.; visualization, VA.S., J.S., AA.S., A.V., M.S., and S.K.U.; writing—original draft preparation, A.S., AB.S., V.S., VA.S., J.S., and AA.S.; writing—review and editing, A.S., O.P.S., AJ.S., and A.K.S.; supervision, A.K.S.; project administration, A.K.S. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflict of Interest

There exists no conflict of interest amongst the authors regarding the publication of this manuscript.

References

1. Miller, K.A.; Frankel, F.; Takahashr, H.; Vance, N.; Stiegerwald, C.; Edelstein, S. Collected literature on isoflavones and chronic diseases. *Cogent Food Agric.* **2016**, *2*, 1135861, <https://doi.org/10.1080/23311932.2015.1135861>.
2. Tuli, H.S.; Tuorkey, M.J.; Thakral, F.; Sak, K.; Kumar, M.; Sharma, A.K.; Sharma, U.; Jain, A.; Aggarwal, V.; Bishayee, A. Molecular Mechanisms of Action of **Genistein** in Cancer: Recent Advances. *Frontiers in Pharmacology* **2019**, *10*, 1336, <https://doi.org/10.3389/fphar.2019.01336>.
3. Ronis, M.J.J. Effects of soy containing diet and isoflavones on cytochrome P450 enzyme expression and activity. *Drug Metab. Rev.* **2016**, *48*, 331-341, <https://doi.org/10.1080/03602532.2016.1206562>.
4. Sak, K. Epidemiological Evidences on Dietary Flavonoids and Breast Cancer Risk: A Narrative Review. *Asian Pac. J. Cancer Prev.* **2017**, *18*, 2309-2328, <https://doi.org/10.22034/APJCP.2017.18.9.2309>.
5. Spagnuolo, C.; Russo, G.L.; Orhan, I.E.; Habtemariam, S.; Daglia, M.; Sureda, A.; Nabavi, S.F.; Devi, K.P.; Loizzo, M.R.; Tundis, R.; Nabavi, S.M. **Genistein** and Cancer: Current Status, Challenges, and Future Directions. *Advances in Nutrition* **2015**, *6*, 408-419, <https://doi.org/10.3945/an.114.008052>.
6. Kim, S.-H.; Kim, C.-W.; Jeon, S.-Y.; Go, R.-E.; Hwang, K.-A.; Choi, K.-C. Chemopreventive and chemotherapeutic effects of genistein, a soy isoflavone, upon cancer development and progression in preclinical animal models. *lar* **2014**, *30*, 143-150, <https://doi.org/10.5625/lar.2014.30.4.143>.
7. Ganai, A.A.; Farooqi, H. Bioactivity of Genistein: A review of in vitro and in vivo studies. *Biomed. Pharmacother.* **2015**, *76*, 30-38, <https://doi.org/10.1016/j.biopha.2015.10.026>.
8. Uifălean, A.; Schneider, S.; Ionescu, C.; Lalk, M.; Iuga, C.A. Soy Isoflavones and Breast Cancer Cell Lines: Molecular Mechanisms and Future Perspectives. *Molecules* **2016**, *21*, 13, <https://doi.org/10.3390/molecules21010013>.
9. Tuli, H.S.; Tuorkey, M.J.; Thakral, F.; Sak, K.; Kumar, M.; Sharma, A.K.; Sharma, U.; Jain, A.; Aggarwal, V.; Bishayee, A. Molecular Mechanisms of Action of **Genistein** in Cancer: Recent Advances. *Frontiers in Pharmacology* **2019**, *10*, 1336, <https://doi.org/10.3389/fphar.2019.01336>.
10. Cassidy, A. Potential Risks and Benefits of Phytoestrogen-Rich Diets. *Int. J. Vitam. Nutr. Res.* **2003**, *73*, 120–126, <https://doi.org/10.1024/0300-9831.73.2.120>.
11. Wang, X.; Wu, J.; Chiba, H.; Umegaki, K.; Yamada, K.; Ishimi, Y. Puerariae radix prevents bone loss in ovariectomized mice. *J. Bone Miner. Metab.* **2003**, *21*, 268-275, <https://doi.org/10.1007/s00774-003-0420-z>.
12. Wong, K.H.; Li, G.Q.; Li, K.M.; Razmovski-Naumovski, V.; Chan, K. Kudzu root: Traditional uses and potential medicinal benefits in diabetes and cardiovascular diseases. *J. Ethnopharmacol.* **2011**, *134*, 584-607, <https://doi.org/10.1016/j.jep.2011.02.001>.
13. Vitale, D.C.; Piazza, C.; Melilli, B.; Drago, F.; Salomone, S. Isoflavones: estrogenic activity, biological effect and bioavailability. *Eur. J. Drug Metab. Pharmacokinet.* **2013**, *38*, 15-25, <https://doi.org/10.1007/s13318-012-0112-y>.
14. Masilamani, M.; Wei, J.; Sampson, H.A. Regulation of the immune response by soybean isoflavones. *Immunol. Res.* **2012**, *54*, 95-110, <https://doi.org/10.1007/s12026-012-8331-5>.
15. Adlercreutz, H. Phyto-oestrogens and cancer. *Lancet Oncol.* **2002**, *3*, 364-373, [https://doi.org/10.1016/s1470-2045\(02\)00777-5](https://doi.org/10.1016/s1470-2045(02)00777-5).
16. Mandy, M.L.; Ying, H.; Jeffrey, W. Developing Phytoestrogens for Breast Cancer Prevention. *Anti-Cancer Agents Med Chem.* **2012**, *12*, 1306-1313, <https://doi.org/10.2174/187152012803833062>.
17. Setchell, K.D.R.; Brown, N.M.; Lydeking-Olsen, E. The Clinical Importance of the Metabolite Equol—A Clue to the Effectiveness of Soy and Its Isoflavones. *J. Nutr.* **2002**, *132*, 3577-3584, <https://doi.org/10.1093/jn/132.12.3577>.
18. Jayagopal, V.; Albertazzi, P.; Kilpatrick, E.S.; Howarth, E.M.; Jennings, P.E.; Hepburn, D.A.; Atkin, S.L. Beneficial Effects of Soy Phytoestrogen Intake in Postmenopausal Women With Type 2 Diabetes. *Diabetes Care* **2002**, *25*, 1709-1714, <https://doi.org/10.2337/diacare.25.10.1709>.
19. Liang, J.; Tian, Y.-X.; Fu, L.-M.; Wang, T.-H.; Li, H.-J.; Wang, P.; Han, R.-M.; Zhang, J.-P.; Skibsted, L.H. Daidzein as an Antioxidant of Lipid: Effects of the Microenvironment in Relation to Chemical Structure. *J. Agric. Food Chem.* **2008**, *56*, 10376-10383, <https://doi.org/10.1021/jf801907m>.
20. Kampkötter, A.; Chovolou, Y.; Kulawik, A.; Röhrdanz, E.; Weber, N.; Proksch, P.; Wätjen, W. Isoflavone daidzein possesses no antioxidant activities in cell-free assays but induces the antioxidant enzyme catalase. *Nutr. Res.* **2008**, *28*, 620-628, <https://doi.org/10.1016/j.nutres.2008.06.002>.

21. Kampkötter, A.; Wiegand, C.; Timpel, C.; Röhrdanz, E.; Chovolou, Y.; Kahl, R.; Wätjen, W. Increased Expression of Catalase in Human Hepatoma Cells by the Soy Isoflavone, Daidzein. *Basic Clin. Pharmacol. Toxicol.* **2008**, *102*, 437-442, <https://doi.org/10.1111/j.1742-7843.2007.00173.x>.
22. Stout, J.M.; Knapp, A.N.; Banz, W.J.; Wallace, D.G.; Cheatwood, J.L. Subcutaneous daidzein administration enhances recovery of skilled ladder rung walking performance following stroke in rats. *Behav. Brain Res.* **2013**, *256*, 428-431, <https://doi.org/10.1016/j.bbr.2013.08.027>.
23. Sharma, A.; Singh, O.P.; Sharma, A.K.; Kumar, A.; Sharma, A.K. Ab-initio study of vibrational spectra revealed the better reactive potential of Eugenol over Carvacrol: Bioactive compounds derived from *Ocimum tenuiflorum* (Tulsi). *Int. J. Pharm. Res.* **2019**, *11*, 147-151, <https://doi.org/10.31838/ijpr/2019.11.01.014>.
24. Sharma, A.; Singh, O.P.; Sharma, A.K.; Sharma, A.K.; Kumar, A. Density functional studies on the electronic structure, polarizability and thermal properties of eugenol and carvacrol. *Int. J. Pharm. Res.* **2019**, *11*, 171-176.
25. Sharma, A.; Sharma, A.; Singh, O.P.; Sharma, V.; Rani, S.; Sharma, V.; Sharma, J.K.; Sharma, U.; Sharma, B.; Sharma, A.; Sharma, A.K. Comparative Account of Density Functional Studies on the Electronic Structure and Spectroscopic Properties of Gallic Acid and Methyl Gallate Reveal their Immense Pharmacological and Biological Potential. *Indian J. Nat. Sci.* **2022**, *13*, 43452-43462.
26. Pandit, N.T.; Patravale, V.B. Design and optimization of a novel method for extraction of genistein. *Indian J. Nat. Sci.* **2011**, *73*, 184-192, <https://doi.org/10.4103/0250-474x.91583>.
27. Bharanidharan, S.; Myvizhi, P. Frontier molecular orbitals (FMO) and molecular electrostatic potential (MEP) surface of 2-(4-chlorophenyl)-1-((furan-2-yl) methyl)-4,5-dimethyl-1H-imidazole using DFT method. *Int. J. Pure Appl. Math.* **2019**, *119*, 6769-6777.
28. Ebrahimi, H.; Hadi, J.S.; Al-Ansari, H.S. A new series of Schiff bases derived from sulfa drugs and indole-3-carboxaldehyde: Synthesis, characterization, spectral and DFT computational studies. *J. Mol. Struct.* **2013**, *1039*, 37-45, <https://doi.org/10.1016/j.molstruc.2013.01.063>.
29. Beyramabadi, S.A.; Javan-Khoshkholgh, M.; Ostad, N.J.; Gharib, A.; Ramezanzadeh, M.; Sadeghi, M.; Bazian, A.; Morsali, A. Spectroscopic (Ft-Ir, Nmr, Uv-Vis, Fluorescence) and Dft Studies (Molecular Structure, Ir and Nmr Spectral Assignments, Nbo and Fukui Function) of Schiff Bases Derived from 2-Chloro-3-Quinolinecarboxaldehyde. *J. Struct. Chem.* **2018**, *59*, 1342-1352, <https://doi.org/10.1134/S0022476618060136>.
30. Ebrahimi, H.P.; Hadi, J.S.; Almayah, A.A.; Bolandnazar, Z.; Swadi, A.G.; Ebrahimi, A. Metal-based biologically active azoles and β -lactams derived from sulfa drugs. *Bioorg. Med. Chem.* **2016**, *24*, 1121-1131, <https://doi.org/10.1016/j.bmc.2016.01.041>.
31. Demircioğlu, Z.; Albayrak Kaştaş, Ç.; Büyükgüngör, O. The spectroscopic (FT-IR, UV-vis), Fukui function, NLO, NBO, NPA and tautomerism effect analysis of (E)-2-[(2-hydroxy-6-methoxybenzylidene)amino]benzotrile. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2015**, *139*, 539-548, <https://doi.org/10.1016/j.saa.2014.11.078>.
32. Kumar, S.; Saini, V.; Maurya, I.K.; Sindhu, J.; Kumari, M.; Kataria, R.; Kumar, V. Design, synthesis, DFT, docking studies and ADME prediction of some new coumarinyl linked pyrazolylthiazoles: Potential standalone or adjuvant antimicrobial agents. *PLOS ONE* **2018**, *13*, e0196016, <https://doi.org/10.1371/journal.pone.0196016>.
33. Sharma, A.; Sharma, A.; Sharma, V.; Sharma, J.; Singh, O.P.; Ramniwas, S.; Sharma, A.; Sharma, V.; Singh, M.; Sharma, A.K. Bioactive α -Santalol Molecule Derived from *Santalum album*: Endorsing Its Reactive and Inhibitory Potential Against Monkeypox gp158 Protein Established Through Density Functional and Molecular Docking Studies. *Lett. Appl. Nanobiosci.* **2024**, *13*(2), 65.

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