Density Functional Theory Calculations of Structure-Antioxidant Activity of Selected Phenolic Acids and Flavonoids Found in Malaysian Honey

(Pengiraan Teori Fungsi Ketumpatan (DFT) bagi Struktur-Aktiviti Anti-oksida untuk Beberapa Sebatian Fenolik dan Flavonoid yang didapati dalam Madu di Malaysia)

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ABSTRACT

Phenolic acids and flavonoids exist naturally in Malaysian honey and contribute significantly to antioxidant contents. Antioxidants plays an important role in scavenging free radicals and prevent health deterioration. Total antioxidant content is measured using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity. The phenolic acids such as gallic, caffeic, syringic and hydroxybenzoic acids and flavonoids like naringenin, apigenin, kaempferol, catechin and luteolin previously have been identified in Malaysian honey of tualang, gelam and borneo type using high-performance liquid chromatography (HPLC). In order to investigate the structure-antioxidant activity relationships of these phenolic compounds using hydrogen atom transfer (HAT) mechanism, density functional theory (DFT) calculation at B3LYP/6-311++G(d,p) levels of theory was performed. In this work, optimization of the compounds chemical structure and radical forms in gas-phase has been calculated with computation of bond dissociation enthalpy (BDE) as antioxidant descriptor. The finding showed that abstraction of H at different OH groups in the structure of the compound, led to a different scavenging free radical activity thus contribute to the overall variation in the antioxidant properties. Besides that, B ring of flavonoids and unsaturated bond in pyran ring are proposed factors that could lower the BDE values and consequently influence the antioxidant properties of the antioxidant compounds. Hence, DFT calculation with BDE descriptor had been successfully applied to investigate the relationship between structure of phenolic acids and antioxidant activity of Malaysian honey and the interesting results could contribute in future development of antioxidant compound.

Keywords: honey; phenolic acid; flavonoid; density functional theory; bond dissociation energy; structure-antioxidant activity

ABSTRAK

Sebatian fenolik dan flavonoid wujud secara semulajadi dalam madu di Malaysia dan menyumbang kepada kandungan anti-oksida. Anti-oksida memainkan peranan penting dalam menangkap radikal bebas dan menghalang kemerosatan kesihatan. Jumlah anti-oksida biasanya diukur menggunakan aktiviti penangkap radikal DPPH (2,2-diphenyl-1-picrylhydrazyl). Sebatian fenolik seperti asid gallic, caffeic, syringic dan hydroxybenzoic acids dan flavonoids seperti naringenin, apigenin, kaempferol, catechin dan luteolinen telah dikenalpasti dalam madu jenis tualang, gelam dan borneo di Malaysia dengan menggunakan kaedah Kromatografi Cecair Berprestasi Tinggi (HPLC). Bagi menyiasat hubungan struktur-aktiviti anti-oksida bagi sebatian fenolik dengan menggunakan mekanisma pemindahan atom hydrogen (HAT), pengiraan teori fungsi ketumpatan (DFT) pada peringkat teori B3LYP/6-311++G(d,p) telah dijalankan. Dalam kajian ini, struktur kimia sebatian dioptimakan dalam bentuk radikal fasa gas dan dikira dengan pengiraan entalpi penceraian ikatan (BDE) sebagai deskriptor anti-oksida. Didapati bahawa pemisahan H pada kumpulan OH yang berbeza pada struktur bahan tersebut membawa kepada penangkapan radikal bebas yang berbeza dan sekaligus menyumbang kepada keseluruhan variasi dalam ciri-ciri anti-oksida. Selain itu, gelang B dari flavonoid dan ikatan tak tepu dari gelang piran adalah cadangan faktor yang merendahkan nilai BDE yang diperolehi dan seterusnya mempengaruhi sifat anti-oksidan di dalam sebatian anti-oksida. Seterusnya, pengiraan DFT dengan menggunakan BDE telah berjaya digunakan untuk menyiasat hubungan antara struktur asid fenolik dan aktiviti anti-oksida dalam madu di Malaysia dan hasilnya yang menarik boleh menyumbang kepada pembangunan sebatian antioksida pada masa akan datang.

Kata kunci: madu; sebatian fenolik; flavanoid; teori fungsi ketumpatan; entalpi penceraian ikatan; struktur- aktiviti anti-oksida

INTRODUCTION

Honey is a natural product or sweetener that contains phytochemicals like minerals, protein, enzymes, vitamins, organic acids, flavonoids and phenolic acids that are generally beneficial to human health. Many previous studies have pointed out that phenolic acids and flavonoids are the main constituents in honey that contribute to or correlated significantly with antioxidant effects (Chua, 2013; Abu Bakar et al. 2017). Analytically, total antioxidant contents of honey sample can be reliably evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity expressed as IC_{50} of DPPH inhibition (mg/mL) where hydrogen or electron-donating activity of single electron can be investigated (Ferreira et al. 2009).

There are several known mechanisms that describe the reaction of free radicals with antioxidant compounds such as electron transfer-proton transfer (SETPT) and sequential proton loss-electron transfer (SPLET) (Wright, 2001). However, the thermodynamically preferred mechanism that best describe the antioxidant activity in gas phase is hydrogen atom transfer (HAT) (Urbaniak and Molski, 2012; Chen et al. 2015). According to this mechanism, as shown in equation (1), a free radical reacts with one hydrogen atom from phenolic antioxidant and subsequently, the phenolic antioxidant itself forms a radical.

Hydrogen atom transfer (HAT) mechanism:

$$ArOH + X\bullet \rightarrow ArO\bullet + XH \qquad (1)$$
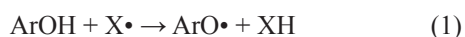
Gallic acid, caffeic acid, syringic acid, hydoxybenzoic acid, naringenin, apigenin, kaempferol, catechin and luteolin are among the phenolic acids and flavonoids identified in Malaysian honey (Moniruzzaman et al. 2016; Seraglio et al. 2016). The quantity of these components is dependent on several factors such as floral and geographical origin, method of processing, handling and storage condition of honey as well as type of honey. Khalil et al. (2011) study of Malaysian honey identified 11 phenolic compounds found in Tualang, Gelam and Borneo types using spectrophotometry along with high-performance liquid chromatography (HPLC) analysis. The DPPH inhibition test of Tualang honey represented by low IC50 values as displayed in Table 1, showed that Tualang honey has the strongest free radical scavenging activity compared to Gelam and Borneo type honeys. Similarly, total phenolic and flavonoid content in Tualang honey is the highest compared to the two type of honey. In addition, HPLC analysis of the honey samples also revealed that several phenolic acids and flavonoids have been detected in the honey samples as listed in Table 1. However, catechin was dominant and commonly found in all of the honey samples where according to Labidi et al. (2018) catechins are powerful antioxidant based on several quantum chemistry calculations. Based on this experimental finding, the aim of the present work is to evaluate structure-antioxidant activity relationships of the selected phenolic compounds identified in Malaysian honey using theoretical approach namely Density Functional Theory (DFT).

METHODOLOGY

The quantum-chemical calculations of the phenolic compounds were carried out using Gaussian 09 program (Frisch et al. 2009). Geometry optimization of the compounds in the gas phase was performed using Density Functional Theory (DFT) calculation at B3LYP functional with 6-311++G (d,p) level of theory. Thus, for all the radical systems, employment of unrestricted B3LYP/6-311++G(d,p) method was carried out (Chen et al. 2015).

This study only employed bond dissociation enthalpy (BDE) as numerical descriptor that is associated with hydrogen atom transfer (HAT) mechanism which reveal stability of the O-H bond in the hydroxyl group. This descriptor is a reaction enthalpy used in measurement of the free radical scavenging activity. The lower BDE value of the H abstraction group characterizes better inactivation of free radical and thus better antioxidant property (Urbaniak and Molski, 2012; Chen et al. 2015; Liang and Kitts, 2014). The following equation has been used in computation of the BDE value:

Bond dissociation enthalpy (BDE):

$$BDE = H_{ArO\bullet} + H_{H\bullet} - H_{ArOH} \qquad (2)$$

in which the three terms on the right-hand side of the equation refers to the enthalpy of the radical, enthalpy of the H atom and enthalpy of the compound respectively.

RESULTS AND DISCUSSION

The BDE for all sites of OH in the studied phenolic compounds has been calculated using equation (2) and hence presented in Table 2. The optimized structures of the corresponding phenolic radicals are illustrated in Figure 1. Gallic acid has three adjacent phenolic OHs. Among the three positions, 4-OH (83.76 kcal/mol) has the smallest BDE value followed by 3-OH (84.59 kcal/mol) and 5-OH (91.55 kcal/mol). Caffeic acid on the other hand, has two adjacent phenolic OHs. Between the 3-OH (90.56 kcal/mol) and 4-OH (78.93 kcal/mol) BDE values, the latter was found to be smaller than that of the former. For syringic acid, the sole OH group located in the middle between two methoxy groups has BDE value at 4-OH of 86.81 kcal/mol. Hence, theoretical calculations of BDE revealed that for gallic, syringic and caffeic acids, 4-OH group (para position) has more ability to donate H atom, representing the primary site that serve as potent radical scavenger, followed by 3-OH and 5-OH.

TABLE 1. Analysis of phenolic acids, flavonoids and antioxidant properties of honey samples

Honey sample	Phenolic acids and flavonoids components detected in the honey sample	IC50 (mg/ml)
Tualang	catechin, gallic acid, syringic acid, naringenin, benzoic acid, and kaempferol	8.60 ± 0.66
Gelam	catechin, benzoic acid, naringenin, luteolin, kaempferol and apigenin	14.36 ± 0.83
Borneo	catechin and caffeic acid	17.51 ± 0.51

TABLE 2. Bond Dissociation Enthalpy (BDE) of Phenolic acids and in the Gas Phase

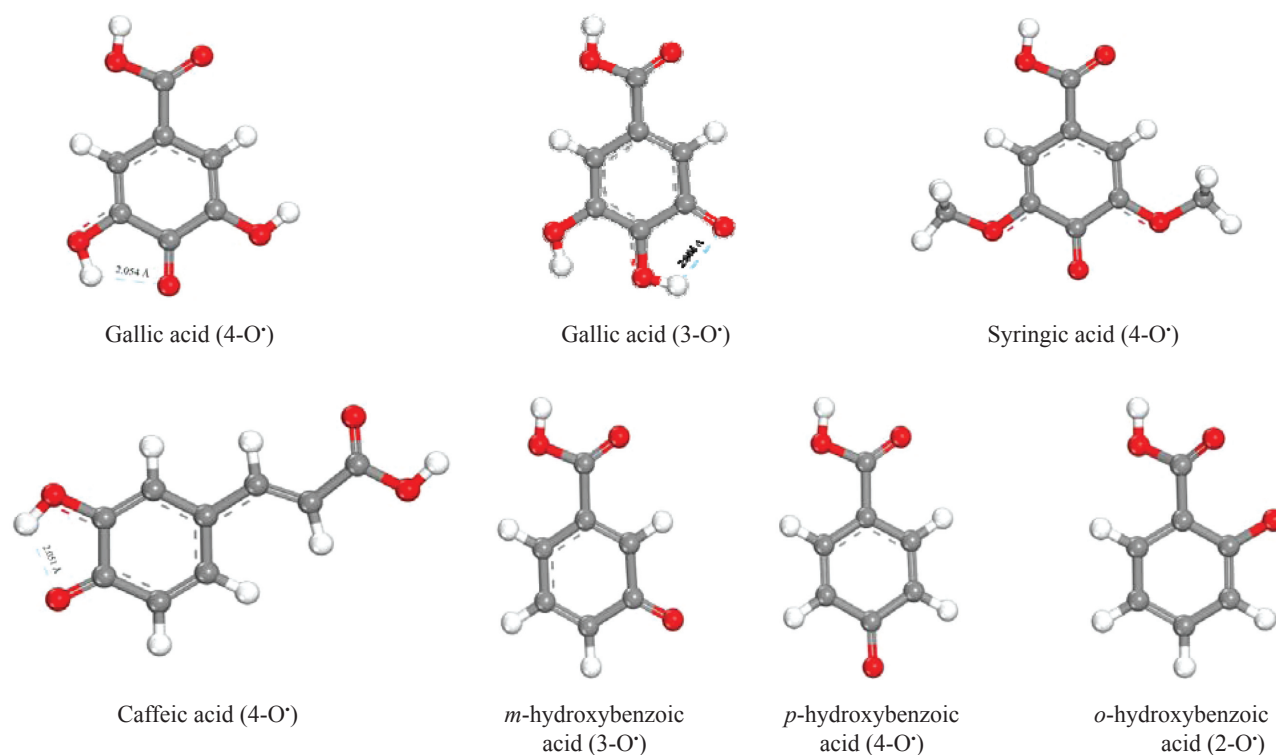| Compound	BDE (kcal/mol)			
	2OH	3OH	4OH	5OH
Gallic acid	−	84.59	83.76	91.55
Caffeic acid	−	90.56	78.93	−
Syringic acid	−	−	86.81	−
2-Hydroxybenzoic acid	92.07	−	−	−
3-Hydroxybenzoic acid	−	91.34	−	−
4-Hydroxybenzoic acid	−	−	92.37	−

FIGURE 1. Optimized structures of phenolic acid radicals calculated at UB3LYP/6-311G++G (d, p) level in gas phase. Oxygen, carbon and hydrogen is denoted by red, grey and white atom, respectively. Blue dash line represents hydrogen bond.

In contrast, BDE values of hydroxybenzoic acid revealed that 3-OH (m-hydroxybenzoic acid) has the smallest value with 91.34 kcal/mol followed by 2-OH (o-hydroxybenzoic acid) and 4-OH (p-hydroxybenzoic acid) with 92.07 kcal/mol and 92.37 kcal/mol, respectively. Obviously, the structure of hydroxybenzoic acid has a moderately deactivating substituent group which is a carbonyl (-COOH), that served as a meta directors in an electrophilic substitution reaction. Thus, it was suggested that stabilization of hydroxybenzoic acid at 3-OH than 2-OH and 4-OH was due to this substituents effect (Bruice 2010). Contrary to gallic, syringic and caffeic acids which has stability at para position, hydroxybenzoic acid, 3-OH (meta position) has greater ability to donate H atom than that of 2-OH (ortho position) and 4-OH (para position). Therefore, the greater the potential of OH group to donate H atom, the more stable the antioxidant radical compounds, thus, lowering the BDE value. Consequently, lower BDEs (Wang et al. 2017) are related to higher antioxidant activity.

Besides delocalization of electrons in the benzene ring and stabilization of the radical antioxidants after abstraction of H atom, other factor that influence the BDE values is hydrogen bond formation between the hydroxyl groups. From optimization of the radical compounds, it was observed that gallic acid at 4-O and 3-O form hydrogen bond interaction with H of adjacent OH group with bond length at 2.054Å and 2.094Å, respectively. Since no hydrogen bond interaction was observed for gallic acid between 5-O and H of the adjacent OH group, stabilization of the compound at 5-O was found to be the lowest as compared to the 4-O and 3-O. Caffeic acid forms hydrogen bond interaction at 4-O with bond length at 2.051Å, however, there is no formation of hydrogen bond observed at 3-O with H of its adjacent OH group. Gallic acid and syringic acid were both a type of hydroxybenzoic compound that has substituents attached at C-3, C-4 and C-5. The only difference is at C-3 and C-5, where gallic acid has hydroxyl groups attached at those carbon positions while syringic acid has methoxy groups. Nonetheless, both compounds have OH group attached at C-4 which is a para position. Calculation of BDE for each of the compounds showed that gallic acid at 4-OH was lower than that of syringic acid. With observation of hydrogen bond interaction found in the former whilst no observation found in the latter, it was suggested that, methoxy substitutions on syringic acid influence the absence of hydrogen bond interaction at 4-O para position. Eventually, stabilization of the syringic acid as compared to the gallic acid was reduced resulting in higher BDE value.

Selected flavonoids which basically have similar structure to phenolic acids are displayed in Figure 2 while the corresponding BDE values of the studied compounds are listed in Table 3. The OH groups of kaempferol are positioned at C-3, C-5, C-7 and C-4' with the smallest BDE found at 3-OH (74.57 kcal/mol) in C ring followed by 4'-OH (85.40 kcal/mol) in B ring and 7-OH (90.94 kcal/mol), 5-OH (109.27 kcal/mol) in A ring. A similar trend was also observed in apigenin and luteolin. The smallest BDE value for apigenin is 4'-OH with 89.73 kcal/mol in B ring whilst the largest is at 5-OH with 106.53 kcal/mol in A ring. For luteolin, the BDE value at 4'-OH, 3'-OH in B ring and 7-OH, 5-OH in A ring are 80.40 kcal/mol, 91.18 kcal/mol and 94.68 kcal/mol, 106.45 kcal/mol, respectively. Nevertheless, the OHs in the B ring for all compounds was found to exhibit smaller BDE value indicating a high antioxidant activity within the moiety compared to the A and C rings. This agree well with finding from other study (Cai et al. 2014) which show B ring dominate the antioxidant property of selected flavonoids. However, with the exception of kaempferol, the OH at C ring has smaller BDE value than in B and A ring. All the three compounds have one unsaturated bond in the pyran ring with one ketone group. The pyran ring of kaempferol however, has an additional OH group at C-3. Even though the BDE of 7-OH was found to be smaller than 5-OH in A ring for kaempferol, apigenin and luteolin, reverse result was observed for catechin and naringenin. The 5-OH was found to has a slightly smaller BDE than 7-OH. Looking back at the structure of catechin and naringenin, both compounds as compared to kaempferol, apigenin and luteolin has no 2,3-double bond in the pyran ring. Furthermore, the pyran ring of catechin does not have a ketone group. Therefore, unsaturated bond in the pyran ring was suggested to be responsible in stabilizing the radical compounds at 7-O. In addition, the effect of this unsaturated bond with the presence of the ketone group in the pyran ring proposed an increase to the delocalization of electron thus increase stabilization at 3-O of kaempferol. Consequently, it can be generalized that OHs group in B ring is the primary site to promote high free radical scavenging activity followed by OHs group in A ring. Interestingly, catechin has C ring as the lowest site that promote donation of H atom than B and A rings, contrary to kaempferol which showed that C ring was the best site followed by B ring and A ring. With regard to the experimental antioxidant findings of Khalil et. al. (2011), it was suggested that variations in the structural activity of OH groups influenced the strength of the free radical scavenging activity and eventually, led towards the strength in antioxidant property of the identified phenolic compounds found in Malaysian honeys.

TABLE 3. Bond Dissociation Enthalpy (BDE) of Flavanoids in the Gas Phase

Compound	BDE (kcal/mol)				
	3OH	5OH	7OH	3'OH	4'OH
Naringenin	–	91.46	92.97	–	89.29
Apigenin	–	106.53	94.63	–	89.73
Kaempferol	74.57	109.27	90.94	–	85.40
Catechin	251.91	88.74	88.94	84.95	84.39
Luteolin	–	106.45	94.68	91.18	80.40

Apigenin (4'-O•)

Naringenin (4-O•)

Catechin (4'-O•)

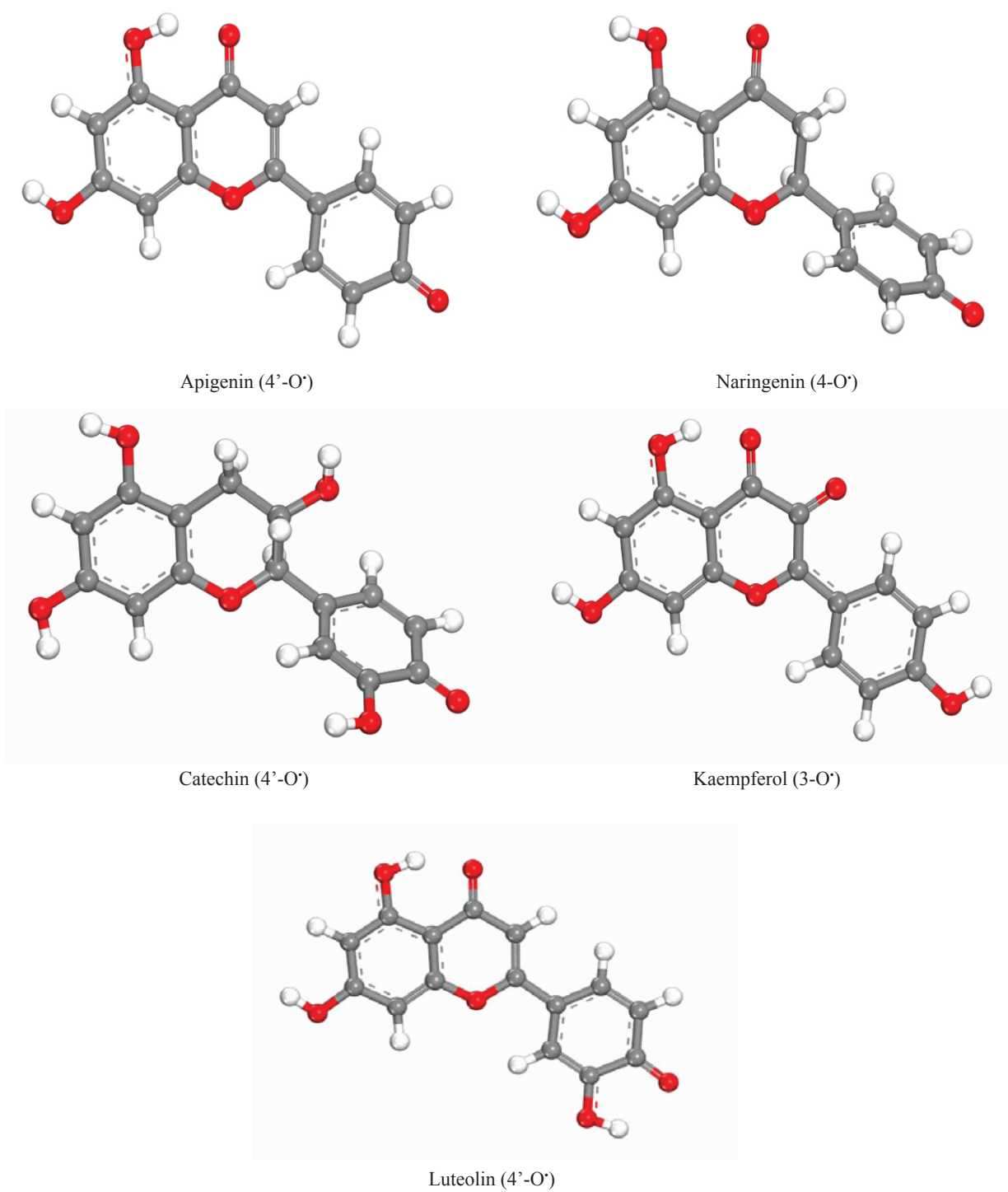Kaempferol (3-O•)

Luteolin (4'-O•)

FIGURE 2. Optimised structures of radical flavanoids calculated at UB3LYP/6-311G++G(d,p) level in gas phase. Oxygen, carbon and hydrogen is denoted by red, grey and white atom, respectively. Blue dash line represents hydrogen bond

CONCLUSION

The present study studied the structure-antioxidant activity of several phenolic compounds and flavanoids found in tualang, gelam and borneo type of Malaysian honey as experimentally determined from previous published work. In this theoretical investigation, DFT method at B3LYP/6-311++G(d,p) level of theory together with BDE descriptor associated with HAT mechanism to evaluate the potential OHs of antioxidants to scavenge free radicals, has been carried out. Phenolic acids at 4-OH or para position as well as intramolecular H-bonding could help to stabilize phenoxy radicals. Similarly, B ring of flavonoids and unsaturated bond in pyran ring are proposed factors that could lower the BDE values. In short, the findings showed that H donation of OH groups at different site of the compounds in addition to the type of substituents attached at carbon atom of the benzene rings are important factors in influencing the antioxidant properties of the phenolic antioxidants. For future work, other type of quantum-chemical descriptors like molecular electrostatic potential (MEPS), ionization potential (IP) and spin density can be used to further explore the phenolic structure-antioxidant activity. Hence, theoretical results obtained in this study could facilitate future development of antioxidant compounds.

REFERENCES

Abu Bakar, M.F., Sanusi, S.B., Abu Bakar, F.I., Cong O.J. & Mian, Z. 2017. Short communication: physicochemical and antioxidant potential of raw unprocessed honey from Malaysian stingless bees. Pakistan Journal of Nutrition 16(11): 888-894.

Bruice, P.Y. 2010. Essential Organic Chemistry. Second edition. Pearson Education, Inc.

Cai, W., Chen, Y., Xie, L., Zhang, H. & Hou, C. 2014. Characterization and density functional theory study of the antioxidant activity of quercetin and its sugar-containing analogues. European Food Research and Technology 238: 121-128.

Chen, Y., Xiao, H., Zheng, J. & Liang, G. 2015. Structure-thermodynamics-antioxidant activity relationships of selected natural phenolic acids and derivatives: An experimental and theoretical evaluation. PLOS ONE 10(3): 1-20.

Chua, L.S., Rahaman, N.L.A, Adnan, N.A. & Eddie Tan T.T. 2013. Antioxidant activity of three honey samples in relation with their biochemical components. Journal of Analytical Methods in Chemistry 2013: 1-8.

Ferreira, I. C. F. R., Aires, E., Barreira, J. C. M. & Estevinho, L. M. 2009. Antioxidant activity of Portuguese honey samples: different contributions of the entire honey and phenolic extract. Food ChemistrY 114: 1438-1443.

Frisch, M.J., Trucks, G.W., Schlegel, H.B. Scuseria, G.E., Robb, M.A., Cheeseman, J.R., Scalmani, G., Barone, V., Mennucci, B., Petersson, G.A., Nakatsuji, H., Caricato, M., Li, X., Hratchian, H.P., Izmaylov, A.F., Bloino, J., Zheng, G., Sonnenberg, J.L., Hada, M., Ehara, M., Toyota, K., Fukuda, R., Hasegawa, J., Ishida, M., Nakajima, T., Honda, Y., Kitao, O., Nakai, H., Vreven, T., Montgomery, J.A., Peralta, Jr., J. E.,Ogliaro, F., Bearpark, M., Heyd, J.J., Brothers, E., Kudin, K. N., Staroverov, V.N., Kobayashi, R., Normand, J., Raghavachari, K., Rendell, A., Burant, J.C., Iyengar, S.S., Tomasi, J., Cossi, M., Rega, N., Millam, J.M., Klene, M., Knox, J.E., Cross, J.B., Bakken,V., Adamo, C., Jaramillo, J., Gomperts, R., Stratmann, R.E., Yazyev, O., Austin, A.J., Cammi, R., Pomelli,C., Ochterski, J.W., Martin, R.L., Morokuma, K., Zakrzewski, V.G., Voth,G. A., Salvador, P., Dannenberg, J.J., Dapprich, S., Daniels, A.D., Farkas, Ö., Foresman, J.B., Ortiz, J.V., Cioslowski, J. & Fox, D.J. 2009. Gaussian 09. Wallingford, C. T. Gaussian, Inc.

Khalil, M.I., Alam, N., Moniruzzaman M., S.A. Sulaiman S.A. & Gan S.H. 2011. Phenolic acid composition and antioxidant properties of Malaysian honeys. Journal of Food Science 76(6): 921-928.

Labidi, N.S., Guerguer, L. & Kacemi, A. 2018. Theoretical evaluation of antioxidant activity of tea catechins. Journal of Materials and Environmental Sciences 9(1): 326-333.

Liang, N. & Kitts, D.D. 2014. Antioxidant property of coffee components: Assessment of methods that define mechanisms of action. Molecules 19: 19180-19208.

Moniruzzaman, M., Sulaiman, S.A. & Gan, H.G. 2016. Phenolic acid and flavanoid composition of Malaysian honeys. Journal of Food Biochemistry 41: 1-8.

Seraglio, S.K.T, Valese, A.C., Daguer, H., Bergamo, G., Azevedo, M.S., Gonzaga, L.V., Fett, R. & Costa, A.C.O. 2016. Development and validation of a LC-ESI-MS/MS method for the determination of phenolic compounds in honeydew honeys with the diluted-and-shoot approach. Food Research International 87: 60-67.

Urbaniak, A., Molski, M. & Malgorzata, S. 2012. Quantum-chemical calculations of the antioxidant properties of trans-p-coumaric acid and trans-sinapinic acid. Computational Methods in Science and Technology 18(2): 117-128.

Wang, J., Tang, H., Hou, B., Zhang, P., Wang, Q., Zhang, B., Huang, Y., Wang, Y., Xiang, Z., Zi, C., Wang, X. & Sheng, J. 2017. Synthesis, antioxidant activity, and density functional theory study of catechin derivatives. RSC Advances 7(85): 54136-54141.

Wright, J. S., Johnson, E. R. & DiLabio, G. A. 2001. Predicting the activity of phenolic antioxidants: theoretical method, analysis of substituent effects, and application to major families of antioxidants. Journal of the American Chemical Society 123: 1173-1183.

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