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Computational study of conformational, electronic and antioxidant properties of prenylated dihydrochalcone

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GRAPHICAL ABSTRACT

Leaves of *Artocarpus lowii*

ABSTRACT

The main aim of this study was to compare the antioxidant properties from theoretical results and experimental results. Theoretical calculations obtained by DMoL3 based on density functional theory (DFT) were used to rationalize the antioxidant activities. The theoretical investigation emphasized on a hydrogen atom transfer (HAT) mechanism and single electron transfer (SET) mechanism. Inhibition of free radicals using 2',4'-dihydroxy-4-methoxy-3'-prenyldihydrochalcone (**1**), 2',4',4-trihydroxy-3'-prenylchalcone (**2**), and 2',4dihydroxy-3',4'-(2,2-dimethylchromene)chalcone (**3**) was examined to determine their antioxidant effects and the structure–activity relationship of chalcone. Density functional theory calculations under the level of generalized gradient approximation (GGA) hybrid with Perdew-Burke-Ernzerhof (PBE) functional and double numerical polarization (DNP) basis set had been utilized to explore the structure, molecular properties and antioxidant abilities of the three chalcone. The bond dissociation enthalpy (BDE), ionization potential (IP), frontier molecular orbital energy gap, molecular electrostatic potential surface (MEPS), and spin density were investigated. They were compared with the previous experimental results. Scavenging activity determined by half maximal inhibitory concentration (IC50) values of the three chalcone increased from compound **2**, followed by **3** and **1**. The calculations showed that compound **2** owns the lowest ionization potential and lowest band energy, which agree well with the experimental results of antioxidant activity determined by IC_{50} values.

Keywords: chalcone, antioxidant activity, density functional theory, bond dissociation energy, ionization potential

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1. INTRODUCTION

There are many types of diseases that can attack human body such as heart attack and others. Many diseases cause researchers to further study on many types of cure. Thus, in this study, the properties and importance of chalcone is studied in computational way since chalcone is well known as the best cure for cancer. Furthermore, chalcone is said to be used in traditional medicine which means that it is important and interesting to be explored further [1]. Three structures from a new prenylated dihydrochalcone are chosen for further study which are 2',4'-dihydroxy-4-methoxy-3'-prenyldihydrochalcone (**1**), 2',4',4-trihydroxy-3'-prenylchalcone (**2**) and 2',4-dihydroxy-3',4'-(2,2-dimethylchromene)chalcone (**3**) [2].

Chalcones or 1,3-diaryl-2-propen-1-one are natural compounds from plants, fruits and vegetables that form a group of open-chain flavonoids. In open-chain flavonoids, two aromatic rings are linked by a three-carbon α,β-unsaturated carbonyl system [3]. These three samples were isolated from leaves of *Artocarpus lowii* [2]. The genus *Artocarpus* of the family Moraceae is represented by approximately 50 species and most of them are cultivated at the tropical rain forest of Malaysia, Indonesia and Philippines. Eleven of them are recognized as edible fruits [4].

Artocarpus lowii King is a rare species which it can be founded in the lowland forest of Malaysia. "miku" is a local name for the *Artocarpus lowii* King while *Artocarpus lowii* represents the three cultivated species which are "sukun", "nangka" and "cempedak". The previous study of phytochemical work on the genus of *Artocarpus* has revealed that this genus is rich with numerous phenolic compounds especially flavonoids [2].

Antiplatelet, antimicrobial, antioxidant, anti-inflammatory, antiproliferative, cytotoxicity, tyrosinase inhibitory activities, free radical scavenging are the fascinating biological properties that can be obtained from the flavonoids [2].

Furthermore, the cytotoxicity of chalcone is said can against some tumor cell lines such as B16 murine melanoma and HCT 116 human colon cancer [5]. This means that chalcone also can acts as antitumor. A pevious study reports that chalcone involves in treatment of cancer chemotherapy by act as microtubule-binding agent [6]. All these examples show that chalcone has many benefits and advantages especially to human.

Computational methods will be used in this study in order to determine which compound gives the highest antioxidant properties. It will be explained more by clarifying the role of hydroxyl group of compounds using a quantum calculation. DFT calculations will be used in order to simulate the structure and obtain the HOMO/LUMO properties which is related well with the activity of the electrons in the compound. Other electronic properties also will be calculated which are bond dissociation enthalpy (BDE) and ionization potential for investigating the antioxidant activity of chalcone.

2. EXPERIMENTAL

All calculations were performed with the DMol3 module. In the geometry optimization, the generalized gradient approximation (GGA) hybrid with Perdew-Burke-Ernzerhof (PBE) functional was employed with the double numerical polarization (DNP) basis sets. Frequency calculations were performed to confirm that the optimized structure is a true minimum (no imaginary frequencies). In addition, to better understand the antioxidant nature of the molecules studied here, the following properties were calculated: (i) energy of the highest occupied molecular orbital (HOMO); (ii) energy of the lowest unoccupied molecular orbital (LUMO); (iii) ionization potential (IP); (iv) hydroxyl bond dissociation energy (BDE); and (v) spin density.

The ionization potential (IP) was calculated as the energy difference between the neutral molecule and its respective cation free-radical. On the other hand, the chalcone radical stability is usually calculated by the hydroxyl bond dissociation energies (BDE). The BDE values were calculated as the energy difference between the neutral molecule and its respective semiquinone plus the hydrogen radical. Molecular electrostatic potential (MEP) surfaces were also obtained using DMol3 module.

3. RESULTS AND DISCUSSION

3.1 Conformational Analysis

Geometry optimization for all studied structures was made in gas medium using the GGA/PBE method together with DNP basis set as shown in Table 1. From the optimized structure, only compound 1 that does not have intramolecular hydrogen bond at 2'OH and oxygen of carbonyl group. This also play a significant role in determining the antioxidant strength of chalcone.

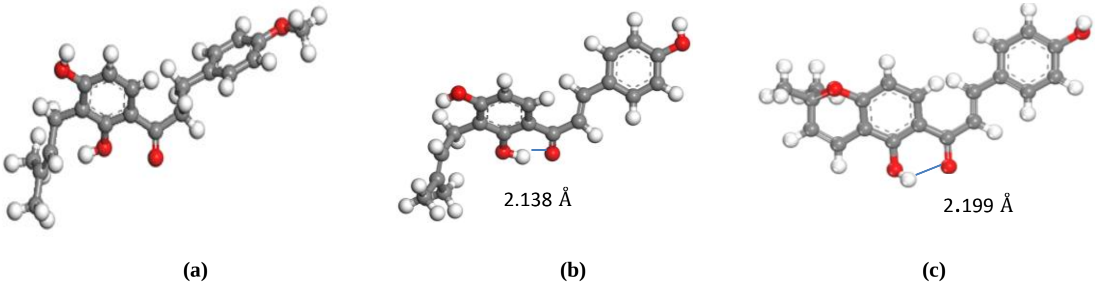


Figure 1 Optimized structure of (a) 2',4'-dihydroxy-4-methoxy-3'-prenyldihydrochalcone, (b) 2',4',4-trihydroxy-3'-prenylchalcone and (c) 2',4-dihydroxy-3',4'-(2,2-dimethylchromene)chalcone.

After optimization, the dihedral angle each of three structures is obtained as shown in Table 2. The higher the π electron delocalization, the more favour towards planar structure which is 180˚ [3]. Based on Table 2, all three optimized structure favour planar conformation with highest value of 178˚ for compound 2, 176˚ for compound 3 and 172˚ for compound 1. The dihedral angle was taken from α-β-C1-C2 positions. The planar behaviour is important since the absence of planar will weaken the electronic distribution of [7]. Compound 1 exhibit the lowest planarity must be due to the absence of double bond compared to in compound 2 and 3 in which this double bond will help to delocalize electron throughout the system and then produce stable chalcone radical.

The most stable conformer for compound 1, compound 2, and compound 3 is found at -1114.168 hartree with dihedral angle of 172˚, -1073.975 hartree with dihedral angle of 178˚, and -1072.520 hartree with dihedral angle of 176˚. The energy difference between compound 2 and 3 is very low, of the order of 1.455 hartree. At this dihedral angle and computational level, these two molecules show planar structure and the planar conformation of the rings A and B allows good electronic delocalization among the rings.

After optimization, harmonic vibration frequency calculation has been done. From harmonic vibration frequency analysis, all conformation was confirmed to not have any imaginary frequency. Positive harmonic vibration frequency indicate that compounds is in absolute minimum energy [9]. Table 3 (a), (b) and (c) display some of the results for structure 1, 2 and 3 respectively which shows that there is no imaginary frequency. Thus, it is confirmed that compounds are in their minimized structure and the next steps to study the properties of chalcone can be proceed.

3.2 Frontier Molecular Orbital Theory

Molecular orbital theory states that electron is not possess only on certain bond, but it spread throughout the entire molecule. Thus, it is important to study the distribution of electron at highest occupied molecular orbital and lowest unoccupied molecular orbital. Moreover, it is suitable to study molecular orbital theory since density functional theory that is used in this study is focusing on electron density rather than wavefunction that is been focusing by other method such as in Hartree-Fock equations.

Table 1 Calculated HOMO, LUMO Energy, Band Gap and IC50

Compound	E(HOMO) (Ha)	E(LUMO) (Ha)	Band Gap [E(LUMO) - E (HOMO)]	IC50 (mM)
1	-0.16841	-0.11413	0.05428	0.24
2	-0.17238	-0.12625	0.04613	0.03
3	-0.18448	-0.13706	0.04742	0.15

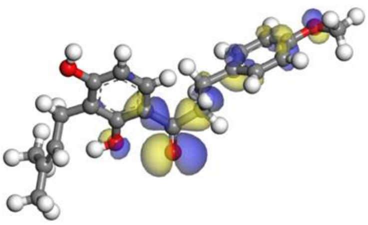
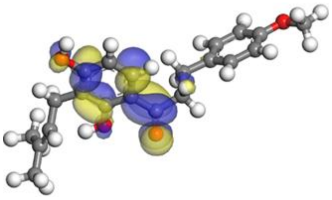
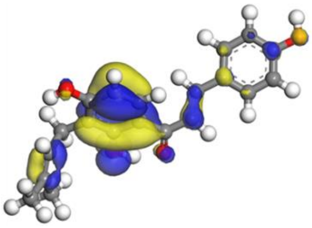
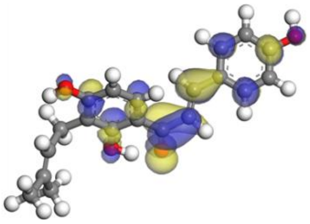
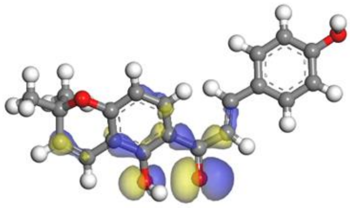
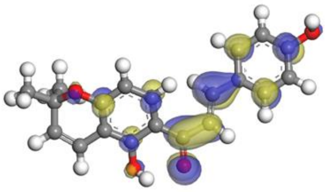
The electron donating ability of the molecule is characterized by the HOMO energy values. A higher HOMO energy values always increase the electron donating ability which in turn accelerates the antioxidant activity [8]. Based on Table 1, compound **3** shows the highest HOMO energy followed by compound **2** and **3**. This is different with the experimental data in which compound **2** has the strongest electron donating ability, followed by compound **3** and **1**. This is might be due to the molecular orbital theory provides just an approximation and the approximations are not quantitatively reliable. The Koopman's theorem does not take into account electron correlation and orbital relaxation effects [8]. Next, from the difference between LUMO and HOMO energy we also can conclude about chemical activity of the molecule. The lower ΔE(LUMO – HOMO) is connected with lower activity of the molecule. ΔE(ELUMO – EHOMO) informs how easy the transition from ground state to excited state may occur [9]. Thus, it can be said that the lower the band gap, the highest its antioxidant properties. Based on Table 1, compound **2** has the smallest value of band gap which may indicate that compound **2** has good antioxidant properties, followed by compound **3** and **1** [10].

Table 1 also gives the IC50 of chalcone structures obtained by carry out the experiment. Based on Table 1, compound **2** has the strongest antioxidant activity followed by compound **3** and **1**. This correlates with the band gap data in Table 4.4 since low value of IC_{50} indicates high antioxidant properties. Thus, the sequence of high antioxidant is as follow compound **2** > **3** > **1** with band gap value of 0.05428 Ha, 0.04613 Ha, 0.04742 Ha and IC50 value of 0.24 mM, 0.03 mM and 0.15 Ha for compound **1**, **2** and **3** respectively. The validity of Koopman theorem was verified for this part. Furthermore, the lowest ability to scavenge free radical by compound 1 can be said due to their lack of double bond. The absence of double bond can make the phenoxy radical become instable which means it is less favored to become phenoxy radical compared to other compounds [3]. In addition, another factor that may influences a compound to have high ability to scavenge the free radical is the presence of electron donating nature of substituents which is in this case is hydroxyl group [11]. Thus, it is correlate with this study in which compound **2** has more hydroxyl group compared to **1** and **3**.

Because the hydrogen abstraction reaction involves electron transfer for bond dissociation energy calculation, the HOMO composition of a phenolic compound can provide a qualitative data to identify its active site for the scavenging radical activity [12]. Hence, the HOMO distribution reveals which chemical groups in a molecule are easily attacked by free radicals [9]. From Table 2, it concludes find the HOMO of compound **1** is mainly distributed in the B-ring, while the LUMO allocated in the A-ring. For compound **2** and **3**, HOMO distribution is highly in A-ring and LUMO distribution is seem evenly distributed both in A-ring and B-ring. So, the higher ability to respond to the functional site is mainly focused on B-ring for compound **1** and A-ring for compound **2** and **3**. Based on Table 2, the electron density concentrates on the oxygen from the hydroxyl group and methoxy groups, phenyl ring and carbonyl bond. It can be concluded that the O-H bond is the most probable place of a free radical reaction in the whole molecule in the studied compounds. Moreover, in this case, the most nucleophilic positions were determined by the main HOMO contributions of the phenol moiety. For compound

1 and **3**, 2’-OH might be the more probable electron donating sites while for compound **2**, 2’-OH is the most easily attacked sites, but also has slightly distribution on 4’-OH sites and 4-OH sites.

Table 2 HOMO and LUMO distribution of chalcones

Compound	HOMO	LUMO
1		
2		
3		

3.3 Molecular Electrostatic Potential Surface

In this study, the electrostatic potential (ESP), electron density (ED) and molecular electrostatic potential (MESP) maps have been mapped as shown in Figure 2. MESP correlates the total charge distribution with dipole moment, electronegativity, and partial charges and site of chemical reactivity of a molecule. It provides a visual method to understand the relative polarity of a molecule and serves as a useful quantity to explain hydrogen bonding, reactivity and structure-activity relationship of molecules. Moreover, MEPS is the potential energy of a proton at a location near a molecule which is a good guide in assessing the molecules reactivity towards positively or negatively charged reactants [13]. Negative electrostatic potential (red sites) corresponds to an attraction of the proton by the concentrated electron density in the molecules (from lone pairs, pi-bonds, etc.). Positive electrostatic potential (blue sites) corresponds to repulsion of the proton by the atomic nuclei in regions where low electron density exists and the nuclear charge is incompletely shielded.

The gradient of colors on MEPS represents electron distribution. From these images, all oxygen moieties showed a negative potential (red) placed at the phenol, ether groups, and methoxy group. Next, the positive potential (blue) located at all hydrogen atoms throughout the structures. This means that areas with a high probability of finding an electron are shaded red which is in this case oxygen sites. Moreover, as the probability of finding an electron decreases the color changes to white, and then blue where the probability is smallest. Therefore, MEPS is also an important tool to study the antioxidant behavior.

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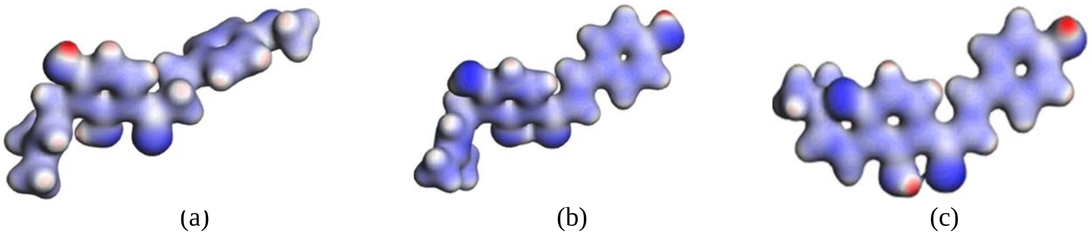

Figure 2 Molecular electrostatic potential surface map of (a) compound **1**, (b) compound **2** and (c) compound **3** (blue: low electronegativity, white: medium electronegativity and red: high electronegativity).

3.4 Bond Dissociation Energy

Bond dissociation energy (BDE) of each of chalcone has been calculated using hydrogen atom transfer mechanism. BDE values is used as molecular descriptor in an effort to elucidate the radical scavenging activity of compounds under investigation. BDE has been calculated for all site of OH as display in Table 3. Based on Table 3, compound **2** has three type of bond dissociation energy since it has three probable active sites. These three-site got BDE slightly difference by ~2 kcal/mol. Next, it is obvious that compound **2** exhibit the highest BDE followed by compound 3 and compound **1**. Difference BDE between compound **2** and **1** is ~123 kcal/mol and compound **2** and **3** is ~50 kcal/mol. This suggest it is due to compound **1** does not have intramolecular hydrogen bond of 2'-OH with oxygen of carbonyl group compared to compound **2** and **3** that exhibit intramolecular hydrogen bond by 2.138 and 2.199 respectively. The absence of intramolecular hydrogen bond can make the hydrogen at 2'-OH easier to be removed.

Table 3 Bond Dissociation Energy and Ionization Potential

Compound	**Bond Dissociation Energy (kcal/mol)**			**Ionization Potential (kcal/mol)**
	2'OH	4'OH	4OH	
1	77.848	82.361	-	52.941
2	200.383	200.119	202.081	24.664
3	159.898	-	200.019	43.994

The presence of intramolecular hydrogen bond can be supported by bond length of 2'OH. The longer the 2'OH means there may exhibit intramolecular hydrogen bond since that hydrogen atom involve is stretched to form bond with oxygen of carbonyl group [7]. It is correlate with the data obtained in which bond distance of 2'OH is the shorter among these three compounds as shown in Table 4.2. Moreover, BDE for compound 2 is larger than compound 3 is maybe due to their bond properties. For example, 2'O-H bond distance of compound 3 is longer than compound 2 by 0.04 Å. Longer bond is said to exhibit weaker bond [14]. This may explain the reason why BDE compound 2 higher than compound 3. Furthermore, other reason that may supported the high BDE of compound 3 compared to compound 2 is the length of their intramolecular hydrogen bond. The shorter intramolecular hydrogen bond exhibit more stronger stabilizing effect thus much difficult to remove its hydrogen atom [7]. It is obvious that compound 2 exhibit longer intramolecular hydrogen bond compared to compound 3.

Another antioxidant descriptor with another mechanism has been calculated which is ionization potential with single electron transfer mechanism. The results obtained are display in Table 3. The ionization potential data has lower value than DPPH free radical, thus it suggests that these chalcone favour single electron transfer mechanism. The data obtained show strong correlation with experimental value in which compound 2 exhibit lower ionization potential, followed by compound 3 and then compound 1.

3.5 Spin Density

For study of the most probable site, the spin density at oxygen radical formed has been analyses as shown in Table 4. Spin density characterize distribution of non-paired electron and stability of radical formed after form of the molecule. BDE often attributed to π electron delocalization, leading to the stabilization of the radicals obtained after H-abstraction. It is assumed that if π electron delocalization exists in the parent molecule, it also exists in the corresponding radical. Therefore, a close relationship between the BDE and spin density is to be expected. A more delocalized spin density in the radical stabilizes the latter, thus favouring radical formation. Therefore, lower SD values indicate better antioxidants [6].

For compound 3, the active site is at 2'OH since HOMO density focus on this part. This correlate with spin density value in which 2'OH exhibit lower spin density value with 0.305 e/Au^3 at 2'OH while high spin density value in 4OH with value 0.42 e/Au^3. For compound 2, the 4'OH is their active site since it has lowest spin density among the three OH group with value of 0.442 e/Au^3. A correlation sequence can be seen in compound 2, for BDE 4'OH < 2'OH < 4OH and spin density 4'OH < 2'OH < 4OH. Moreover, spin density for compound 1 does not match the BDE value in which should be 2'OH active site has lower spin density that 4OH. We could not figure out a logical explanation for the observation at the moment since HOMO density is concentrated more on 2'OH instead of 4'OH. However, there is not much difference between spin density value of 2'OH and 4'OH in which they differ only by 0.048 e/Au^3.

Table 4 Spin density of chalcone radical

Compound	**Spin density (e/Au^3)** **2'**	**4'**	**4**
1	0.493	0.445	-
2	0.498	0.442	0.485
3	0.305	-	0.42

Thus, it can be concluded that active site for compound 1and 3 is 2'OH and 4'OH for compound 2. Based on BDE value, the highest antioxidant properties is compound 1, followed by compound 3 and compound 2. This is difference with the experimental value in which compound 2 exhibit strongest antioxidant properties followed by compound 3 and compound 1. This is due to during experiment, they use DPPH free radical in which the BDE value for DPPH free radical is 80 kcal/mol. Another important thing that must be consider in study antioxidant is the BDE chalcone must be lower than a given threshold in which in this case BDE of DPPH free radical [3]. Thus, the hydrogen atom transfer mechanism used in calculation of BDE is not a favour mechanism for this three chalcone. This may be a reason why the computational data does not correlate with experimental one.

4. CONCLUSION

The antioxidant mechanism of 2',4'-dihydroxy-4-methoxy-3'-prenyldihydrochalcone (compound 1), 2',4',4-trihydroxy-3'-prenylchalcone (compound 2), and 2',4-dihydroxy-3',4'-(2,2-dimethylchromene)chalcone (compound 3) were evaluated in this study. Based on the results obtained, the hydroxyl group is accountable for antioxidant abilities. Hydroxyl groups and double bonds contribute to a decrease in the band gap value and the ionization potential (IP). The prevalent spin density contributions of these groups are determinant for the highest stability of the free-radicals due to more resonance structures. This suggests that for all investigated compounds, the hydrogen atom mechanism is not favoured whereas single electron transfer is favoured. Quantum chemical calculations confirmed that high antioxidant activity of these three considered compounds. Based on calculated properties such as the HOMO/LUMO band gap and ionization potential showed that compound 2 shows a higher antioxidant potential than compound 3 and compound 1. This correlates with the experimental data (Table 1) which stated that compound 2 has the lowest IC50 value. Moreover, it can be concluded that the band gap, bond properties and ionization potential (IP) are consistent with antioxidant properties while the bond dissociation energy is not consistent with antioxidant properties. Therefore, in conclusion, the quantum chemical approach is a useful tool to determine the antioxidant ability of chalcone and its derivatives.

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