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Structure-activity relationship and MM2 energy minimized conformational analysis of quercetin and its derivatives in the DPPH• radical scavenging capacity

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ABSTRACT

Antioxidant activity of quercetin (**1**) and its derivatives (**2-15**) was evaluated by using DPPH assay and IC_{50} values were calculated. Dihedral angles α of C3-C2-C1'-C6' chain and β of O1-C2-C-1'-C2' chain between AC and B rings of these flavones were determined by using MM2 energy minimized structures. Structure-activity relationship study revealed that quercetin (**1**), quercetin-5-methyl ether (**2**), quercetin-3'-methyl ether (**3**) and quercetin-3',5-dimethyl ether (**4**) displaying a high antioxidant activity (IC_{50} = 47.20-119.27 μM) possess similar dihedral angles (α 11.1-11.5º and β 6.3-6.6º). Mono- and/or di-methoxy substituent(s) at 3' and 5 positions of the flavone are most suitable for the preservation of the antioxidant capacity while retaining conformational geometry.

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1. Introduction

Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), superoxide (O_2•–), hydroxyl radicals (OH•), etc. are generated during oxygen metabolism in biological systems. Imbalance between generation and elimination of ROS leads cellular aging, mutagenesis, carcinogenesis, immunodeficiency syndrome, diabetes, coronary heart diseases, neurodegenerative diseases, etc. [1-3]. Dietary flavonoids are considered as powerful antioxidants since they act as radical scavengers, metal chelators and enzyme inhibitors, and produce the beneficial health effects [4-7].

In several publications, the antioxidant activity of flavonoids have been evaluated using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) [8], hydroxyl radical (HO•) [9], superoxide ($O_2^{\bullet-}$) [10], peroxyl radical (ROO•) [11], and hypochlorite (HOCl/OCl^-) [12], and structure-activity relationships (SAR) of flavonoids in antioxidant activity have been documented [1, 11-27]. Hydrogen or electron-donating properties of flavonoids are considered to be the basis of their antioxidant activity [1, 25].

Structural requirement in flavonoids for the hydrogen-donation by single-electron transfer includes *ortho*-dihydroxyl substituent in the B ring and C2-C3 double bond in conjugation with C-4 carbonyl group in the C ring, which expands electron delocalization for radical stabilization and determines the co-planarity of the hetero ring. Quercetin, a pentahydroxy flavone (**1**), seems to be a paradox which satisfies these structural variations and so efficiently captures free radicals thereby exhibiting high antioxidant activity. In case of absence of a catecholic structure in B ring, the antioxidant activity is compensated by 3- and/or 5-hydroxyl substituent(s). Blocking or removing the 3-OH group decreases antioxidant property. The dihedral angle of the B ring with respect to remaining structure in flavones is considered for strong influence on radical scavenging activity [18, 27]. However, the roles of chemical structure and underlying molecular phenomena have stayed elusive [23, 28].

The nature of rapid conjugation with glucuronosyltransferases and sulfotransferases present in the small intestine halts quercetin (**1**) to reach the malignant organs through gastrointestinal tract in oral therapy [29]. When the hydroxyl groups in polyphenols are methylated, the resulting compounds are much less prone to glucuronidation and sulphation, and hence are more metabolically stable increasing their bioavailablity. These underlying facts promoted us to commence a research program to study the SAR of quercetin derivatives for possible enhancement of antioxidant capacity.

2. Experimental

Chemicals and equipments

Quercetin (**1**), DPPH and gallic acid were purchased from Sigma-Aldrich and were used as received. Quercetin derivatives (**2**-**15**) used in this work were previously synthesized in our laboratory [30]. Spectrophotometric analysis was performed with a Cary 60 UV-Visible spectrophotometer (Agilent Technologies).

DPPH radical scavenging assay

The DPPH assay was performed according to the procedure described by Brand-Williams *et al.* with a slight modification [31]. To generate free DPPH• radical, 11.7 mg of DPPH in methanol (300 mL) was stirred overnight at 0 °C and used immediately. Sample solutions of the compounds **1**-**15** of different concentrations (5, 25, 50, 100, 250 and 500 μM in acetone) were prepared. Each sample solution (0.5 mL) was mixed with freshly prepared DPPH• solution (2.5 mL). A control solution was prepared by mixing acetone (0.5 mL) and DPPH• solution (2.5 mL). The content was shaken well, kept in dark at room temperature for 30 min and then absorbance was measured at 517 nm against the blank solution consisting acetone (0.5 mL) and MeOH (2.5 mL). The percentage of inhibition was calculated by the equation:

$$I\ (\%) = (1 - A_{sample} / A_{control}) \times 100$$

where, A_{sample} and $A_{control}$ are the absorbance values of the reaction mixture with and without sample, respectively.

Thus obtained data of % inhibitions at different concentrations were computed to calculate IC_{50} values by employing the equation:

$$IC_{50} = (50\ \mu M - c) / m$$

where, IC_{50} = concentration causing 50% inhibition of absorbance, c = intercept, m = slope of a linear curve describing dependence of % inhibition with concentration.

Computational analysis

Energy minimized structure of quercetin (**1**) and synthesized quercetin derivatives (**2**-**15**) were produced by using Molecular Mechanics Part 2 (MM2) calculation, Cambridgesoft's Chem3D Pro 12.0.2.1076. Thus optimized geometry of the structures were studied for determining the dihedral angles between the planes of the AC-ring and the B-ring employing the angels of C3-C2-C1'-C6' (α) and O1-C2-C1'-C2' (β).

3. Results and Discussion

DPPH assay measures the antioxidant capacity of a compound in terms of its ability to donate hydrogen atom to the free DPPH• radical. The DPPH• radical, which shows absorption at 517 nm, is reduced to the corresponding hydrazine when it reacts with hydrogen donors. DPPH assay is considered a valid and easy assay to evaluate the SAR of antioxidants [32].

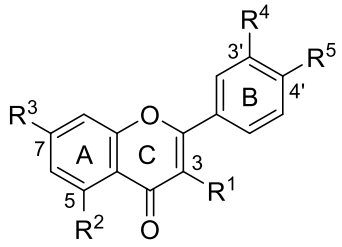
Some of the compounds used in this study were not completely soluble in MeOH; therefore, standard procedure was slightly modified by using acetone as a dissolving solvent to prepare the sample solutions. In the DPPH assay, six different concentrations of each compound were used in order to obtain IC_{50} value. Linear regression curve of concentration *versus* percentage of inhibition for each compound was plotted where R^2 values were found nearly equal to 1. The slope and intercept values of linear regression curve were used to obtain the IC_{50} value and the result is presented in Table 1.

The IC_{50} value of quercetin **(1)** in DPPH assay was reported by several groups and it ranged from 1.82 to 119.60 μM [26, 33-39]. In this study, compared to the parent quercetin **1** (IC_{50} = 47.20 μM), its monomethylated derivatives **2** (IC_{50} = 52.24 μM) and 3 (IC_{50} = 52.45 μM) have retained the antioxidant capacity in the DPPH assay (Table 1). The dimethylated derivative 4 also displayed antioxidant property with IC_{50} value of 119.27 μM. The antioxidant capacity was gradually reduced as the number of substituents other than hydroxyl group is increased. Although 3,5-diOH and/or 3',4'-diOH groups are important for scavenging of DPPH• radical; however, this study showed that one (or two) of these hydroxyl group(s) can be replaced with methoxy group(s). This conclusion is also supported by Moalin *et a*l., who have reported that substitutions of methoxy group at 5 and/or 7 position(s) in ring A of quercetin **(1)** retain the antioxidant capacity in ABTS assay [27]. Acetylation of free hydroxyl groups reduced the antioxidant capacity; however, quercetin 3,3',4',5-tetraacetate **(5)** and quercetin 3,3',4',5,7-pentaacetate **(6)** derivatives have exhibited a mild antioxidant activity with IC_{50} values of 516.26 and 790.57 μM, respectively. These results also indicated that a free 7-OH group in a flavone has a crucial role. Remaining quercetin derivatives (**7**-**15**) were found to be lost their DPPH• radical scavenging capacity displaying IC_{50} value >2000 μM.

Olejniczak and Potrzebowski have reported that a small perturbation of geometry has a great influence on bond orders in ring B in density functional theory (DFT) calculation of quercetin (**1**) [40]. And, the change in conformers may significantly change the susceptibility for the formation of radicals and consequently alters the biological property. Although molecular structure of quercetin (**1**) (and its derivatives as well) can be represented by exact conformation; however, the presence of many flexible hydroxyl groups and hydrogen bonding networks in the system leads a wrong conclusion in the crystal lattice provided by X-ray diffractometry (XRD). Later, Filip *et al.* have performed the conformational calculation of quercetin (**1**) by employing the molecular mechanics (MM) level of theory that combined with previous XRD and solid state NMR data [41, 42]. It was concluded that small changes of conformation and hydrogen bonding pattern greatly influence the bond order parameters of quercetin (**1**). The authors have further mentioned that no matter how precisely the conformational analysis is performed, the most important is finding of the most probable molecular conformation. The MM2 energy minimization calculation can identify the more stable conformation [43]. Therefore in the present study, the MM2 calculations of the compounds **1**-**15** were performed.

In the case of quercetin (**1**), the orientation of the hydroxyl groups at 3 and 3' carbon atoms in a planar arrangement of A, B and C rings produces *anti* and *syn* conformers. Herein, energy minimized structures of compounds **1**-**15** were computed by keeping 3 and 3' substituents at *anti* fashion. The optimal conformations of the compounds obtained are depicted in Figure 1 and their dihedral angles are given in Table 1.

Table 1: IC_{50} values of quercetin scaffolds (**1**-**15**) in DPPH• free radical scavenging activity.



Compound	Substituents					Dihedral angles		IC_{50} (μM)
	R^1	R^2	R^3	R^4	R^5	α°	β°	
Quercetin (**1**)	OH	OH	OH	OH	OH	11.5	6.6	47.20
Quercetin 5-methyl ether (**2**)	OH	OMe	OH	OH	OH	11.2	6.4	52.24
Quercetin 3'-methyl ether (**3**)	OH	OH	OH	OMe	OH	11.1	6.3	52.45
Quercetin 3',5-dimethyl ether (**4**)	OH	OMe	OH	OMe	OH	11.2	6.3	119.27
Quercetin 3,3',4',5-tetraacetate (**5**)	OAc	OAc	OH	OAc	OAc	9.2	4.6	516.26
Quercetin 3,3',4',5,7-pentaacetate (**6**)	OAc	OAc	OAc	OAc	OAc	7.3	3.9	790.57
Quercetin 3,3',4',7-tetraacetate (**7**)	OAc	OH	OAc	OAc	OAc	7.3	1.7	>2000
Quercetin 3,3',4',7-tetrabenzyl-5-methyl ether (**8**)	OBn	OMe	OBn	OBn	OBn	3.4	1.3	>2000
Quercetin 3,4',7-tribenzyl ether (**9**)	OBn	OH	OBn	OH	OBn	2.8	0.9	>2000
Quercetin 3,3',4',7-tetrabenzyl ether (**10**)	OBn	OH	OBn	OBn	OBn	2.7	1.1	>2000
Quercetin 3,4',7-tribenzyl-3',5-dimethyl ether (**11**)	OBn	OMe	OBn	OMe	OBn	2.6	0.9	>2000
Quercetin 3,4',7-tribenzyl-3'-methyl ether (**12**)	OBn	OH	OBn	OMe	OBn	2.3	0.8	>2000
Quercetin 3,3',4',5,7-pentamethyl ether (**13**)	OMe	OMe	OMe	OMe	OMe	1.7	0.5	>2000
Quercetin 3,3',4',7-tetramethyl ether (**14**)	OMe	OH	OMe	OMe	OMe	1.5	0.4	>2000
Quercetin 3,4',7-trimethyl ether (**15**)	OMe	OH	OMe	OH	OMe	1.4	0.3	>2000

The dihedral angles α = 11.5º and β = 6.6º were found in MM2 energy minimization calculation of quercetin (**1**), which exhibited a high antioxidant property (IC_{50} = 47.20 μM) (Table 1, Figure 1). Interestingly, in comparison with quercetin (**1**), quercetin-5-methyl ether (**2**) (α 11.2º and β 6.4º), quercetin-3'-methyl ether (**3**) (α 11.1º and β 6.3º) and quercetin-3',5-dimethyl ether (**4**) (α 11.2º and β 6.3º) showed similar dihedral angels and they were also highly efficient in scavenging of DPPH• radical with the IC_{50} values of 52.24, 52.45 and 119.27 μM, respectively. The decrease in dihedral angles α and β decreased the antioxidant property as shown by quercetin-3,3',4',5-tertaacetate (**5**) (α = 9.2º, β = 4.6º, IC_{50} = 516.26 μM) and quercetin-3,3',4',5,7- pentaacetate (**6**) (α = 7.3º, β = 3.9º, IC_{50} = 790.57 μM). The MM2 calculation of remaining compounds (**7**-**15**) showed that they exist in more or less planar conformation. Therefore, a slightly twisted conformation between rings B and C with dihedral angles α 11.5-7.3º and β 6.6-3.9º plays a pivotal role in preserving the antioxidant capacity of parent compound quercetin (**1**) and perfect planarity is merely not necessary [27]. While modifying the structure of a flavone in search of the molecule with enhanced antioxidant activity, preservation of its planarity is therefore a must.

4. Conclusion

In conclusion, we have evaluated the DPPH• free radical scavenging capacity of quercetin (**1**) and its derivatives (**2**-**15**). Quercetin (**1**) and its methoxyl derivatives **2**, **3** and **4** were found as highly efficient antioxidants. The acetyl derivatives **5** and **6** exhibited a mild antioxidant capacity while other remaining derivatives (**7**-**15**) were found to be inefficient. Dihedral angles α of C3-C2-C1'-C6' chain (11.5-7.3º) and β of O1-C2-C-1'-C2' chain

(6.6-3.9º) between AC and B rings as can be found in quercetin (**1**) are suitable for the preservation of antioxidant capacity. Mono- and/or di-methoxy substituent(s) at 3' and 5 positions of the flavone are most suitable for the preservation of the antioxidant capacity. Free 7-OH group of a flavone also plays a crucial role in preserving of the antioxidant capacity.

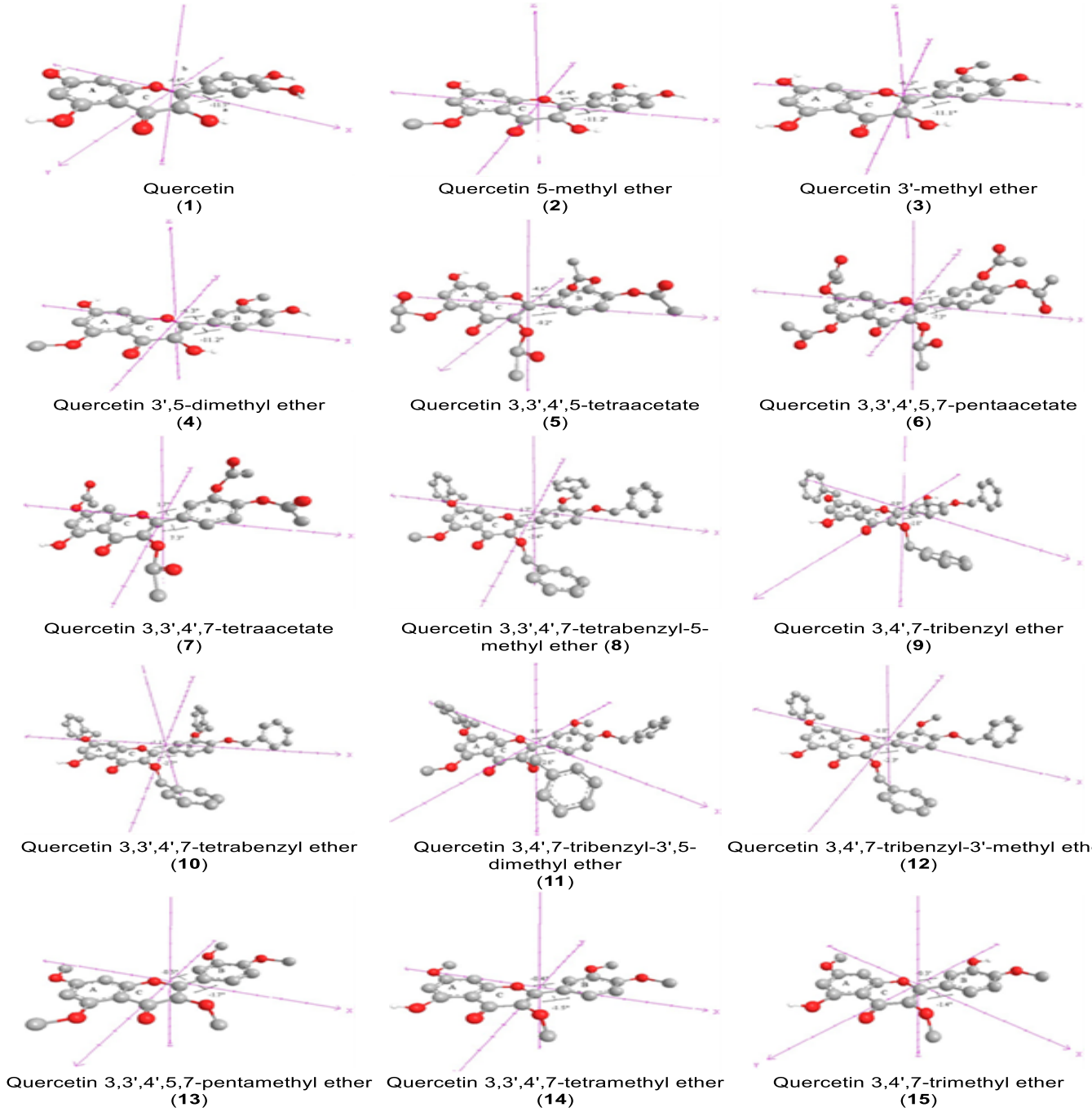


Fig. 1: Three dimensional energy minimized structure of quercetin (**1**) and its derivatives (**2-15**) with their dihedral angles α and β.

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