



## A structure–activity relationship study of flavonoids as inhibitors of *E. coli* by membrane interaction effect



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### ARTICLE INFO

#### Article history:

Received 5 June 2013

Received in revised form 24 July 2013

Accepted 29 July 2013

Available online 9 August 2013

#### Keywords:

Flavonoids

Antibacterial activity

*E. coli*

Membrane interaction

Fluorescence polarization

QSAR

### ABSTRACT

Flavonoids exhibit a broad range of biological activities including antibacterial activity. However, the mechanism of their antibacterial activity has not been fully investigated. The antibacterial activity and membrane interaction of 11 flavonoids (including 2 polymethoxyflavones and 4 isoflavonoids) against *Escherichia coli* were examined in this study. The antibacterial capacity was determined as flavonoids > polymethoxyflavones > isoflavonoids. Using fluorescence, it was observed that the 5 flavonoids rigidified the liposomal membrane, while the polymethoxyflavones and isoflavonoids increased membrane fluidity. There was a significant positive correlation between antibacterial capacity and membrane rigidification effect of the flavonoids. A quantitative structure–activity relationship (QSAR) study demonstrated that the activity of the flavonoid compounds can be related to molecular hydrophobicity (CLogP) and charges on C atom at position 3 (C3). The QSAR model could be used to predict the antibacterial activity of flavonoids which could lead to natural compounds having important use in food and medical industry.

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### 1. Introduction

Flavonoids are widely present in the plant kingdom exhibiting a broad range of biological activities, including antibacterial, antifungal, antiviral, anti-allergic, anti-inflammatory, antiproliferative and antioxidant activities [1–4]. Due to growing public concern regarding the negative effects of antimicrobial drugs on human health and the environment, there is a demand for novel antimicrobials. Flavonoids, extracted from plants or obtained as byproducts at low cost from waste residues, for example of the citrus industry, have potential value in food and pharmaceutical industries. Previous studies have reported on isolation and identification of flavonoids possessing antibacterial activity rather than on mechanism [5–7]. Since the ability of flavonoids to interact with membranes can greatly affect their bioactivities [8], the antibacterial activity of flavonoids may be attributed to their common mode of action on membranes. It has been reported that sophoraflavanone G demonstrated antibacterial activity by reducing membrane fluidity of bacterial cells [9]. A study also demonstrated that galangin caused a significant increase in potassium loss from *Staphylococcus aureus* cells, which may be attributed to either direct damage to the cytoplasmic membrane or indirect damage effected through autolysis/weakening of the cell wall and consequent osmotic

lysis [10]. Therefore, it is important to characterize the relationship between antibacterial activity of flavonoids and membrane interaction.

Structurally, most flavonoids are derived from the parent compound exhibiting a diphenylpropane (C6–C3–C6) skeleton. Taking into account the chemical nature of the molecule, and the positions of moieties substituting rings A, B, and C, the flavonoids are divided into different groups. Depending on the position at which the ring B is attached to the benzo- $\gamma$ -pyrone core of the molecule, flavonoids (ring B is bound at position 2 of ring C) and isoflavonoids (ring B is bound at position 3 of ring C) are distinguished. The antibacterial activity of flavonoids has been suggested to be related to their chemical structure, especially the number and positions of methoxyl and hydroxyl groups [11,12]. Therefore, the quantitative structure–activity relationship (QSAR) of flavonoids is an important area of study. The QSAR approach has been efficiently used for the study of biological mechanisms of various reactive chemicals by which the molecular structures or properties are quantitatively correlated with variations in biological activity [13].

In this study, the inhibitory activities of 11 commercially available flavonoids against *Escherichia coli*, one of the most common pathogens found in food were evaluated by microbroth dilution method. Flavonoids (Fig. 1) were chosen according to various hydroxyl groups, methoxyl groups or other substituents in the structure. To determine the mechanism involved in the antimicrobial action, large unilamellar vesicles (LUVs) formed by mixtures of 1,2-dipalmitoyl-sn-glycerophosphatidylcholine (DPPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol (DPPG) phospholipids were used as structural models

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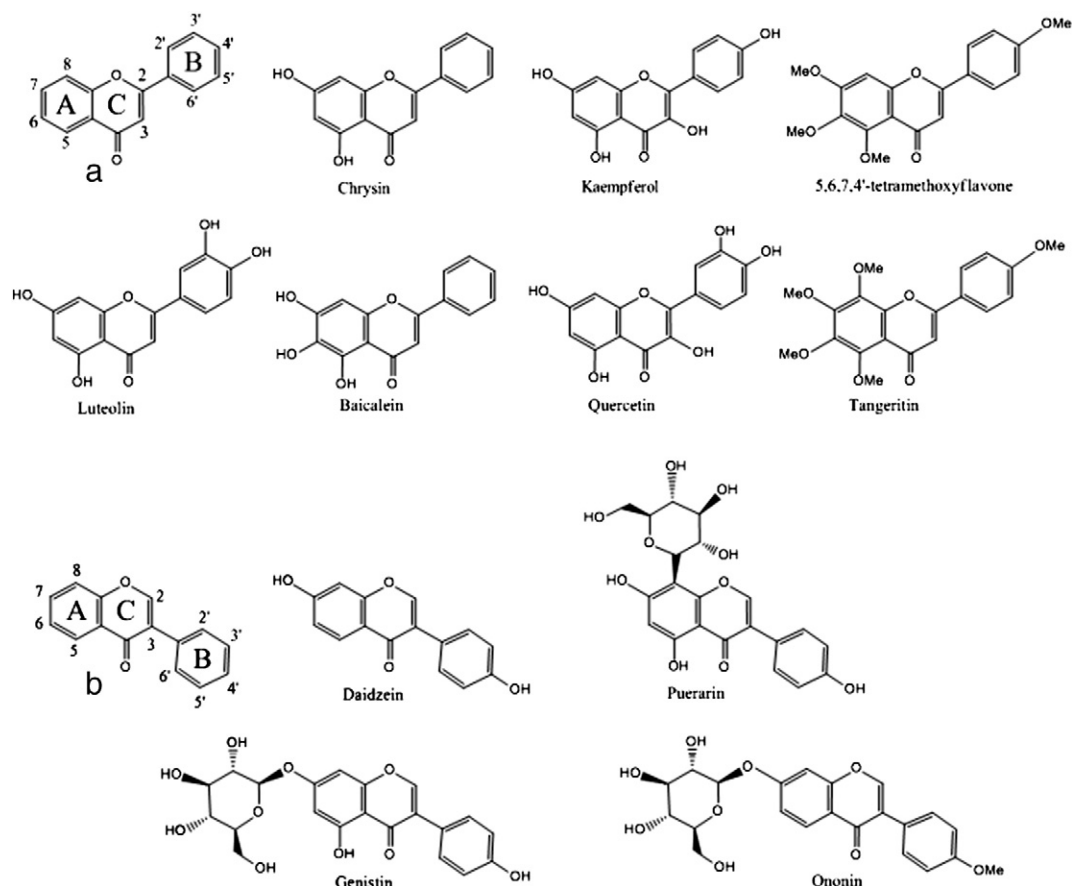


Fig. 1. Structures of flavonoids used in this study.

of biological membranes, as it was reported that LUVs of DPPC/DPPG mixtures could be used to simulate the membrane of Gram-negative bacteria [14]. The interaction of flavonoids with the model membrane was assessed by fluorescence polarization measurements. The observed antibacterial capacities and the relationship between antibacterial activity and membrane interaction of the tested flavonoids were further evaluated through QSAR method.

## 2. Materials and methods

### 2.1. Bacterial strain

*E. coli* ATCC25922 was purchased from China Center of Industrial Culture Collection.

### 2.2. Chemicals

Flavonoids including baicalein, chrysin, daidzein, genistin, kaempferol, puerarin, quercetin, ononin, and luteolin, were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China). Tangeritin was purchased from National Institutes for Food and Drug Control (Beijing, China) and 5,6,7,4'-tetramethoxyflavone from ChromaDex Co. (Irvine, US). Phospholipids: 1,2-dipalmitoyl-sn-glycerophosphatidylcholine (DPPC) and 1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol (DPPG) were obtained from AVT Co., Ltd. (Shanghai, China). The fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH) was from Sigma Aldrich Co. (Shanghai, China). The flavonoid stocks were prepared in dimethyl sulfoxide (DMSO) and the DPH stock solutions were prepared in N,N-dimethylformamide. All other chemicals were of analytical grade.

### 2.3. Antibacterial activity

The antibacterial activity was measured as MIC<sub>50</sub> which is defined as the concentration that inhibits the growth of 50% of organisms. The MIC<sub>50</sub> of each flavonoid was evaluated according to the microbroth dilution method performed in 96-well micro-plates described by Mandalari et al. [15] with minor modifications. Briefly, *E. coli* was cultured in Mueller-Hinton Broth (MHB) and grown overnight at 37 °C with shaking (150 rpm). A 100 µL bacterial suspension containing 10<sup>8</sup> cfu/mL of the bacteria (density matching the turbidity of a 0.5 McFarland standard) was added to each well. Then, 100 µL of each dissolved flavonoid stock was twofold serially diluted and transferred to each well with the test performed in a final volume of 200 µL. The same tests were performed for sterility control (MHB) and growth control (MHB + bacteria). The plates were covered and incubated for 16 h at 37 °C with shaking (220 rpm). The absorbance at 600 nm was measured using a microplate reader (Multiskan Spectrum, Thermo) to determine the bacterial growth. The inhibition ratio (%) was calculated as follows:

$$\% \text{ inhibition} = 100\% - \left[ \frac{\text{Absorbance of test sample} - \text{Absorbance of control}}{\text{Absorbance of control}} \right] \times 100.$$

### 2.4. Liposome preparation

LUVs formed by mixtures of DPPC and DPPG were prepared by the method of Wrobel et al. [16] with minor modifications. Briefly, lipids (DPPC/DPPG = 1/2, V/V) were dissolved in a methanol chloroform

(3:7, v/v) solution, then a mixture containing 500  $\mu\text{L}$  lipids and 687.5  $\mu\text{L}$  of the DPH stock ( $2 \times 10^{-5}$  mol/L) was dried under vacuum in a round-bottomed flask. The molar ratio of DPH to total membrane lipids was 1:500. The dry films of lipids and DPH were sonicated in 1 mL phosphate-buffered saline (PBS) buffer at 45 °C for 15 min. The suspension was then passed 19 times through 2 stacked polycarbonate filters (pore size 100 nm) using a LipoFast extruder (Avestin, Ottawa, Canada) and diluted to 50 mL in PBS buffer. The final concentration of lipids in the liposome was 0.135 mM.

### 2.5. Fluorescence spectroscopy

The ability of flavonoids to interact with membranes was evaluated according to the method of Arora, Byrem, Nair and Strasburg [17] with minor modifications. Briefly, an aliquot of the flavonoid solution was added to the liposome suspensions to achieve final concentration of 50  $\mu\text{M}$  for each, followed by incubation at 40 °C for 40 min. The volume of DMSO was adjusted to be less than 0.5% (v/v) of the total volume, which did not influence membrane fluidity. Fluorescence polarization measurements were carried out with a FP-6500 Spectrofluorometer (Jasco, Japan). The excitation and emission wavelengths were 360 nm and 430 nm, respectively (slit width 5 nm). The fluorescence polarization values (FP) of the samples were calculated as:

$$FP = (I_{VV} - GI_{VH}) / (I_{VV} + 2GI_{VH})$$

where  $I_{VV}$  and  $I_{VH}$  are the vertical and horizontal fluorescence intensities respectively, when the sample is excited with vertically polarized light. The grating correction factor  $G = I_{HV} / I_{HH}$  corrects the polarizing effects of the monochromator.

### 2.6. Data analysis

All the experiments were performed in triplicate and the data presented represent the mean values.

### 2.7. QSAR study

The geometric optimization of the 11 flavonoids was initially performed by Sybyl 7.3 software (Tripos Inc., USA). Then the stable geometric structures of these compounds were further optimized using the DFT calculations at the level of Becke's 3-parameter hybrid functional (B3LYP) and 6-31G basis set. The quantum-chemical parameters for each molecule were performed by Gaussian 03 program package. The molecular properties and topological descriptors were calculated applying the Molconn-Z module (Tripos Inc., USA). Biological activity data determined as  $\text{MIC}_{50}$  values were first transformed into  $\text{pMIC}_{50}$  values ( $-\log \text{MIC}_{50}$ ) and used as dependent variables in the QSAR study.

The correlation analysis was performed by SPSS (version 19) to select the descriptors affecting the antibacterial activity. The descriptors with higher correlation to  $\text{pMIC}_{50}$  and lower inter-correlation ( $|r| < 0.7$ ) were selected for the multiple linear regression analyses to establish the QSAR model. The best QSAR model was selected on the basis of the highest square of the correlation coefficients ( $R^2$ ), adjusted squared correlation coefficient ( $R^2_{\text{adj}}$ ), Fisher ration value (F) and significance level of the model p. The model was additionally validated by leave-one-out cross-validation. For a reliable model, the square of cross-validation coefficient ( $Q^2$ ) should be greater than 0.5. In addition, the dataset of the 11 flavonoids was randomly divided into a training set (9 compounds) and a test set (2 compounds) to obtain a QSAR model with reliable external validation. The QSAR model is of high predictive ability, only if the square of predictive correlation coefficient ( $R^2_{\text{pred}}$ ) for the test set is greater than 0.6 [18].

## 3. Results and discussion

### 3.1. Antibacterial activity

The tested flavonoids exhibited varying levels of antibacterial activity against *E. coli* (Table 1). Flavonoids inhibited the growth of *E. coli* more intensely than isoflavonoids. The highest inhibitors were kaempferol, quercetin and chrysin, with  $\text{MIC}_{50}$  values of 25  $\mu\text{g}/\text{mL}$ , 35.76  $\mu\text{g}/\text{mL}$  and 36.72  $\mu\text{g}/\text{mL}$ , respectively. The isoflavonoids ononin and puerarin, with  $\text{MIC}_{50}$  values of 712.5  $\mu\text{g}/\text{mL}$  and 1500  $\mu\text{g}/\text{mL}$ , had little effect on bacterial inhibition, indicating that the basic structure of a flavonoid is more suited for antibacterial activity than that of an isoflavonoid. The relative antibacterial activities were determined as: kaempferol > quercetin > chrysin > luteolin > baicalein > tangeritin > 5,6,7,4'-tetramethoxyflavone and daidzein > genistin > ononin > puerarin.

Kaempferol and quercetin showed the highest antibacterial ability, and quercetin exhibited stronger inhibitory activity than luteolin, with the only structural difference between these 2 compounds being that quercetin has a hydroxyl group at position 3 in the C ring, while luteolin has none. This indicated that hydroxyl group at position 3 in the C ring is important to the antibacterial activity of flavonoids, which is in agreement with previous studies reporting that the absence of the hydroxyl group at position 3 in flavonoids decreases their antioxidant and antimicrobial abilities [19,20].

It has been reported that methoxy groups decrease the antibacterial activity of flavonoids [12]. In this study, tangeritin and 5,6,7,4'-tetramethoxyflavone, the 2 polymethoxyflavones, showed lower antibacterial activity than the flavonoids, indicating that 4 or 5 additional methoxy groups in the A ring and B ring inhibit the growth of *E. coli*. However, the methoxyl group at C-8 in the A ring appears to increase antibacterial activity. Tangeritin showed higher activity than 5,6,7,4'-tetramethoxyflavone, both having a similar structure except for the methoxyl group at C-8 in the A ring. This result is consistent with previous studies reporting that the presence of a lipophilic group at position 8 improves the antibacterial and antifungal activity of flavonoids [21,22].

Among the 4 isoflavonoids, daidzein exhibited the highest inhibitory ability, suggesting that the addition of the glucoside at C-7 and C-8 in the A ring decreases the antibacterial activity of isoflavonoids.

### 3.2. Membrane interactivity

The membrane interactivity of flavonoids was evaluated by DPH polarization differences from controls (treated without flavonoids). An increase in FP value indicates a decrease of membrane fluidity. As shown in Table 1, the 5 flavonoids rigidified the model membrane, in the order kaempferol > chrysin > quercetin > baicalein > luteolin; whereas the polymethoxyflavones and isoflavonoids increased membrane fluidity,

**Table 1**  
Antibacterial activity of flavonoids and fluorescence polarization of model membranes treated with flavonoids.

Types of flavonoids		$\text{MIC}_{50}$ ( $\mu\text{g}/\text{mL}$ )	$\text{pMIC}_{50}$	$\Delta\text{FP}$
Flavonoid	Chrysin	36.72	3.84	$0.0151 \pm 0.0050$
	Kaempferol	25.00	4.06	$0.0178 \pm 0.0030$
	Quercetin	35.76	3.93	$0.0041 \pm 0.0019$
	Baicalein	70.94	3.58	$0.0055 \pm 0.0030$
	Luteolin	67.25	3.63	$0.0006 \pm 0.0020$
Polymethoxyflavone	Tangeritin	137.1	3.43	$-0.0091 \pm 0.0001$
	5,6,7,4'- Tetramethoxyflavone	156.3	3.34	$-0.0134 \pm 0.0007$
Isoflavonoid	Daidzein	120.0	3.33	$-0.0518 \pm 0.0002$
	Puerarin	1500	2.44	$-0.0677 \pm 0.0010$
	Genistin	238.0	3.26	$-0.0188 \pm 0.0014$
	Ononin	712.5	2.58	$-0.0633 \pm 0.0003$

in the order puerarin > ononin > daidzein > genistin > 5,6,7,4'-tetramethoxyflavone > tangeritin.

The membrane interaction effects of flavonoids were influenced by the number and the position of hydroxyl groups. The hydroxyl group at C-3 in the C ring is important for decreasing membrane fluidity, with potency being kaempferol > chrysin and quercetin > luteolin. This result corroborates a previous study that concluded the hydroxyl group at C-3 is the primary determinant for significant membrane interaction [23]. The flavonoids decreased the membrane fluidity while the polymethoxyflavones increased fluidity. It is likely that the difference can be attributed to higher saturation of the liposomes with polymethoxyflavones compared to flavonoids (at the same concentration) resulting in domains or rafts formation in the membrane.

In our study, an increase in polarization was observed for most tested flavonoids, while for isoflavonoids the polarization decreased. It was reported that flavonoids incorporate into the interior of the lipid bilayers and increase the ordering and dynamics in the membrane interior, while isoflavonoids which do not contain hydrophobic prenyl chains interact with the polar region of lipid bilayer or penetrate its polar/apolar interface with the hydrophobic core not directly affected [24,25]. The difference between flavonoids and isoflavonoids on the effect of fluidity of liposomal membranes may be related to the different locations of the molecules in lipid bilayer which is related to the different chemical structures of these compounds. It has been suggested that the basic structure of a flavonoid is more suitable for membrane rigidification than that of an isoflavonoid [23]. However, some research has reported the opposite effect; isoflavonoids caused an increase in fluorescence polarization, indicating a decrease in membrane fluidity [4,17]. This contradiction may be due to differences in model membrane composition used in experiments. Our study used DPPC/DPPG liposome which have relatively rigid membranes, whereas those two studies used liposome consisting of unsaturated 1-stearoyl-2-linoleoylphosphatidylcholine (SLPC) or 1-palmitoyl-2-oleoylphosphatidylcholine (POPC), which makes the membranes relatively fluid.

As shown in Fig. 2, there is a significant positive correlation between antibacterial capacity and membrane rigidification effect of the flavonoids ( $r = 0.921$ ), indicating that flavonoids exert their antibacterial action by reducing membrane fluidity. Kaempferol, located deeply in the hydrophobic core of the lipid bilayer, decreased the membrane fluidity most while exhibiting the highest antibacterial capacity, while puerarin and ononin, 2 isoflavonoids with glycosidic linkages, had little antibacterial effect. It was observed that flavanone hesperidin, due to its glycoside moiety, is located at the level of polar head whereas hesperetin interacts better with acyl chains and adopts a more planar

conformation [26]. Functions of membrane enzymes and receptors, as well as the reaction efficacy of membrane components could be modulated by fluidity changes, leading to the multiple biological effects of flavonoids [27]. The cell membrane could be a direct target for antibacterial action of flavonoids, suggesting that membrane interaction could be an important mechanism of the antibacterial activities of flavonoids.

### 3.3. QSAR study

The model can be obtained using the  $\text{pMIC}_{50}$  as the dependent variable and calculated properties as independent variables. In this work, multiple regression analysis was used for variable selection to obtain the best equation. The number of variables in the multiple regression procedure was limited to two, considering the rule that the number of compounds in the data set should be three to six times the number of parameters in the model [28]. The best QSAR equation was obtained for 9 compounds in training set as follows:

$$\text{pMIC}_{50} = 2.563 + 0.469\text{CLogP} + 2.529\text{C3}$$

$$R^2 = 0.903, R^2_{\text{adj}} = 0.870, R^2_{\text{pred}} = 0.840, Q^2 = 0.818, F = 27.780, p < 0.001.$$

The correlation matrix between antibacterial capacity and the respective calculated properties is shown in Table 2. A regression equation is of no relevance when the variables are mutually interrelated by simple or multiple correlations or either, they are not orthogonal [29]. The correlation matrix showed that the properties are independent; indicating that the model in this study is strong.

The agreement between predicted  $\text{pMIC}_{50}$  values and experimental ones is satisfactory (Fig. 3). The predicted activity of the QSAR model was further evaluated by the test set. The predicted deviations of luteolin and ononin (0.39 and 0.06, respectively) are small and the predictive correlation coefficient  $R^2_{\text{pred}}$  reached 0.840. The results demonstrate that the QSAR model is strong and predictive, and can be used to explain the antibacterial mechanism of flavonoids.

In QSAR analysis, hydrophobic, electronic and steric are the 3 major types of interactions that the modeler must deal with [30]. In our QSAR model, the 2 selected descriptors were CLogP and C3. CLogP is a measure of hydrophobicity (a large value of CLogP represents higher hydrophobicity) while C3 is the net charge of C atom at position 6 on C ring relating to electronic properties. This model showed that increasing CLogP value and C3 value leads to an increase in antibacterial capacity against *E. coli*.

The correlation coefficient of CLogP versus  $\text{pMIC}_{50}$  ( $R = 0.702$ ) indicates that CLogP has the higher contribution to the  $\text{pMIC}_{50}$ , implying that hydrophobicity plays a major role in the antibacterial activity of flavonoids. In Table 3, it was observed that compounds with values of CLogP above 2 such as chrysin, baicalein, and kaempferol (CLogP of 3.56, 3.00, 2.08, respectively) are the most active, while compounds with low values of CLogP, such as, puerarin, ononin and genistin (CLogP of 0.02, 0.77, 0.91, respectively) are less active. It has been reported that 5-hydroxyflavanones and 5-hydroxyisoflavanones with one, two or three additional hydroxyl groups at the 7, 2 and 4 positions inhibited the growth of *Streptococcus mutans* and *Streptococcus sobrinus* [31]. A study concluded that flavonoids lacking hydroxyl groups on the

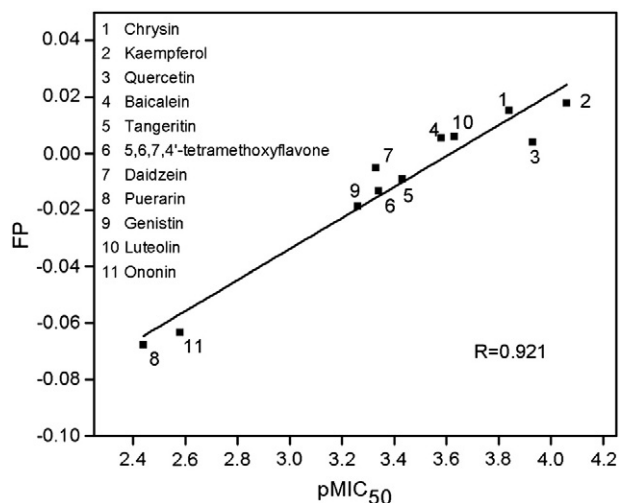


Fig. 2. Correlation between the antibacterial activity ( $\text{pMIC}_{50}$  values) of the flavonoids and the corresponding fluorescence polarization of model membranes treated with flavonoids.

Table 2  
Correlation matrix for descriptors included in the QSAR model.

	$\text{pMIC}_{50}$	CLogP	C3
$\text{pMIC}_{50}$	1.000		
CLogP	0.702	1.000	
C3	0.316	-0.376	1.000

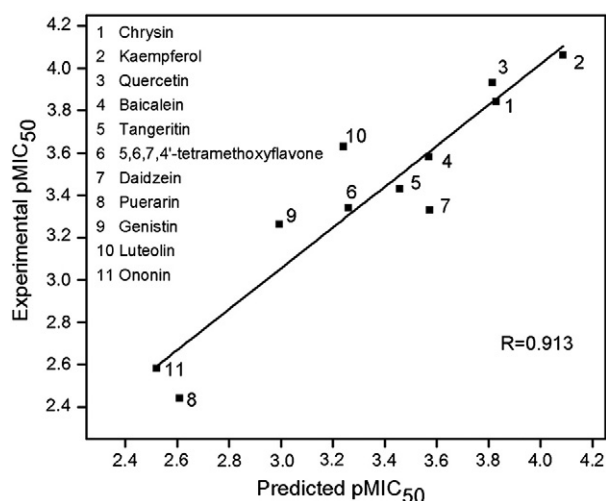


Fig. 3. Experimental versus predicted pMIC<sub>50</sub> for the model.

B ring are more active against micro-organisms than those with hydroxyl groups [32]. These results may be due to the negative correlation between the relative hydrophobicity of flavonoids and the numbers of hydroxyl groups in their structures. Flavonoids rich in hydroxyl groups have lower hydrophobicity and are more difficult to partition into lipid membranes.

As the charge in C3, the other descriptor in the QSAR model, may represent a measure of extension of the electronic delocalization of the molecule, it is concluded that electronic effects have an important role in the antibacterial activity of flavonoids. Table 3 illustrates that kaempferol and quercetin with the highest positive charges on C3 exhibit the highest antibacterial activity. Although the addition of hydroxyl groups weakens the hydrophobicity of flavonoids, it increases the charge of atoms. This could explain why other studies also found the effect that as hydroxyl groups increased so did antimicrobial activity [12,33]. The relative high hydrophobicity but low C3 values of tangeritin and 5,6,7,4'-tetramethoxyflavone resulting from the addition of methoxyl groups in A and B rings and the absence of hydroxyl groups contributed to mid-range antibacterial activity in comparison to the other compounds. When comparing tangeritin to 5,6,7,4'-tetramethoxyflavone, the activities of these polymethoxyflavones are mainly determined by hydrophobicity, as their charges on C are similar. Tangeritin is more active because of the methoxyl group at C-8 in the A ring. This confirms that the antibacterial activity and structure relationship of *E. coli* can be explained by the obtained QSAR model.

Hydrophobicity is a basic parameter used to describe the ability of drugs to interact with biological membranes [4] and the C atom at

position 3 in the C ring has a major role. The two selected descriptors in the QSAR model further indicate that interactions with membranes play an essential role in antibacterial activity of flavonoids. It was reported that antibacterial activities against *E. coli* of epicatechin gallate (ECg) and epigallocatechin gallate (EGCg) are much stronger than those of epicatechin (EC) and epigallocatechin (EGC), which is in good agreement with the order of the amounts of catechins incorporated into lipid membranes, as the hydrophobicity of the molecules were increased by the galloyl moiety [34]. A QSAR study related to the inhibitory effect of 10 natural phenolic compounds on *Fusarium verticillioides*, indicated that the antifungal activity increased with hydrophobicity [35]. These results suggest that the bioactivity of these compounds is due to their ability to partition into lipid membranes.

#### 4. Conclusion

The antibacterial capacity and membrane rigidification effect of flavonoids were positively correlated. The QSAR model demonstrated that hydrophobicity and electronic property are key factors in the antibacterial activity of flavonoids. Higher values for CLogP and high positive charges on C3 lead to an increase in antibacterial activity. These variables further confirmed that cell membrane could be one of the direct modes for antibacterial action. To our knowledge, this study constitutes the first contribution of the QSAR model to predict the inhibition activity of flavonoids against *E. coli*. In addition, the obtained model based on molecular descriptors could be used to predict the activity of flavonoids and to direct research related to the use of compounds with high antibacterial capacity in the food and medical industry.

#### Acknowledgements

This research was supported by the National Natural Science Foundation of China (No. 31171756) and the project was also sponsored by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry.

#### References

- [1] H. Cheong, S.-Y. Ryu, M.-H. Oak, S.-H. Cheon, G.-S. Yoo, K.-M. Kim, Studies of structure activity relationship of flavonoids for the anti-allergic actions, *Arch. Pharm. Res.* 21 (1998) 478–480.
- [2] V. García-Mediavilla, I. Crespo, P.S. Collado, A. Esteller, S. Sánchez-Campos, M.J. Tuñón, J. González-Gallego, The anti-inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein, and down-regulation of the nuclear factor kappaB pathway in Chang liver cells, *Eur. J. Pharmacol.* 557 (2007) 221–229.
- [3] T.P.T. Cushnie, A.J. Lamb, Antimicrobial activity of flavonoids, *Int. J. Antimicrob. Agents* 26 (2005) 343–356.
- [4] A.B. Hendrich, Flavonoid-membrane interactions: possible consequences for biological effects of some polyphenolic compounds, *Acta Pharmacol. Sin.* 27 (2006) 27–40.
- [5] G. Brahmachari, N.C. Mandal, S.K. Jash, R. Roy, L.C. Mandal, A. Mukhopadhyay, B. Behera, S. Majhi, A. Mondal, A. Gangopadhyay, Evaluation of the antimicrobial potential of two flavonoids isolated from *Linnophila* plants, *Chem. Biodivers.* 8 (2011) 1139–1151.
- [6] M.A. Alvarez, N.B. Debattista, N.B. Pappano, Antimicrobial activity and synergism of some substituted flavonoids, *Folia Microbiol.* 53 (2008) 23–28.
- [7] A. Basile, S. Giordano, J.A. López-Sáez, R.C. Cobiánchi, Antibacterial activity of pure flavonoids isolated from mosses, *Phytochemistry* 52 (1999) 1479–1482.
- [8] P.I. Oteiza, A.G. Erlejman, S.V. Verstraeten, C.L. Keen, C. Fraga, Flavonoid-membrane interactions: a protective role of flavonoids at the membrane surface? #233, *s. G. Clin. Dev. Immunol.* 12 (2005) 19–25.
- [9] H. Tsuchiya, M. Iinuma, Reduction of membrane fluidity by antibacterial sophoraflavanone G isolated from *Sophora exigua*, *Phytomedicine* 7 (2000) 161–165.
- [10] T.P.T. Cushnie, A.J. Lamb, Detection of galangin-induced cytoplasmic membrane damage in *Staphylococcus aureus* by measuring potassium loss, *J. Ethnopharmacol.* 101 (2005) 243–248.
- [11] H. Tsuchiya, M. Sato, T. Miyazaki, S. Fujiwara, S. Tanigaki, M. Ohyama, T. Tanaka, M. Iinuma, Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*, *J. Ethnopharmacol.* 50 (1996) 27–34.

Table 3

Calculated results using the QSAR model.

	Flavonoids	ClogP	C3	pMIC <sub>50</sub> (expt)	pMIC <sub>50</sub> (pred)	Residue (pred)	
Training set	Chrysin	3.56	−0.159	3.84	3.83	0.01	
	Kaempferol	2.08	0.217	4.06	4.09	−0.03	
	Quercetin	1.50	0.217	3.93	3.82	0.11	
	Baicalein	3.00	−0.158	3.58	3.57	0.01	
	Tangeritin	2.80	−0.165	3.43	3.46	−0.03	
	5,6,7,4'-tetramethoxyflavone	2.39	−0.167	3.34	3.26	0.08	
	Daidzein	2.08	0.014	3.33	3.57	−0.24	
	Puerarin	0.02	0.015	2.44	2.61	−0.17	
	Genistin	0.91	0.002	3.26	2.99	0.27	
	Test set	Luteolin	2.31	−0.160	3.63	3.24	0.39
		Ononin	0.77	−0.159	2.58	2.52	0.06

- [12] L.E. Alcaráz, S.E. Blanco, O.N. Puig, F. Tomás, F.H. Ferretti, Antibacterial activity of flavonoids against methicillin-resistant *Staphylococcus aureus* strains, *J. Theor. Biol.* 205 (2000) 231–240.
- [13] R. dos Santos, C.A. Kuhn, R.A. Yunes, Molecular structure and QSAR study on antispasmodic activity of some xanthoxyl derivatives, *Arch. Pharm.* 339 (2006) 227–237.
- [14] D. de Sousa Neto, M. Tabak, Interaction of the meso-tetrakis (4-N-methylpyridyl) porphyrin with gel and liquid state phospholipid vesicles, *J. Colloid Interface Sci.* 381 (2012) 73–82.
- [15] G. Mandalari, R.N. Bennett, G. Bisignano, D. Trombetta, A. Saija, C.B. Faulds, M.J. Gasson, A. Narbad, Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel, a byproduct of the essential oil industry, *J. Appl. Microbiol.* 103 (2007) 2056–2064.
- [16] D. Wrobel, M. Ionov, K. Gardikis, C. Demetzos, J.-P. Majoral, B. Palecz, B. Klajnert, M. Bryszewska, Interactions of phosphorus-containing dendrimers with liposomes, *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1811 (2011) 221–226.
- [17] A. Arora, T.M. Byrem, M.G. Nair, G.M. Strasburg, Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids, *Arch. Biochem. Biophys.* 373 (2000) 102–109.
- [18] S. Zhang, L. Wei, K. Bastow, W. Zheng, A. Brossi, K.-H. Lee, A. Tropsha, Antitumor agents 252. Application of validated QSAR models to database mining: discovery of novel tylophorine derivatives as potential anticancer agents, *J. Comput. Aided Mol. Des.* 21 (2007) 97–112.
- [19] G. Sichel, C. Corsaro, M. Scalia, A.J. Di Bilio, R.P. Bonomo, In vitro scavenger activity of some flavonoids and melanins against O<sub>2</sub>-dot, *Free Radic. Biol. Med.* 11 (1991) 1–8.
- [20] E. Tripoli, M.L. Guardia, S. Giammanco, D.D. Majo, M. Giammanco, Citrus flavonoids: molecular structure, biological activity and nutritional properties: a review, *Food Chem.* 104 (2007) 466–479.
- [21] H.-Q. Li, L. Shi, Q.-S. Li, P.-G. Liu, Y. Luo, J. Zhao, H.-L. Zhu, Synthesis of C(7) modified chrysin derivatives designing to inhibit  $\beta$ -ketoacyl-acyl carrier protein synthase III (FabH) as antibiotics, *Bioorg. Med. Chem.* 17 (2009) 6264–6269.
- [22] L. Liu, X. Xu, D. Cheng, X. Yao, S. Pan, Structure–activity relationship of citrus polymethoxylated flavones and their inhibitory effects on *Aspergillus niger*, *J. Agric. Food Chem.* 60 (2012) 4336–4341.
- [23] H. Tsuchiya, Structure-dependent membrane interaction of flavonoids associated with their bioactivity, *Food Chem.* 120 (2010) 1089–1096.
- [24] N. Poklar Ulrih, A. Ota, M. Šentjurc, S. Kure, V. Abram, Flavonoids and cell membrane fluidity, *Food Chem.* 121 (2010) 78–84.
- [25] B. Łania-Pietrzak, A.B. Hendrich, J. Żugaj, K. Michalak, Metabolic O-demethylation does not alter the influence of isoflavones on the biophysical properties of membranes and MRP1-like protein transport activity, *Arch. Biochem. Biophys.* 433 (2005) 428–434.
- [26] J. Londoño, T.B. Creczynski-Pasa, V.R. de Lima, M.A.S. Silva, F.S. Murakami, C. Jaramillo, J.R. Ramirez-Pineda, Clean recovery of flavonoids from citrus peel: studying lipid–flavonoid interactions as a tool for understanding the antioxidant activity, *Planta Med.* 74 (2008) S183.
- [27] H. Tsuchiya, M. Nagayama, T. Tanaka, M. Furusawa, M. Kashimata, H. Takeuchi, Membrane-rigidifying effects of anti-cancer dietary factors, *Biofactors* 16 (2002) 45–56.
- [28] V. Agrawal, S. Srivastava, P. Khadikar, QSAR study on phosphoramidothioate (Ace) toxicities in housefly, *Mol. Divers.* 8 (2004) 413–419.
- [29] J. Lameira, C.N. Alves, V. Moliner, E. Silla, A density functional study of flavonoid compounds with anti-HIV activity, *Eur. J. Med. Chem.* 41 (2006) 616–623.
- [30] C. Hansch, W.E. Steinmetz, A.J. Leo, S.B. Mekapati, A. Kurup, D. Hoekman, On the role of polarizability in chemical–biological interactions, *J. Chem. Inf. Comput. Sci.* 43 (2002) 120–125.
- [31] K. Osawa, H. Yasuda, T. Maruyama, H. Morita, K. Takeya, H. Itokawa, Isoflavonones from the heartwood of *Swartzia polyphylla* and their antibacterial activity against cariogenic bacteria, *Chem. Pharm. Bull.* 40 (1992) 2970–2974.
- [32] M.M. Cowan, Plant products as antimicrobial agents, *Clin. Microbiol. Rev.* 12 (1999) 564–582.
- [33] M. Sato, S. Fujiwara, H. Tsuchiya, T. Fujii, M. Iinuma, H. Tosa, Y. Ohkawa, Flavones with antibacterial activity against cariogenic bacteria, *J. Ethnopharmacol.* 54 (1996) 171–176.
- [34] M. Kamihira, H. Nakazawa, A. Kira, Y. Mizutani, M. Nakamura, T. Nakayama, Interaction of Tea catechins with lipid bilayers investigated by a quartz–crystal microbalance analysis, *Biosci. Biotechnol. Biochem.* 72 (2008) 1372–1375.
- [35] J.S. Dambolena, J.A. Zygodlo, H.R. Rubinstein, Antifumonisin activity of natural phenolic compounds: a structure–property–activity relationship study, *Int. J. Food Microbiol.* 145 (2011) 140–146.