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THEORETICAL & COMPUTATIONAL CHEMISTRY | RESEARCH ARTICLE

Prediction of anticancer activities of cynaroside and quercetin in leaf of plants *Cynara scolymus* L and *Artocarpus incisa* L using structure–activity relationship

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Abstract: Natural products from plants are an alternative resource in the search for anti-cancer drugs and can have a direct impact on eliminating cancer cells and also reduce cancer side effects. Recently, we have isolated a few flavonoid quercetin and cynaroside from leaf of *cynara scolymus* L and *artocarpus incisa* L in Vietnam, with cytotoxic activity relatively strong in Hela cancer cells. The flavonoid compound is a search target, research and development of anti-cancer agents in clinical use. To clarify the important nature of the activity, the subject QSAR studies on cancer Hela cell line use the multiple linear regression (MLR) gradually, partial least square regression (PLS) and artificial neural network. The MLR and PLS models showed good correlation values of $R^2 = 0.938$, $R^2_{pred} = 0.903$, and $R^2 = 0.943$, $R^2_{pred} = 0.912$, respectively. The MLR model shows the level of importance of atomic charge descriptors. Also, artificial neural network architecture I(6)-HL(4)-O(1) is built with RMSE = 0.00345, $R^2 = 0.993$, $R^2_{pred} = 0.971$ using the atomic charge descriptors selected in the MLR model such as neurons of input layer and the anti-cancer activity such as neuron of output layer. The anti-cancer activities of the flavonoids and isoflavonoids in the test group and

Bui Thi Phuong Thuy

ABOUT THE AUTHORS

Scientific research for us is important and necessary in order to improve and expand the knowledge. Since 1990s, our role of scientific research had become to be important in an university, so we had some projects in QSAR field as:

Studying the process of extracting and purifying the total alkaloid extract from Dichroa febrifuga Lour and the screening process for alkaloid from the extract of leaves of Dichroa febrifuga Lour. Using artificial neural network for screening process of alkaloids from Dichroa febrifuga Lour. During this duration, we had a number of scientific works in the field of computer applications in chemistry. For example, the QSAR in design for new drugs.

In 1990s, we had a project of Ministry of Education and Training in the field of computer applications in the chemistry with the topic: Study of quantitative relationship between the structure and activity of the group antimalarial compounds, anti-cancer, anti-HIV, anti-fungal, and anti-bacterial.

PUBLIC INTEREST STATEMENT

The studies for *Cynara scolymus L* showed that its ingredients from flowers, leaves, stems, roots are very effective in healing and for food. The flavonoid compounds were extracted from *Cynara scolymus L* working in the treatment of some diseases such as liver, bile, cardio, antioxidants and reduce cholesterol in blood, especially HIV anti-virus (Loi, 2006).

In Vietnam, *Artocarpus Incisa L* scattered only has been planted in the orchard of the Vietnamese family. *Artocarpus Incisa L* is the kind of big trees. The compound groups in leaves *Artocarpus Incisa L* were determined quantitatively; the results showed that the leaves of *Artocarpus Incisa L* contain substances: flavonoids, saponins, anthranoid, tannin, reducing sugar, acid amines and polysaccharides. The water extract from the leaves of *Artocarpus Incisa L* showed that the blood pressure is lowered and decreased heart rate in mice. The water extract from leaves of *Artocarpus Incisa L* effects on cancer cells of the pancreas (Loi, 2006).

compounds *quercetin* and *cynaroside* isolated from *cynara scolymus* L and *artocarpus incisa* L are compared with experimental data and those from references.

Subjects: Computational and Theoretical Chemistry; Medicinal & Pharmaceutical Chemistry; Organic Chemistry

Keywords: $QSAR_{MLR}$ and $QSAR_{PLS}$ model; neural network $QSAR_{ANN}$ model; anticancer activities Hela

1. Introduction

Natural products from plants are of interest in searching for new anti-cancer drugs and can have a direct effect on HeLa cancer cells and reduce side effects. Recently, we have isolated a few flavonoids from leaf of *cynara scolymus* L and *artocarpus incisa* L (Loi, 2006) and tested their *in vitro* activities pointed out the relatively strong impacts for cancer cells HeLa (Singh, Kaur, & Silakari, 2014). These flavonoids from leaf of *cynara scolymus* L (Apóstolo, Brutti, & Llorente, 2005; Fritsche, Beindorff, Dachtler, Zhang, & Lammers, 2002; Zhu, Zhang, & Lo, 2004) and *artocarpus incisa* L (El Senousy, Farag, Al-Mahdy, & Wessjohann, 2014) were also tested biologically in the treatment of some diseases such as liver, bile, cardio, antioxidants and reduce cholesterol in blood, especially HIV anti-virus (Abbasi & Samadi, 2014; Moreira, Castelo-Branco, Monteiro, Tavares, & Beltramini, 1998). Flavonoids are polyphenolic compounds in most plants (Mahapatra, Bharti, & Asati, 2015; Priyadarsini et al., 2010; Ziberna, Fornasaro, Čvorović, Tramer, & Passamonti, 2014). The flavonoids have been shown their activities and role of food within flavonoids in the cancer inhibition are widely studied (Gavin & Durako, 2012; Lee, Boyce, & Breadmore, 2012; Pawlikowska-Pawlęga et al., 2014).

In recent years, the methods of quantum chemistry calculations are widely applied to the study of chemical properties and seeking new drugs. The field of new drug design by computer tools has become an important tool nowadays. Study on quantitative relationships between structure and activity (QSAR) of natural compounds is of concern for new drug researchers and pharmaceutical manufacturing facilitators. In Vietnam, there are a number of works of scientists from universities and institutions published in the journal (Phuong Thuy & Tat, 2012a, 2012b). The previous studies of 3-aminoflavonoid substances have focused on the basis of semi-empirical calculation (Tat, 2009a). These studies have shown a way for designing new drugs efficiently with the assistance of computers. The QSAR model can be predicted the biological activity of new drugs from the atomic charges in the molecule. This method allows for the identification of an active-central location of molecule.

The set of flavones and isoflavones is known to have an important activity against cervical cancer cells (Chen, 2008; Liao et al., 2005; Liao, Chen, Qian, Shen, & Zheng, 2008). This group is currently of interest for researching in different directions such as the synthesis and metabolizing of natural products or extracting them from plants (Loi, 2006). The consideration of the quantitative relationship between the structure of flavones and isoflavones with activity against cancer is an important issue in searching for the flavone and isoflavone derivatives to be valid.

In this work, we report the use of semi-empirical quantum calculations and construction of quantitative structure–activity relationship (QSAR) models using 32 flavone and isoflavone derivatives (Chen, 2008; Liao et al., 2005, 2008). The flavones and isoflavones are constructed and optimized by means of molecular mechanics MM+. The atomic charge descriptors resulting from Parametric Model number 3 (PM3) method are used to build the multivariate QSAR models such as multiple linear regression (MLR), partial least squares regression (PLS), and artificial neural network (ANN). Anti-cancer activities GI_{50}/μM of flavones and isoflavones in the test group and the new flavonoids quercetin and cynaroside isolated from the leaves of *cynara scolymus* L and *artocarpus incisa* L are predicted from QSAR models and compared with those from experimental data.

2. Computational details

2.1. Materials and means

To ensure the accurate level of QSAR models, structural data and anticancer activities GI_{50}/μM for Hela cells (GI_{50} is the concentration for 50% of maximal inhibition of cell proliferation) for flavones and isoflavones are taken from the data source of Wang and et al. (Chen, 2008; Liao et al., 2005, 2008) as pointed out in Figure 1 and Table 1. The anti-cancer activities are transformed into following value pGI_{50}

$$pGI_{50} = -\log \quad GI_{50} \tag{1}$$

The atomic charge parameters on molecules are calculated by means of program HyperChem v8.0 (HyperChem Release 8.03, 2008). The multiple linear regression ($QSAR_{MLR}$) and the partial least squares regression $QSAR_{PLS}$ models are built with program Origin 2015 (Tat, 2009b). The artificial neural network ($QSAR_{ANN}$) models are constructed with Visual Gene Developer 1.7 (Jung & McDonald, 2011).

2.2. Isolated technology of quercetin and cynaroside

2.2.1. Chemicals and equipment

In this work, we use the chemicals and the equipment for isolating and purifying two flavonoids quercetin and cynaroside before determining the substance structures by ^{1}H-NMR and ^{13}C-NMR spectrum:

- Silica gel with the particle size in range 0.04–0.06 mm was used for ordinary and Rp18 phase chromatography.
- Thin-layer chromatography was implemented by the thin plate DC-Alufolien F254 (Merck) for the ordinary phase and Rp18 F254s (Merck) for the reverse-phase chromatography.
- Solvents were used for the isolation processes: hexane, petroleum ether, chloroform, methanol, ethyl acetate, ethanol, acetone, distilled water.
- Reagent was used to trace out the compound coloration on plate: using H_2SO_4/EtOH; $FeCl_3$/EtOH.
- UV handheld lamps, 254 and 365 nm UVITEC effect.
- Vacuum Evaporators Buchi-111.
- Water Bath cooker JULABO 461.
- Infrared heating equipment SCHOTT.
- Chromatography column with diameter range 2–5.5 cm.
- Analytical Balances AND HR 200.

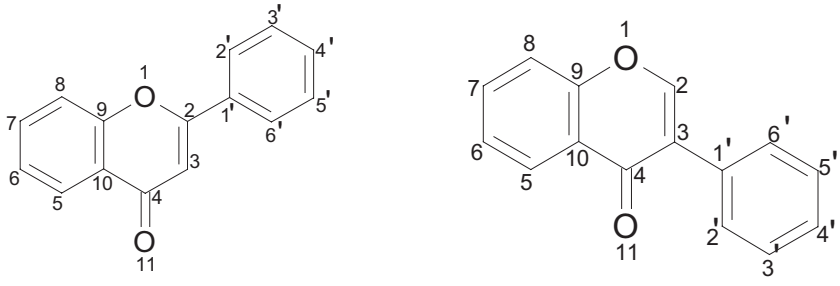
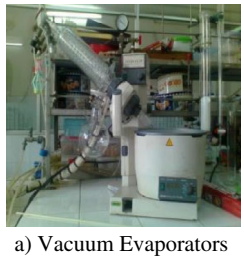


Figure 1. Molecular skeleton: (a) flavone and (b) isoflavone.

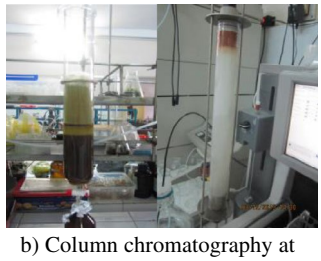
Table 1. Molecular structure and anticancer activities GI_{50} (μM) of flavones and isoflavones (Chen, 2008; Liao et al., 2005, 2008).

Substance		Name	Substitutive site	Substitutes *R*	GI_{50} (μM)
Training set for establishing QSAR models					
Fla1	1a-1	Flavone	C_3	$-OCH_2CCH_3=NOH$	2,0
Fla3	2a-3	Flavone	C_7	$-OCH_2CCH_3=NOH$	2,0
Isofla4	3a-4	Isoflavone	C_7	$-OCH_2CCH_3=NOH$	9,8
Fla5	4a	Flavone	C_3	$-OCH_2CCH_3=NOCH_3$	2.0
Fla6	5a	Flavone	C_3	$-OCH_2CCH_3=NOCH_3$	0.9
Fla7	6a	Flavone	C_7	$-OCH_2CCH_3=NOCH_3$	2.2
Isofla8	7a	Isoflavone	C_7	$-OCH_2CCH_3=NOCH_3$	8.5
Fla10	8a	Flavone	C_3	$-OCH_2C(4\text{-}F\text{-}C_6H_4)=NOH$	2,1
Fla11	9a	Flavone	C_3	$-OCH_2C(4\text{-}CH_3O\text{-}C_6H_4)=NOH$	2,0
Fla13	10a	Flavone	C_6	$-OCH_2C(4\text{-}F\text{-}C_6H_4)=NOH$	1,6
Fla14	11a	Flavone	C_3	$-OCH_2C(4\text{-}CH_3O\text{-}C_6H_4)=NOH$	1,0
Fla17	12a	Flavone	C_7	$-OCH_2C(4\text{-}CH_3O\text{-}C_6H_4)=NOH$	2,0
Isofla18	13a	Isoflavone	C_7	$-OCH_2C(C_6H_5)=NOH$	9,0
Isofla19	14a	Isoflavone	C_7	$-OCH_2C(4\text{-}F\text{-}C_6H_4)=NOH$	7,8
Isofla20	15a	Isoflavone	C_7	$-OCH_2C(4\text{-}CH_3O\text{-}C_6H_4)=NOH$	7,6
Fla21	16a	Flavone	C_3	$-OCH_2C(C_6H_5)=NOCH_3$	1,6
Fla22	17a	Flavone	C_3	$-OCH_2C(4\text{-}F\text{-}C_6H_4)=NOCH_3$	2,0
Fla23	18a	Flavone	C_3	$-OCH_2C(4\text{-}CH_3O\text{-}C_6H_4)=NOCH_3$	2,0
Fla24	19a	Flavone	C_6	$-OCH_2C(C_6H_5)=NOCH_3$	2,4
Fla25	20a	Flavone	C_6	$-OCH_2C(4\text{-}F\text{-}C_6H_4)=NOCH_3$	2,3
Fla26	21a	Flavone	C_6	$-OCH_2C(4\text{-}CH_3O\text{-}C_6H_4)=NOCH_3$	2,0
Fla27	22a	Flavone	C_7	$-OCH_2C(C_6H_5)=NOCH_3$	6,6
Fla28	23a	Flavone	C_7	$-OCH_2C(4\text{-}F\text{-}C_6H_4)=NOCH_3$	2,7
Fla29	24a	Flavone	C_7	$-OCH_2C(4\text{-}CH_3O\text{-}C_6H_4)=NOCH_3$	2,5
Isofla30	25a	Isoflavone	C_7	$-OCH_2C(C_6H_5)=NOCH_3$	8,2
Isofla31	26a	Isoflavone	C_7	$-OCH_2C(4\text{-}F\text{-}C_6H_4)=NOCH_3$	6,4
Test set for validating QSAR models					
Fla2	1b	Flavone	C_6	$-OCH_2CCH_3=NOH$	1,2
Fla9	2b	Flavone	C_3	$-OCH_2C(C_6H_5)=NOH$	1,8
Fla12	3b	Flavone	C_6	$-OCH_2C(C_6H_5)=NOH$	0,8
Fla15	4b	Flavone	C_7	$-OCH_2C(C_6H_5)=NOH$	2,0
Fla16	5b	Flavone	C_7	$-OCH_2C(4\text{-}F\text{-}C_6H_4)=NOH$	2,0
Isofla32	6b	Isoflavone	C_7	$-OCH_2C(4\text{-}CH_3O\text{-}C_6H_4)=NOCH_3$	7,3

Figure 2. Separate equipment for two flavonoids quercetin and cynaroside.



a) Vacuum Evaporators



b) Column chromatography at atmospheric and high pressure

c) Thin-layer chromatography

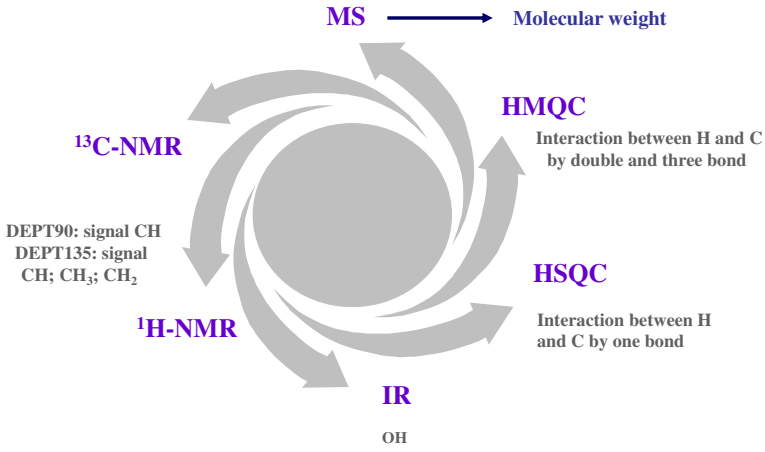


Figure 3. General diagram of structure identification for substances quercetin and cynaroside.

2.2.2. Isolation and identification of flavonoids

To isolate and purify the flavonoid compounds, we used the techniques of thin-layer and column chromatography, as exhibited in Figure 2. After isolating the compounds, they were identified with the structure using different spectrums:

- Melting temperature carried out on Electrothermal IA 9000 series, using unadjusted capillary.
- Column chromatography with silica gel for ordinary-phase, reverse-phase chromatography Rp 18 and Sephadex techniques combined with thin-layer chromatography.
- Substances were detected by ultraviolet light at wavelengths 254 and 365 nm or reagent used is liquid H_2SO_4/EtOH or $FeCl_3$/EtOH.
- Nuclear magnetic resonance spectrum (NMR) ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) implemented on Bruker AM500 FT-NMR Spectrometer (Figure 3).

2.3. Constructing QSAR models

The fitness models were proved using different validations. To validate QSAR models, the method was carried out: (1) leave-one-out cross-validation technique, (2) validation was implemented by dividing randomly the 32 compounds into training and test group. The developed model should be capable enough making accurate and reliable predictions of anticancer activities of new substances. So, the QSAR models that are constructed from a training set should be validated using the divided compounds in test group for testing the predictability of the developed models. The validation methods depicted the reliability of the developed models for their applicability on a group of new compounds, and confident level of predictibility can thus be resolved (Tat, 2009). For all the new-constructed models, the multiple-determined coefficient (R^2) and leave-one-out cross-validation value (R^2_{pred}) for the training set were evaluated by (OriginLab Corporation, 2015; Tat, 2009). However, additionally, test set was used for calculation of (R^2_{pred}) values. The acceptability and the predictability of the model are determined based on statistical parameters such multiple-correlation coefficient (R^2) of training group and test group and cross-validation coefficient (R^2_{pred}), Fisher value (F), and standard deviation (SD). Multiple linear models were set (Tat, 2009).

$$Y = \sum_{i=1}^{k} a_i x_i + b \quad (2)$$

where Y is the biological activity pGI_{50} (the dependent variable), x_i is the atomic charge parameters (independent variable). Check the results from the model with experimental data based on single-factor analysis of variance (ANOVA). The correlation coefficient R^2, absolute relative error (ARE, %) and mean of absolute relative error (MARE,%) were calculated according to the Equation (3-5) (Tat, 2009).

$$R^2 = 1 - \frac{\sum_{i=1}^{N}(Y_i - \hat{Y}_i)^2}{\sum_{i=1}^{N}(Y_i - \bar{Y})^2} \quad (3)$$

Here Y is pGI_{50} experimental value and $\hat{Y}$ is pGI50 predicted value; $\bar{Y}$: pGI_{50} is mean value; The statistical error values ARE,% are determined by

$$ARE, \% = 100|(pGI_{50,exp} - pGI_{50,pred})/pGI_{50,exp}| \quad (4)$$

where $pGI_{50,exp}$ and $pGI_{50,pred}$ are experimental and prediction activities. The average value of absolute relative error MARE,% is used to assess the global uncertainty of QSAR model using the formula:

$$MARE, \% = \frac{100}{N}\left|\frac{(pGI_{50,exp} - pGI_{50,pred})}{pGI_{50,exp}}\right| \quad (5)$$

with *N* as number of activity values.

3. Results and discussion

3.1. Calculation of charge parameters

The molecular structures were built and optimized by means of MM+ molecular mechanics. The atomic charge parameters on molecules were calculated by semi-empirical quantum chemistry method SCF PM3 using the optimized molecules. The program HyperChem 8.05 (HyperChem Release 8.03, 2008) was used for all these calculations. The atomic charge parameters were used to build the multiple linear regression ($QSAR_{MLR}$), partial least squares regression ($QSAR_{PLS}$), and artificial neural network ($QSAR_{ANN}$) model.

3.2. Constructing $QSAR_{MLR}$ and $QSAR_{PLS}$ model

Before conducting the $QSAR_{MLR}$ and $QSAR_{PLS}$ modeling, the activity values GI_{50}(μM) are transformed into the values pGI_{50} to adapt the statistical properties. The activity values pGI_{50}(μM) are the most appropriate value. The $QSAR_{MLR}$ models were established using the relationship of the atomic charge predictors and biological activities pGI_{50} (Tat, 2009). The change of values R^2, R^2_{pred} and SE (standard error) in the $QSAR_{MLR}$ models with the atomic charge predictors, respectively, are pointed out in Table 2.

To have those $QSAR_{MLR}$ models, the atomic charge descriptors were selected using stepwise regression algorithm. The selection process for atomic charge descriptors is based on the change of

Table 2. The $QSAR_{MLR}$ models (*k* from 1 to 10) with change of values R^2, R^2_{pred} and SE

k	The atomic charge predictors in models	R^2	SE	R^2_{pred}
2	O_1, C_7	0.816	0.139	0.765
3	O_1, C_4, C_6	0.860	0.124	0.800
4	O_1, C_2, C_4, C_5	0.901	0.107	0.829
5	O_1, C_2, C_4, C_5, $C_{3'}$	0.924	0.096	0.873
6	O_1, O_{11}, C_3, C_4, C_6, C_7,	0.938	0.089	0.903
7	O_{11}, C_4, C_6, C_8, C_9, $C_{2'}$, $C_{6'}$	0.959	0.074	0.879
8	O_{11}, C_3, C_6, C_7, C_8, C_9, C_{10}, $C_{3'}$	0.970	0.065	0.696
9	O_1, O_{11}, C_3, C_4, C_6, C_7, C_8, C_9, C_{10}	0.978	0.057	0.563
10	O_1, O_{11}, C_3, C_6, C_7, C_8, C_9, $C_{2'}$, $C_{3'}$, $C_{6'}$	0.978	0.059	0.358

the statistical values R^2, SE, R^2_{pred}, and *F*-stat. The $QSAR_{MLR}$ models were cross-validated using leave-one-out (LOO) technique to determine R^2_{pred}. The 10 fitness models are shown in Table 2. The $QSAR_{MLR}$ models (with *k* from 2 to 10) that are arranged in an orderly change of values R^2 and R^2_{pred}. From the models in Table 2, the $QSAR_{MLR}$ models (with *k* from 5 to 7) are shown the greater values of R^2_{pred} than others.w

In particular, the $QSAR_{MLR}$ model with $k = 6$ with value R^2 of 0.938 gave the highest value R^2_{pred} of 0.903. So we selected the best models (with *k* of 5, 6, and 7) to determine the contribution percentage of atomic charges. The valuable contribution percentages MP_mx_k,%, GMP_mx_k,% and the statistical values of these models (with *k* of 5, 6, and 7), respectively, are exhibited in Table 3.

The valuable contribution percentages MP_mx_k,% of independent variables in each model $QSAR_{MLR}$ (with *k* of 5, 6, and 7) were determined from the contribution percentages Px_k,% of variables in each case, respectively (Tat, 2009). This value is determined by the total value of contribution C_{total} of variables in a substance (Chen, 2008). So the average contribution percentage MP_mx_k,% of each variable is defined by the formula (6) and the results are depicted in Table 3.

$$MP_mx_k,\% = \frac{1}{N}\sum_{j=1}^{N}\left(100.\left|b_{m,i}x_{m,i}\right|/C_{total}\right) \text{ with } C_{total} = \sum_{j=1}^{k}\left|b_{m,k}x_{m,k}\right| \quad (6)$$

where *N* the total number of cases, *m* number of variables. The global average contribution percentage GMP_mx_k,% of each independent variable for three models is determined by the formula (7):

$$GMP_mx_k,\% = \frac{1}{n}\sum_{n=1}^{3} MP_mx_k \quad (7)$$

with *n* number of models.

Table 3. Statistical values and valuable contribution percentages MP_mx_k,% and GMP_mx_k,% for atomic charges in the models $QSAR_{MLR}$ (with *k* of 5, 6, and 7)

Variable	$QSAR_{MLR}$			MP_mx_k,%			GMP_mx_k,%
x_k	*k* = 5	*k* = 6	*k* = 7	*k* = 5	*k* = 6	*k* = 7	
R^2	0.9243	0.9382	0.9589				
R^2_{adj}	0.9053	0.9186	0.9429				
SE	0.0957	0.0887	0.0743				
R^2_{pred}	0.873	0.903	0.879				
Constant	−0.9332	6.7116	4.714				
O_1	−101.2076	−42.3105	-	57.6024	24.6289	-	27.4104
O_{11}	-	−8.1592	−32.8026	-	18.6316	21.4621	13.3646
C_2	−15.4264	-	-	13.4176	-	-	4.4725
C_3	-	3.0139	-	-	4.2160	-	1.4053
C_4	−6.8735	−19.0370	−60.0703	15.1206	42.4467	38.3868	31.9847
C_5	−7.9686	-	-	2.0583	-	-	0.6861
C_6	-	6.6117	20.8772	-	6.5716	5.7785	4.1167
C_7	-	4.6038	-	-	3.5052	-	1.1684
C_8	-	-	16.9016	-	-	5.6960	1.8987
C_9	-	-	95.4205	-	-	22.5970	7.5323
C_2	-	-	−24.4720	-	-	2.4973	0.8324
C_3	−16.1166	-	-	11.8011	-	-	3.9337
C_6	-	-	−25.4219	-	-	3.5824	1.1941

The contribution percentages $GMP_m x_k$,% in Table 3 display the important level of atomic charges in flavones and isoflavones. For 3 $QSAR_{MLR}$ models, the important level of atomic charges are that arranged by the values $GMP_m x_k$,%: $C_4 > O_1 > O_{11} > C_9 > C_2 > C_6 > C_3$. The atom positions C_4, O_1, O_{11} are considered such as the most important positions in the molecules. Besides those atoms are in carbonyl group $C_4 = O_{11}$ and atom O_1 has free electron pair conjugating with π electronic bond $C_2 = C_3$, and $C_4 = O_{11}$ to form a conjugate system. The carbonyl group $C_4 = O_{11}$ has fully reactive natures of carbonyl substance. So, these important atoms are demonstrated quantitatively using the $GMP_m x_k$ values,% and this is also consistent with the verdicts from experimental evaluation (Lee et al., 2012; Liao et al., 2005, 2008). Also, the atomic position C_6 is also an important position and is explored for attaching the new substitutes (Chen, 2008; Liao et al., 2005, 2008). The atomic positions C_9 and C_3 also represent the important impacts for biological activities GI_{50}, but the C_9 atom is not vacant position so should not be selected for attaching the new substitutes. So the C_6 is vacant position can be chosen to add the new substitutes to sample flavone in Table 1 or new flavonoid. Similarly, the position $C_{3'}$ is also empty and can be utilized to add the new substitutes. Those can hope to constitute a new compound with higher activity. From this orientation, a new flavonoid isolating from the leaf of *artocarpus incisa* L was selected such as sample substance to design new drugs with high activity. This is carried out in below discussion.

The $QSAR_{PLS}$ model is also built from the atomic charges, in which those were selected for the $QSAR_{MLR}$ model (Tat, 2009). The six variables O_1, O_{11}, C_3, C_4, C_6, and C_7 are also used to build the $QSAR_{PLS}$ models. The present results of biological activities are depicted in R^2 values in which those are consistent with experimental data. The partial least squares ($QSAR_{PLS}$) model exhibited in the form:

$$Y = 5.168 - 20.643 \times O_1 - 0.358 \times C_3 - 7.892 \times C_4 + 0.425 \times C_6 - 0.583 \times C_7 - 3.465 \times O_{11} \quad (8)$$

with $n = 26$; $R^2 = 0.943$; SE = 0.360; $R^2_{pred} = 0.912$.

3.3. Building $QSAR_{ANN}$ model

The $QSAR_{ANN}$ model is built by the neuro-fuzzy technique with the genetic algorithms using program Visual Gene Developer v1.7 (Jung & McDonald, 2011). The artificial neural network architecture consists of three layers I(6)-HL(4)-O(1); the input layer I(6) includes six neurons as parameters O_1, O_{11}, C_3, C_4, C_6, and C_7; the neuron on output layer O(1) is biological activity pGI_{50}; the hidden layer HL(4) consists of four neurons. This multi-layer neural network employing backpropagation algorithm is used to train the network. The transfer function is sigmoid on each node of the network; the neural network parameters include the training rate of 0.7 and learning rate of 0.7; the goal monitoring error MSE = 0.000816 with 10,000 iteration. After training the neural network, R^2 value is 0.993 and R^2_{pred} of 0.971 while for $QSAR_{MLR}$ model, the value R^2 is 0.938 and R^2_{pred} of 0.903.

3.4. Prediction of biological activity for new substance

The predictability of the models $QSAR_{MLR}$, $QSAR_{PLS}$ and $QSAR_{ANN}$ are evaluated carefully using the leave-one-out (LOO) technique to determine the value R^2_{pred}; the flavonoids were divided randomly from the data in Table 1 into the training group of 26 compounds and the test group of six compounds. The biological activities of six flavonoids in the test group in Tables 1 and 2 new flavonoids isolated from the leaves of *cynara scolymus* L and *artocarpus incisa* L (Loi, 2006) are predicted from models $QSAR_{MLR}$, $QSAR_{PLS}$, and $QSAR_{ANN}$.

The predicted values of biological activities for those are compared with experimental values, as presented in Table 4. The substance cynaroside is isolated from the leaf of *cynara scolymus* L (Loi, 2006) and its structure is identified using the different spectra such as: ^{1}H-NMR (500 MHz, DMSO, δ ppm): δ 6.39 (s, H_3); 6.44 (d, J = 2 Hz, H_6); 6.78 (d, J = 2 Hz, H_8); 5.08 (1H, d, J = 7,5 Hz, $H_{1''}$); 7.41 (d, J = 8.5 $H_{2'}$); 6.90 (d, J = 8,5 Hz); 7.44 (dd, J = 8.5 Hz, J = 2); 3.489 (1H, m, $H_{2''}$); 3.476 (1H, m, $H_{4''}$); 3.466(1H, m, $H_{3''}$); 3.500(1H, m, $H_{5''}$); 3.725 (1H, dd, J = 12.3; 2.5 H_2, $H_{6''}$); 3.702 (1H, dd, J = 12.4; 6.3 H_2, $H_{6''}$); 12.8 (C_5OH); 3.466–3.725 (7H, m, glucose protons). The associated spectrum was also used to

Table 4. Activities pGI_{50} of test group resulting from models $QSAR_{MLR}$, $QSAR_{PLS}$, and $QSAR_{ANN}$

Substance	Reference	$pGI_{50,exp}$	$pGI_{50,pred}$			ARE (%)		
			$QSAR_{MLR}$	$QSAR_{PLS}$	$QSAR_{ANN}$	$QSAR_{MLR}$	$QSAR_{PLS}$	$QSAR_{ANN}$
Fla2	(Lee et al. 2012; Liao et al., 2005, 2008)	5.9208	6.0079	5.8012	5.8509	1.4719	2.0200	1.1802
Fla9	(Lee et al. 2012; Liao et al., 2005, 2008)	5.7447	5.6915	5.6082	5.7407	0.9252	2.3758	0.0698
Fla12	(Lee et al. 2012; Liao et al., 2005, 2008)	6.0969	5.7587	5.8416	5.8136	5.5478	4.1875	4.6463
Fla15	(Lee et al. 2012; Liao et al., 2005, 2008)	5.6990	5.6511	5.6518	5.7124	0.8402	0.8275	0.2357
Fla16	(Lee et al. 2012; Liao et al., 2005, 2008)	5.6990	5.6514	5.6549	5.7188	0.8350	0.7746	0.3466
Isofla32	(Lee et al. 2012; Liao et al., 2005, 2008)	5.1367	5.0917	5.0830	5.1115	0.8767	1.0457	0.4900
Cynaroside	This work	5.3260	5.1910	5.6317	5.3186	2.5350	5.7393	0.1388
Quercetin	This work	5.3790	4.5858	5.5355	5.3591	14.7455	2.9094	3.9388
					MARE (%)	3.4722	2.4850	1.3808

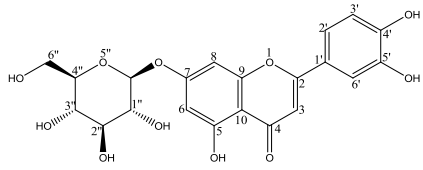
have more structural information such as: ^{13}C-NMR (DMSO, δ ppm, 125 MHz), DEPT: δ 164.5(C_2); 105.3(C_3); 181.785 (C_4); 161.2 (C_5); 100.2 (C_6);162.9 (C_7); 95 (C_8); 156.9 (C_9); 103.1 (C_{10}); 121.4 ($C_{1'}$); 113.6 ($C_{2'}$); 145.7 ($C_{3'}$); 149.9 ($C_{4'}$); 115.9 ($C_{5'}$); 119.2 ($C_{6'}$); 100.0 ($C_{1''}$); 76.4 ($C_{2''}$); 77.2 ($C_{3''}$); 69.5 ($C_{4''}$); 78 ($C_{5''}$); 60.6 ($C_{6''}$). Interaction of C and H in spectrum HMBC and HSQC are also pointed out: H_6-C_5-C_7-C_8-C_{10}; H_8-C_6-C_7-C_9-C_{10}; $H_{2'}$-C_2-$C_{1'}$-$C_{3'}$-$C_{4'}$-$C_{6'}$; $H_{5'}$-$C_{1'}$-$C_{3'}$-$C_{4'}$-$C_{6'}$; $H_{6'}$-C_2-$C_{1'}$-$C_{2'}$-$C_{4'}$-$C_{5'}$.

The substance quercetin is isolated from the leaf of *artocarpus incisa* L (Loi, 2006) and its structure is also identified using the spectrum ^{1}H-NMR (DMSO-d_6, 500 MHz, δ ppm) combining with spectrum HSQC, HMBC: δ 6.26 (1H, d, J = 1.5 Hz, H_6); δ 6.52 (1H, s like *t*, H_8); δ 7.82 (1H, d, J = 1,5 Hz, $H_{2'}$); δ 7.68 (1H, dd, J = 8.5 và 2 Hz, $H_{5'}$); δ 6.98 (1H, d, J = 8.5 Hz, $H_{6'}$); δ 12.16 (1H, s). Also, using spectrum ^{13}C-NMR (DMSO-d_6, 125 Hz) combining with spectra DEPT, HSQC, HMBC: δ_C 146.9 (C_2); δ_C 136.6 (C_3); δ_C 176.5, (C_4); δ 162.2 (C_5); δ 99.1 (C_6); δ 164.9 (C_7); δ 94.4 (C_8); δ 157.7 (C_9); δ 104.0 (C-10); δ_C 121.4 ($C_{1'}$); δ 116.1 ($C_{2'}$); 145.7 ($C_{3'}$); δ 148.2 ($C_{4'}$); δ 115.6 ($C_{5'}$); δ_C 123.7 ($C_{6'}$).

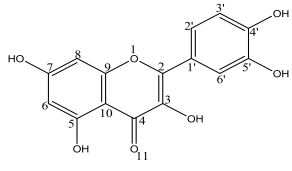
Figure 4. The molecular structures of: (a) cynaroside and (b) quercetin.

a) leaf of *cynara scolymus* L [1]

b) leaf of *artocarpus incisa* L [1]



Cynaroside with $GI_{50,exp}$ (μM) = 4.72 ± 0.280



Quercetin with $GI_{50,exp}$ (μM) = 4.18 ± 0.327

Table 5. The anti-cancer activities GI_{50} (μM) of five new flavonoids (*n*) designing from the vacant positions C_6 và $C_{3'}$ on quercetin, resulting from $QSAR_{ANN}$ model

New substance	Substitutes at C_6	Substitutes at $C_{3'}$	GI_{50} (μM)	Method in this work
Quercetin	-H	-H	4.18 ± 0.327	*in vitro* test on Hela
Fla-1(n)	$-OCH_2CONHCH_3$	-OH	0.1539	$QSAR_{ANN}$
Fla-2(n)	$-OCH_2CONHC_6H_4F$	-H	0.1487	$QSAR_{ANN}$
Fla-3(n)	-OH	$-OCH_2CONHCH_3$	0.1247	$QSAR_{ANN}$
Fla-4(n)	$-OCH_2CH_3C{=}NOH$	-OH	0.1233	$QSAR_{ANN}$
Fla-5(n)	$-OCH_2CONHC_6H_4OCH_3$	-H	0.1174	$QSAR_{ANN}$

The molecular structures of substances cynaroside and quercetin are shown in Figure 4.

After isolation of two new flavonoids cynaroside and quercetin, their activities pGI_{50} were conducted to test *in vitro* toxicity on Hela cells in the laboratory of molecular biology, university of natural sciences. The activity values pGI_{50} of two these flavonoids were also predicted from the models $QSAR_{MLR}$, $QSAR_{PLS}$, and $QSAR_{ANN}$, as shown in Table 4. Those were compared with experimental activities and with each other based on the average value of the absolute relative error MARE,%. The predictability of the model $QSAR_{MLR}$ is lower than models $QSAR_{PLS}$ and $QSAR_{ANN}$, respectively, as given in Table 4.

The $QSAR_{ANN}$ model has the valuable error MARE,% of 1.3808. This is smaller than values MARE,% of both models $QSAR_{MLR}$ and $QSAR_{PLS}$. So, the predictability of $QSAR_{ANN}$ model is better than models $QSAR_{MLR}$ and $QSAR_{PLS}$. After using the models $QSAR_{MLR}$, $QSAR_{PLS}$, and $QSAR_{ANN}$ to predict the biological activities pGI_{50} of six compounds in test group and two new flavonoids quercetin and cynaroside, the accurate level of the predicted results is exhibited in the acceptable errors within the uncertainty of experimental measurements. Thus, the models $QSAR_{MLR}$, $QSAR_{PLS}$ and $QSAR_{ANN}$ are good adaptable for predicting the biological activities of new substances.

In this work, we selected the new flavonoid quercetin with the vacant positions C_6 and $C_{3'}$ such as sample compound for designing five new compounds. The substitutes were attached to two vacant positions C_6 and $C_{3'}$, as shown in Table 5. The new designed compounds were also predicted with the biological activities pGI_{50} using the $QSAR_{ANN}$ model. Then, the predictive activities pGI_{50} were recovered in the original form GI_{50} (μM), as given Table 5.

The predicted results pGI_5 for new substances are transformed into values GI_{50} (μM) and compared with experimental activity of sample quercetin, as depicted in Figure 5. Thus, the five new compounds designing from the C_6 and $C_{3'}$ positions on quercetin displayed stronger activity GI_{50} (μM) than sample quercetin. Herein, the new designed compounds will promise to forward a designing plan for the new pharmaceutical products from natural products.

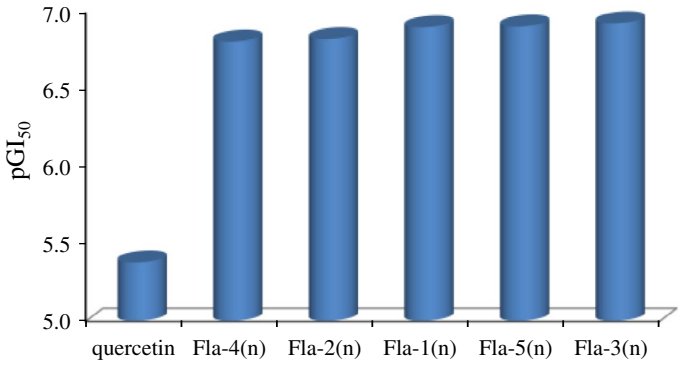


Figure 5. Comparison between values pGI_{50} of five new flavonoids with quercetin.

4. Conclusion

We used the quantum chemistry calculations, multivariable regression and artificial neural network to construct successfully the quantitative relationships between the partial atomic charges and anti-cancer activities GI_{50} (μM) of flavonoids. The models $QSAR_{MLR}$ showed six important sites O_1, O_{11}, C_3, C_4, C_6, and C_7 on flavonoids.

The $QSAR_{MLR}$ model found out the most important positions C_6 and $C_{3'}$ to add the new substitutes to create five new flavonoids with higher activity of quercetin isolating from the leaf of *artocarpus incisa* L. The $QSAR_{ANN}$ model with architecture I(6)-HL(4)-O(1) is better predictable for flavonoids.

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