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3D-QSAR Modeling of Anti-oxidant Activity of some Flavonoids

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The anti-oxidant activities for a diverse set of flavonoids as TEAC (Trolox equivalent anti-oxidant capacity) assay were subjected to 3D-QSAR (3-dimensional quantitative structural-activity relationship) studies using CoMFA (comparative molecular field analysis) and CoMSIA (comparative molecular similarity indices analysis). The obtained results indicated superiority of CoMSIA model over CoMFA model. The best CoMSIA model was developed by using hydrogen-bond donor (H-bond donor) and electrostatic field components. This model gave the cross-validated correlation coefficient, Q^2 = 0.512, correlation coefficient, R^2 = 0.950, standard error of prediction, SE = 0.284, and F = 47.3, for training set, and R^2 = 0.922 and SE = 0.286, for test set indicating robustness and high prediction power of the developed model. The contour maps of electrostatic and H-bond donor fields of CoMSIA model provide interpretable and fruitful relationship between chemical structures and their anti-oxidant activities giving useful insight for designing new compounds with higher activity.

Keywords: 3D-QSAR, Anti-oxidant activity, CoMSIA, CoMFA, Flavonoids

INTRODUCTION

Flavonoids are a broad class of low molecular weight, secondary plant phenolics that are characterized by the flavan nucleus. In the human diet, they are most concentrated in fruits, vegetables, teas and cocoa [1]. Flavonoids show many biological and pharmacological effects [2] and have been used as anti-inflammatory [1,3], anti-allergic [3], anti-microbial [1], estrogenic [1,4] anti-HIV [5,6] and anti-cancer agents [7,8]. Recent interests in natural anti-oxidants have been stimulated by potential health benefits arising from the anti-oxidant activity of flavonoids [9-11]. Imbalance between generation and elimination of reactive oxygen species (ROS) such as peroxide, peroxyl radicals and hydroxyl radicals, leading to enhanced ROS level, may result in oxidative stress. Anti-oxidants can protect cells against damaging effects through formation of phenoxy radicals, which combine with ROSs, and terminate the unwanted free radical chain reaction in cells [12].

Anti-oxidants prevent some serious diseases like cancer, diabetes and immunodeficiency due to their capacity to inhibit oxidation of lipids, proteins and nucleic acids [13,14]. Flavonoids like many other poly phenols are very effective radical scavengers (chain-breaking antioxidants) since they donate easily their hydrogens or electrons to free radicals. Cytochromes P450 (CYPs), as hemoproteins, are the terminal oxidase enzymes in electron transfer chains. Hydroxylation of flavonoids by CYP1A isozymes yields dihydroxylated derivatives that retain the flavan nuclear structure. Flavonoids can inhibit various types of P450 isozymes, including CYP1A. Interactions between flavonoids and cytochromes P450 (in oxidative stress), are a complex process and are reviewed elsewhere [17]. The propensity of a flavonoid to inhibit free-radical mediated events is governed by its chemical structure. Structure-activity relationship (SAR) studies of flavonoids have indicated the importance of the number and location of the

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phenolic OH groups on their effective radical scavenging activity [15-17]. Moreover, it was indicated that methoxy groups introduce unfavorable steric effects and decrease anti-oxidant activity, whereas double bonds and carbonyl functional groups in the heterocycles or polymerization of the nuclear structure increase activity by affording a more stable flavonoid radical through conjugation and electron delocalization [16].

There are some methods to evaluate anti-oxidant activities of chemicals, *in vitro,* such as TEAC, DPPH and TRAP test. Trolox equivalent anti-oxidant capacity (TEAC) assay is one of the common experimental methods measuring the concentration of Trolox solution (in molar) with an equivalent anti-oxidant potential to a standard concentration of the compound under investigation. TEAC reflects the H-donating ability of anti-oxidants to scavenge the radical cation 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)(ABTS). Therefore H-donating ability of anti-oxidants can be measured quantitatively by spectrophotometric methods [18,19].

Due to the correlation between the structure and anti-oxidant activity of organic compounds, QSAR approaches could be used for modeling, clarifying the radical scavenging mechanisms of anti-oxidants, and predicting their activity without using any chemicals and instruments [19]. In addition, developing QSAR models is fruitful to design and synthesize the specific compounds with a considerable anti-oxidant activity. In the recent years, researchers have used various descriptors for modeling and prediction of anti-oxidant activities of some chemicals. For example, Abreu *et al*. reported some QSAR models for predicting the radical scavenging capacity of diaryl benzothiophens derivatives using the partial least squares (PLS) method. They indicated that presence of i. electronegative and polarizable atoms in the structure could increase anti-oxidant activity of the studied chemicals [21]. In addition, we performed QSAR studies on the radical scavenging activities of various types of anti-oxidant families using multiple linear regressions (MLR) and a multilayer perceptron neural network (MLP-NN), separately. The obtained model had the statistics of correlation coefficient R^2 = 0.968 and cross-validated correlation coefficient Q^2 = 0.898 for the MLP-NN model, R^2 = 0.902 and Q^2 = 0.862 for the MLR model. The obtained result indicated that the proposed models can be successfully used for prediction of radical scavenging activities of new antioxidants [21]. Recently, application of 3D-QSAR modeling has been rapidly increased. Classical QSAR correlates biological activities of chemicals with their physicochemical properties or indicator variables encoding certain structural features of a molecule [22,23], while 3D-QSAR approaches consider interaction of compounds with their surrounding media besides structural, geometrical and electronic parameters, so represent better and interpretable models. Comparative molecular field analysis (CoMFA) and comparative molecular similarity analysis (CoMSIA) are two effective computer-aided 3D-QSAR techniques deriving a correlation between a set of biologically active molecules and their 3D shape, electrostatic and hydrogen bonding characteristics and employ both interactive graphics and statistical techniques. There are some reports about prediction of anti-oxidant activity of chemicals by using CoMFA and CoMSIA methods. For example, Ramalkshmi *et al*. developed some models by using CoMFA method to investigate the anti-oxidant capacity of polyphenols and reported R^2 = 0.84 [24]. In addition Jing *et al*. subjected 21 anthocyanins to CoMSIA and CoMFA analyses and reported R^2 = 0.998 and R^2 = 0.997 for these models, respectively [25]. In another work, Chen *et al*. performed the CoMSIA analysis on a set of 27 curcumin analogues with the radical scavenging activities resulting in a significant Q^2 value of 0.784 for CoMSIA. [26]. The main aim of the present work is developing the 3D-QSAR models in order to correlate anti-oxidant activity of a diverse set of flavonoids to their structural parameters and driving models that can predict the anti-oxidant activities of other flavonoids.

The data set consists of experimental anti-oxidant activities of a diverse set of flavonoid families including flavone, flavonol, flavanone, anthocyanin and anthocyanidin taken from the work of Rice-Evance [19]. The names of the studied flavonoids and their experimental and predicted values of TEAC (mM) are shown in Table 1.

EXPERIMENTAL

Data Set

The anti-oxidant activities of these chemicals were

Table 1. The Experimental, CoMSIA Estimated, and their Residual Values of TEAC (mM)

No.	Name	Experimental	CoMSIA	Residual
1	Epicatechingallate	4.90	5.02	0.12
2	Epigallocatechingallate	4.80	4.67	-0.13
3	Quercetin	4.70	4.39	-0.31
4[a]	Delphinidin	4.44	3.66	-0.78
5	Cyanidin	4.40	3.92	-0.48
6	Epigallocatechin	3.80	3.45	-0.35
7[a]	Keracyanin	3.25	3.08	-0.17
8	Myricetin	3.10	3.42	0.32
9[a]	Gallic acid	3.01	3.21	0.20
10	Idein	2.90	3.51	0.61
11	Morin	2.55	2.73	0.18
12	Gallic acid methyl ester	2.44	2.29	-0.15
13	Catechin	2.40	2.85	0.45
14	Rutin	2.40	2.14	-0.26
15	Apigenidin	2.35	2.15	-0.20
16[a]	Peonidin	2.22	2.33	0.11
17	Luteolin	2.10	2.48	0.38
18	Malvidin	2.06	1.97	-0.09
19	Taxifolin	1.90	2.12	0.22
20[a]	Oenin	1.78	1.58	-0.20
21	Luteolin-4'-glucoside	1.74	1.82	0.08
22	Naringenin	1.53	1.69	0.16
23	Apigenin	1.45	1.69	0.24
24	Chrycin	1.43	1.14	-0.29
25	Hesperitin	1.37	1.29	-0.08
26	Kaempferol	1.34	1.24	-0.10
27[a]	Pelagonidin	1.30	1.43	0.13
28	Hesperidin	1.08	1.14	0.05
29	Luteolin-3',7-glucoside	0.79	0.45	0.34
30	Narirutin	0.76	0.93	0.17

[a]Refers to the chemicals in the test set.

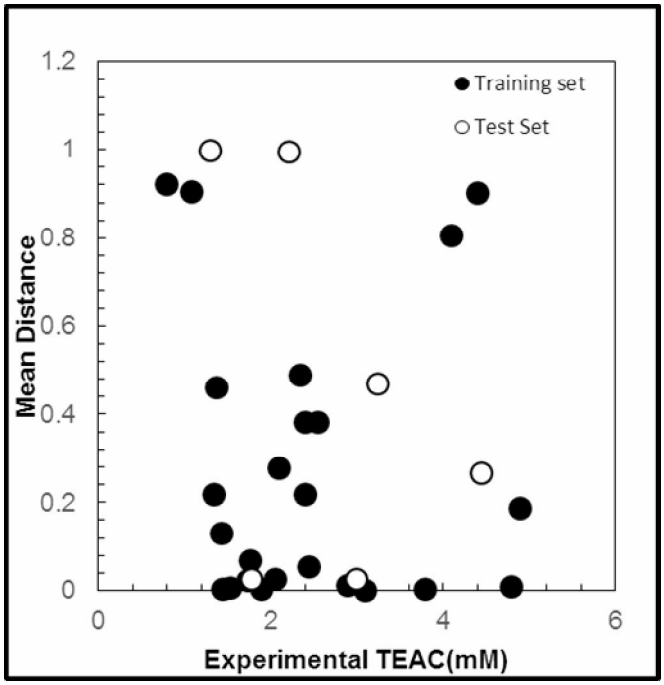

Fig. 1. Scatter plot of normalized mean distance of chemicals *versus* experimental anti-oxidant values.

determined by TEAC assay and values were reported as the concentration of Trolox solution. The values of TEAC were ranged from 0.76-4.9 for narirutin and epicatechingallate, respectively.

The data set was divided into training and test set based on y sorting method (24 molecules in training set and 6 molecules in the test set). In this way the structural diversity and wide range of activity in dataset were included in test set. To explore the way of molecules to cover the determined structural space, diversity analysis was performed for the data set based on decrypted algorithm by Luan *et al.* [27] and the calculated mean distances of samples in descriptors space were plotted versus the corresponding experimental data (Fig. 1). As show in this figure, the structures of the compounds are diverse in both sets and the training set with a broad representation of the chemistry space was adequate to ensure models' stability and the diversity of test set can prove the predictive capability of the model. The 3D structures for all compounds were designed and their geometry were optimized using Hyperchem7.

CoMFA and CoMSIA analyses

CoMFA is a versatile and powerful method in rational drug design and related applications. CoMFA samples the steric (Lennard-Jones) and electrostatic (Coulombic) fields surrounding a set of ligands and constructs a 3D-QSAR model by correlating these 3D steric and electrostatic fields with the corresponding biological activities. Partial least squares (PLS) analysis with a cross validation procedure is employed to select relevant components from the large set of CoMFA data to build up the best QSAR equation [28].

CoMSIA is an extension of CoMFA methodology and differs only in the implementation of the fields [29,30]. In CoMSIA approach, hydrophobic, H-bond donor and H-bond acceptor similarity fields are calculated in addition to the steric and electrostatic fields, providing a better interpretation of the correlation between the 3D-structures of the studied molecules and their activities. These fields are evaluated by PLS analysis similar to CoMFA. The obtained CoMFA and CoMSIA contour maps are used as visual guides for designing new and more potent radical scavenging compounds.

All molecular modelings and 3D-QSAR calculations were constructed using the molecular modeling software package SYBYL-X 1.1 (Tripos Associates, Saint Louis, MO). Partial charge for each atom in all molecules was calculated by Gasteiger-Huckel charge with distance dependent dielectric. The energy minimizations were performed using Tripos force field and conjugate gradient method with convergence criterion of 0.001 kcal mol^{-1} and maximum iteration of 5000. Structural alignment is the most crucial step in the CoMFA and CoMSIA study. The optimized molecules were aligned based on common structure by Distill method as databases align function. The chemical having the most functional groups (Hesperidin) was selected as template structure [31-34].

In CoMFA, a grid box with grid spacing of 2.0 Å was generated around molecules in the training set based on the molecular volume of the structures. A sp^3-carbon atom with a +1.0 unit charge was selected as a probe atom and the cut off for both steric and electrostatic fields was set to 30 kcal mol^{-1}. CoMSIA fields were explored using common probe atom and a box similar to CoMFA analysis. Attenuation factor was set to 0.4. PLS methodology was used for extraction latent variables of the obtaining fields and developing the 3D-QSAR models. For reducing noise and improving efficiency of CoMFA and CoMSIA models, the filtering columns were set to 2.0 and 4.1 kcal mol^{-1}, respectively.

The optimum number of the PLS components (N) used to derive models was defined as the number of the components leading to the highest Q^2 and the lowest SE. The Q^2 values were derived after "leave-one-out" cross-validation test. In this technique one compound is eliminated from the data set randomly in each cycle and the model is built using the rest of the compounds. The model thus formed is used for predicting the activity of the eliminated compound. This process is repeated until all the compounds are eliminated once. Based on the predicting ability of the model, the Q^2 for the model is determined. This parameter is expressed as:

$$Q^2 = 1 - \frac{\sum (Y_{Exp} - Y_{Pre})^2}{\sum (Y_{Exp} - \overline{Y}_{Train})^2} \quad (1)$$

In Eq. (1), Y_{Exp} and Y_{Pre} are the experimental and predicted anti-oxidant activity values, respectively and $\overline{Y}_{Train}$ is the mean of experimental value of the training set compounds. The values more than 0.5 indicate model's robustness.

Moreover the predictive capacity of QSAR models could be judged based on R^2 values, which is calculated according to the following equation [35]:

$$R^2 = 1 - \frac{\sum (Y_{Exp} - Y_{Pre})^2}{\sum (Y_{Exp} - \overline{Y}_{Train})^2} \quad (2)$$

In the above equation, Y_{Exp} and Y_{Pre} indicate the experimental and predicted anti-oxidant activity values of the test set compounds, respectively. The statics of Q^2 defines the goodness of prediction, whereas the R^2 indicates well fitting of the QSAR model. Also, Schüürmannet al. proposed the calculation of Q^2_{Ext} based on the prediction of test compounds from the following equation [34]:

$$Q^2_{Ext^2} = 1 - \frac{\sum (Y_{Exp} - Y_{Pre})^2}{\sum (Y_{Exp} - \overline{Y}_{Test})^2} \quad (3)$$

where Y_{Exp} and Y_{Pre} indicate experimental and predicted anti-oxidant activity values, respectively, and $\overline{Y}_{Test}$ refers to the mean experimental anti-oxidant activity of the test set compounds. The value of Q^2_{Ext} differs from R^2 only in the mean used in the denominator for calculation of the external predictive parameters.

RESULTS AND DISCUSSION

The CoMFA analysis was performed to derive 3D-QSAR model using electrostatic and steric fields. The performance of the models was evaluated based on the statistically significance of models by considering the highest values of Q^2 and R^2 and the lowest SE. Some statics of developed CoMFA model based on the six PLS latent variables are $Q^2 = 0.213$, $R^2 = 0.85$, SE = 0.263 for training set, and $R^2 = 0.083$ and SE = 1.31 for test set. As can be seen, due to consideration of only steric (26.1%) and electrostatic (73.9%) fields, these statistical parameters indicate that CoMFA model was not successful in modeling and prediction of anti-oxidant activities of the studied flavonoids.

Table 2. The Statistical Parameters of CoMSIA Models

Model No.	Model name	Q^2	R^2	SE	Fraction of fields				
					Steric	Electrostatic	Hydrophobic	H-bond donor	H-bond acceptor
1	CoMSIA-ED	0.514	0.950	0.284	-	0.317	-	0.683	-
2	CoMSIA-EH	0.382	0.390	0.850	-	0.587	0.403	-	-
3	CoMSIA-DA	0.412	0.916	0.403	-	-	-	0.712	0.288
4	CoMSIA-HD	0.507	0.815	0.508	-	-	0.285	0.715	-
5	CoMSIA-EDA	0.504	0.873	0.48	-	0.290	-	0.480	0.230
6	CoMSIA-EHD	0.493	0.850	0.504	-	0.280	0.200	0.520	-
7	CoMSIA-SHD	0.462	0.930	0.382	0.193	-	0.180	0.627	-
8	CoMSIA-EHDA	0.385	0.782	0.530	-	0.305	0.165	0.244	0.256
9	CoMSIA-SEHDA	0.198	0.071	0.851	0.08	0.220	0.199	0.290	0.200

In the next step, some CoMSIA models were developed using electrostatic, steric, hydrophobic, H-bond donor and H-bond acceptor fields. Various combinations of these fields to generate hybrid models that establish these models. The results of these models are indicated in Table 2. As can be seen in this table, the best CoMSIA model is developed based on the combination of electrostatic and H-bond donor fields (CoMSIA-ED) (No. 1). The model was built by six PLS latent variables. The predictive ability of this CoMSIA model was surveyed using the test set which provided the statistics of R^2 = 0.922, SE = 0.286 and Q^2_{Ext} = 0.638 indicating the high predictive ability of the developed model. The percentage of the variance explained by H-bond donor and electrostatic fields are 68.3% and 31.7%, respectively.

Comparison of the statistics of CoMFA and CoMSIA models indicate the superiority of CoMSIA model over CoMFA ones. CoMFA analysis does not consider H-bond interactions whereas according to the results of the best CoMSIA model, this field has highest contribution for developing a reliable model, which can better explain the relation between anti-oxidant activity of flavonoids and their structure. Therefore, this CoMSIA model was used for explaining the QSAR study and predicting the anti-oxidant activities of the examined chemicals. The experimental and predicted values of TEAC and their residuals (the differences between the experimental and the predicted values) for the best CoMSIA model are shown in Table 1.

The scattered plot of the experimental and predicted values of TEAC for molecules in the training and test sets are depicted in Fig. 2, which indicate a good correlation between these values. Figure 3 shows the plot of the residuals against the experimental values of the anti-oxidant activities of the studied chemicals. The random distribution

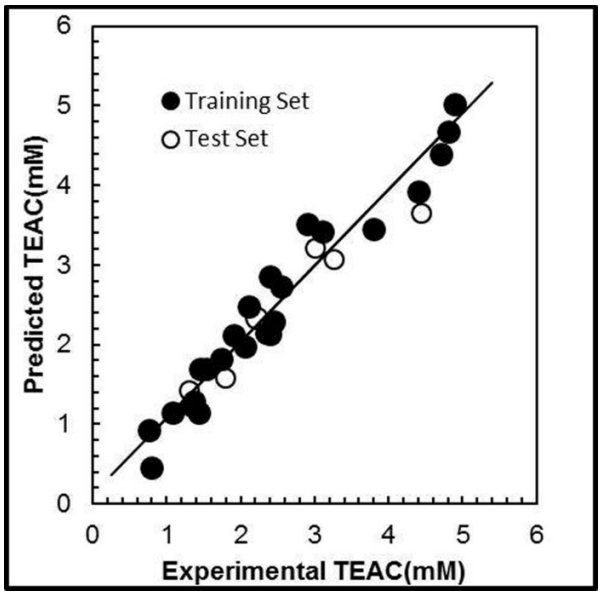

Fig. 2. The CoMSIA plot of the predicted TEAC versus experimental values.

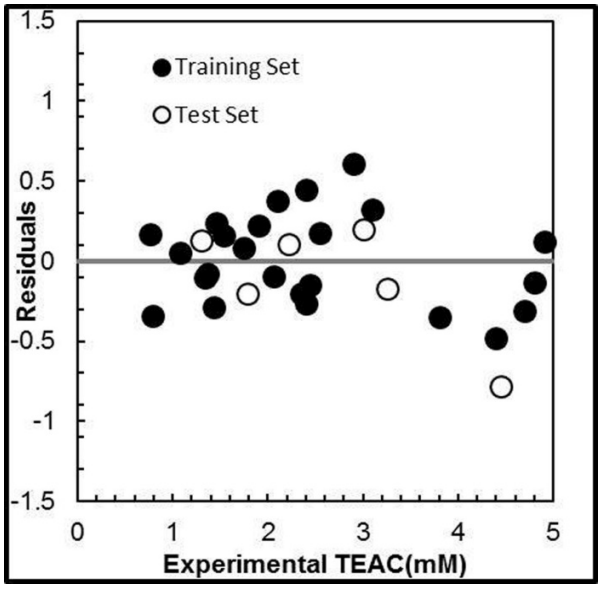

Fig. 3. Plot of residuals *versus* experimental values of TEAC.

of the residuals on both sides of zero line indicates that there is not any systematic error in the developed mode. In the present work, the CoMFA and CoMSIA of anti-oxidant activities of this group of flavonoids have been modeled for the first time.

CoMSIA Contour Maps

The results of CoMSIA model are usually represented as 3D contour maps. These contour maps are useful for exploring and visualizing structure-activity relationship. The contour maps for the best CoMSIA model are shown in

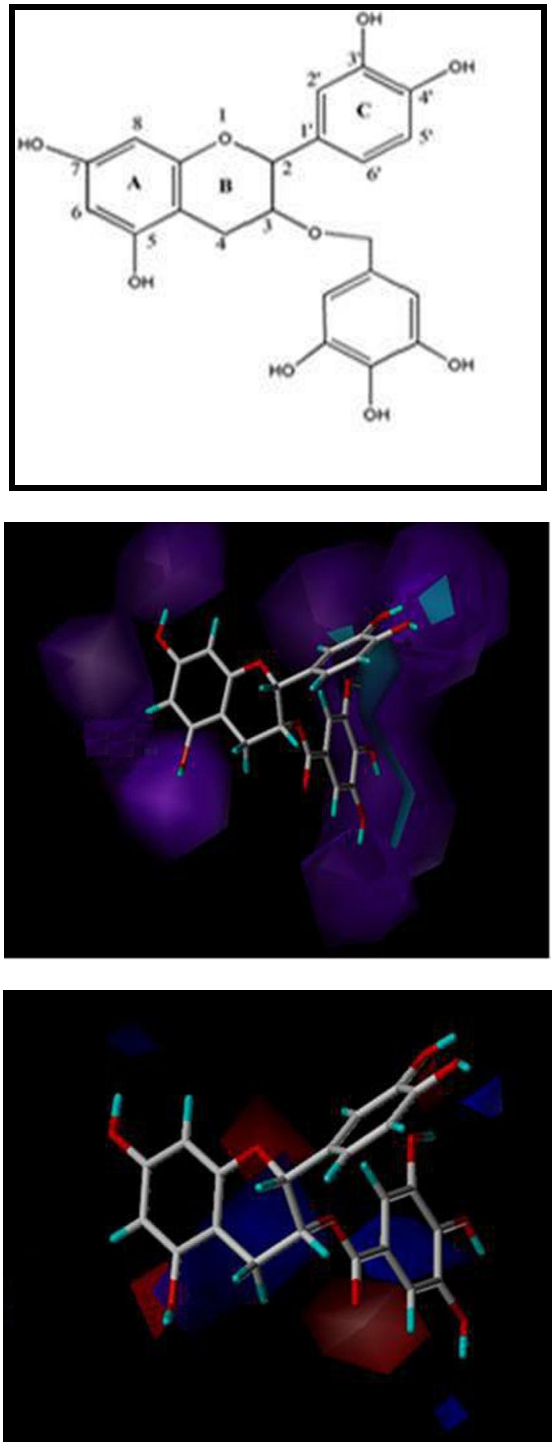

Fig.4. (a) Epicatechingallate as the reference chemical for representing field. CoMSIA hydrogen bond donor contour map; (b) cyan contours represent areas where hydrogen bond donor group is favored and the purple contours shows unfavorable areas for substitution of hydrogen bond donor group. CoMSIA electrostatic contour map; (c) the blue contour indicates region where electropositive substituent is favored and red contour refers to region where electronegative substituent is favored.

Fig. 4 by using the structure of the most active molecule (epicatechingallate (TEAC 4.9 mM)) as the reference structure (Fig. 4a). H-bond donor contour map (Fig. 4b) shows a big purple region on the rings C and A, which are considered as the unfavorable regions for H-bond donor groups for most of the flavonoids. For example, in epigallocatechingallate (TEAC 4.8 mM), substitution of a hydroxyl group on ring C leads to decrease the anti-oxidant activity in comparison with epicatechingallate. Similar effects are observed by insertion of a third hydroxyl group on ring C in myricetin (TEAC 3.1 mM) decreasing its activity in comparison with quercetin (TEAC 4.7 mM). However, a small cyan region near ring C indicates that substitution of H-donor groups on this ring enhances anti-oxidant activity for some flavonoids. This effect is evidenced by comparison of epigallocatechin (TEAC 3.8mM) with catechin (TEAC 2.8 mM). Comparison of the naringenin (TEAC 1.5 mM) structure with narirutin (TEAC 0.76 mM) shows that replacement of glycoside group (with several hydroxyl groups) in the position 7 of ring A has a strong influence on decreasing anti-oxidant activity of narirutin. Similar effects are observed when methoxy group in ring A of hesperetin (TEAC 1.4 mM) is replaced with a glycoside group in hesperidin (TEAC 1.08 mM).

The electrostatic contour map of CoMSIA model in Fig. 4c suggests that for increasing anti-oxidant activities of flavonoids, electronegative substituents (the red contours) should be located on the position 1 of the ring B and position 8 of the ring A for increasing anti-oxidant activities of flavonoids. This figure also indicates that electropositive substituents (the blue contours) should be located on the position 3 of ring B and the position 4' of ring C. For example, glycosylation of the position 3 of ring B in epigallocatechin (TEAC 3.8 mM) enhances activity of epigallocatechingallat to TEAC = 4.8 mM. In addition, insertion of a hydroxyl group on the position of 4' in ring C enhances the TEAC value of 2.5 mM for morin to 3.12 mM for myricetin.

CONCLUSIONS

Most of flavonoid effects are originated from the ability to inhibit lipid peroxidation, chelate redox-active metals and attenuate other processes involving reactive oxygen species as anti-oxidants. The anti-oxidant activities of flavonoids are affected by their structural properties, so developing 3D-QSAR models could be useful. In this study, CoMSIA analysis has been successfully applied to develop an interpretable 3D-QSAR model for prediction of anti-oxidant activity for a set of flavonoids. The obtained statistical parameters illustrate that the established CoMSIA model based on electrostatic and H-bond donor fields, is robust and reliable. The contour maps of fields provide enough information to understand relationships between structural features and anti-oxidant activities of the studied chemicals. Consequently, the present study gives the meaningful structural insights into possible modifications of flavonoids improving anti-oxidant activities for the future works.

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