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**Original scientific paper**

# VITAMIN C EQUIVALENT ANTIOXIDANT CAPACITY PREDICTION FOR SET OF FLAVONES WHICH INFLUENCE FOOD QUALITY

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## ABSTRACT

Flavones are secondary metabolite products in plants and have broad-spectrum effects in both microorganisms and animals with varied structures and functions. The description of antioxidant potential of various chemicals or dietary foods using vitamin C equivalent antioxidant capacity (VCEAC) is preferable compared to other assays. VCEAC values is measure for the antioxidant capacity of the various natural chemicals. The higher the VCEAC value of test compound, the more effective the antioxidant. QSAR (quantitative structure - activity relationships) are theoretical models used to estimate or predict the physicochemical and biologically important activities or properties of biological molecules. A main goal of this investigation was finding QSAR correlation between the experimental VCEAC values and several characteristic calculated descriptors for set of selected flavones. For that reason, physicochemical descriptors (molar refractivity, molar volume, parachor, refractive index, surface tension, density, polarizability and rings plus double bonds equivalent) were selected as independent variables and VCEAC values as dependent values in this QSAR study. According statistical results ($R^2$; $R^2_{adj}$) three-parametric models consist by molar volume, density and polarizability, has been selected as statistically significant. Predicted VCEAC value was compared with the corresponding observed values VCEAC and predictive correlation coefficient ($R_{pre}^2$) was calculated. The obtained predictive correlation coefficient ($R_{pre}^2 = 0.866$) confirms our findings.

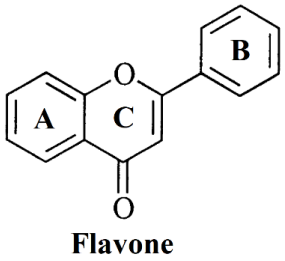
**Key words:** flavones, VCEAC values, antioxidant capacity, QSAR, plants.

## INTRODUCTION

The compounds that can delay, inhibit, or prevent the oxidation of oxidizable matters by scavenging free radicals and diminishing oxidative stress are known as *antioxidants*. Oxidative stress is an imbalanced state where excessive quantities of reactive oxygen species are present at levels more than what is required for normal cell function and overwhelm endogenous antioxidant capacity and repair. Oxidative stress, induced by *reactive oxygen species* (ROS) such as $O_2^{\bullet-}$, $OH^{\bullet}$, or lipid propyl radicals LOO•, can result in damaging of proteins, nucleic acids and lipids, which has been implicated in the pathogenesis of various diseases, including coronary heart disease and some form of cancer (Lucic, 2008).

Flavonoids are naturally occurring compounds, present in the nascent parts of a plant (Shen et all, 2022; Roy et all, 2022). Together with chlorophylls and carotenoids, they are pigments present in almost all plants, and provide fragrance and taste to fruits, flowers, and seeds (D'Arcy 2022). Flavones are a class of flavonoids. Chemical structure has a 15-carbon skeleton, with two phenyl rings (A and B) and one heterocyclic ring (C) (Figure 1).

Flavonoids are low molecular weight polyphenolic phytochemicals, secondary metabolite products in plants produced in secondary pathways that synthesize compounds that are needed in trace amounts (Donadio et al., 2021; Roy et al., 2021). The secondary metabolites regulate primary pathways such as hormones, and coenzymes; during specific stress conditions, act as toxins and antibiotics. Apart from the main role in plants, they are important for human health because of various pharmacological activities. Nowadays is known that flavonoids have broad-spectrum effects in both microorganisms and animals with varied structures and functions.



**Flavone**

Figure 1. Flavone core

Historically, since ancient times, the presence of flavonoids in plants was identified, but their chemical structure was not known until the end of the nineteenth century (Perez-Vizcaino & Fraga, 2018). In the early twentieth century, flavonoids were either isolated in different plants or synthesized under laboratory conditions. Since flavonoids are natural polyphenolic antioxidants, they are capable of combating ROS by scavenging free radicals, chelating metal ions, inhibiting prooxidant enzymes, and activating antioxidant and detoxifying enzymes (Heller et al., 1996; Soobrattee et al., 2005). They are recognized as potential candidates for use as drugs in illnesses such as atherosclerosis, coronary heart diseases, cancer, cardiovascular diseases (Havsteen et al., 2002).

Vitamin C is known as a leading natural nutrient and antioxidant which has been shown to scavenge reactive oxygen species such as superoxide radical anion, singlet oxygen, hydrogen peroxide, and hydroxyl radical (Rock et al., 1996), reactive free radicals normally generated via biological metabolism of oxygen (Camougrand & Rigoulet, 2001). Vitamin C also has been found to have anticarcinogenic effects (Lee et al., 2002). The description of antioxidant potential of various chemicals or dietary foods using vitamin C equivalent antioxidant capacity (VCEAC) is preferable to that used by the other assays (Kim et al., 2002).

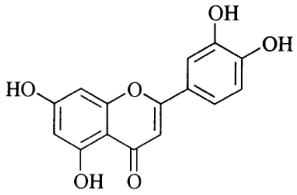
Due to the wide range of biological activities of flavones, their structure–activity relationships (SAR) have generated interest among medicinal chemists, biochemists, agronomists (Bagal et al., 2022). QSAR (quantitative structure - activity relationships) are theoretical models used to estimate or predict the physicochemical and biologically important activities or properties of molecules. Since 1964 and the paper by Hansch and Fujita, this field has been developing into a science highly useful in the process of designing new biologically active compounds having desirable properties by QSAR driven modification of basic chemical structure (Jhanwarb, et al., 2011).

## MATERIALS AND METHODS

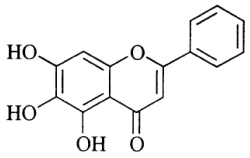
### Materials

Following flavones: luteolin, baicalein, 7,8-dihydroxyflavone, apigenin, diosmin, chrysin, 6-hydroxyflavone, 5-hydroxyflavone, 7-hydroxyflavone and rhoifolin were used in this investigation. The structure of flavones is presented in Table 1.

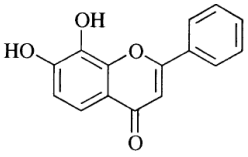
Table 1. Structure of investigated flavones



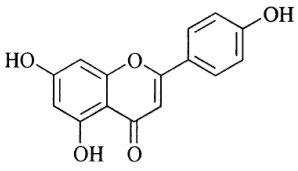

**Luteolin**



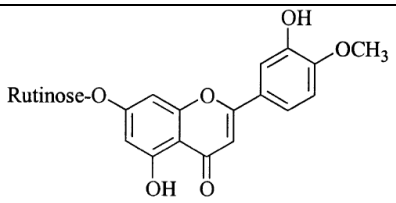

**Baicalein**



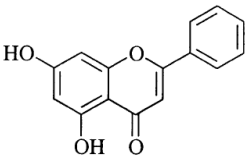

**7,8-Dihydroxyflavone**



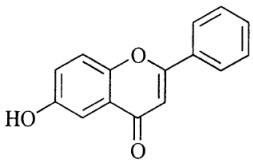

**Apigenin**



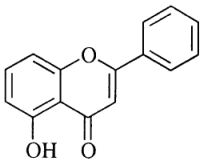

**Diosmin**



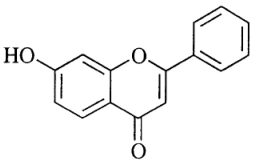

**Chrysin**



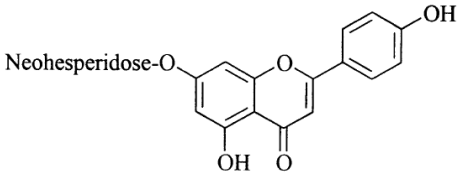

**6-Hydroxyflavone**




**5-Hydroxyflavone**




**7-Hydroxyflavone**




**Rhoifolin**

## Methods

*A. VAEC model*

***Calculation of vitamin C equivalent antioxidant capacity (VCEAC)***

VCEAC (vitamin C equivalent antioxidant capacity) values is measure for the antioxidant capacity of the various natural chemicals. The vitamin C standard curve that relates the concentration of vitamin C to the amount of absorbance reduction caused by vitamin C was obtained using the VCEAC assay using free ABTS radical.

The vitamin C standard curve that relates the concentration of vitamin C to the amount of absorbance reduction caused by vitamin C was obtained using the VCEAC assay using free ABTS radical. The absorbance reduction at 734 nm by all test chemicals also was measured at the concentration level of 100 mg/L. The higher the VCEAC value of test compound, the more effective the antioxidant. The calculation of VCEAC of each flavone was made using vitamin C standard curve (Equation 1) as follows:

$$\mathbf{VCEAC\ (mg/L) = (\Delta\ \mathit{Abs} - \mathit{a})/\mathit{b}} \qquad \text{Equation (1)}$$

where, *a* is y-intercept of vitamin C standard curve, *b* is slope of vitamin C standard curve, *Abs* is the initial absorbance of control minus the resulting absorbance of chemicals tested at

10 min, 734 nm and 37ºC. In Table 2 are presented VCEAC values expressed as mg/L (Dae-Ok & Lee, 2004).
Table 2. VCEAC value

| Flavone | VCEAC | Flavone | VCEAC |
|---|---|---|---|
| Luteolin | 178.3 | Chrysin | 24.9 |
| Baicalein | 118.2 | 6-Hydroxyflavone | 22.4 |
| 7,8-Dihydroxyflavone | 108 | 5-Hydroxyflavone | 11.6 |
| Apigenin | 89.8 | 7-Hydroxyflavone | 5.3 |
| Diosmin | 32.4 | Rhoifolin | 4.2 |

**Physicochemical descriptors**

To obtain the quantitative effect of structural parameters of the flavones on their antioxidant capacity, QSAR analysis with physicochemical descriptors was performed. Three general groups of descriptors were calculated: *(i)* basic macroscopic properties: Molar Refractivity (MR), Molar Volume (MV) and Parachor (Pr); *(ii)* derived macroscopic properties: refractive index (n), and surface tension (ST) and density (D), *(iii)* Polarizability (Pol) and rings plus Double Bonds Equivalent (RDBE). The physicochemical parameters used in this study were calculated using ACD Labs software (Table 3).

Table 3. Calculated physicochemical descriptors for selected flavones

| Flavone | MR | MV | Pr | n |
|---|---|---|---|---|
| Luteolin | 71.73 | 172.9 | 536.6 | 1.767 |
| Baicalein | 69.85 | 174.5 | 521.4 | 1.732 |
| 7,8-Dihydroxyflavone | 67.97 | 176.1 | 506.1 | 1.698 |
| Apigenin | 69.85 | 174.5 | 521.4 | 1.732 |
| Diosmin | 141.7 | 361.8 | 1147.9 | 1.711 |
| Chrysin | 67.97 | 176.1 | 506.1 | 1.698 |
| 6-Hydroxyflavone | 66.08 | 177.7 | 490.9 | 1.666 |
| 5-Hydroxyflavone | 66.08 | 177.7 | 490.9 | 1.666 |
| 7-Hydroxyflavone | 66.08 | 177.7 | 490.9 | 1.666 |
| Rhoifolin | 135.33 | 340.3 | 1089.3 | 1.726 |
| Flavone | **ST** | **D** | **Pol** | **RDBE** |
| Luteolin | 92.5 | 1.654 | 28.43 | 11 |
| Baicalein | 79.5 | 1.548 | 27.69 | 11 |
| 7,8-Dihydroxyflavone | 68.2 | 1.443 | 26.94 | 11 |
| Apigenin | 79.5 | 1.548 | 27.69 | 11 |
| Diosmin | 101.2 | 1.68 | 56.17 | 13 |
| Chrysin | 68.2 | 1.443 | 26.94 | 11 |
| 6-Hydroxyflavone | 58.2 | 1.34 | 26.19 | 11 |
| 5-Hydroxyflavone | 58.2 | 1.34 | 26.19 | 11 |
| 7-Hydroxyflavone | 58.2 | 1.34 | 26.19 | 11 |
| Rhoifolin | 104.9 | 1.69 | 53.65 | 13 |

Molar Refractivity (MR), Molar Volume (MV) and Parachor (Pr); refractive index (n), surface tension (ST) density (D), Polarizability (Pol), rings plus Double Bonds Equivalent (RDBE)

*B. QSAR model*

Multiple linear regression is a common method used in QSAR studies. The QSAR equations were obtained by Equation 2:

$$\mathbf{VCEAC} = a_0 + a_1D_1 + a_2D_2 + a_3D_3 + \ldots + a_nD_n \qquad \text{Equation (2)}$$

where $D_1$, $D_2$, $D_3$ … $D_n$ are descriptors, $n$ is number of descriptors, $a_0$ is intercept, $a_1$, $a_2$, $a_3$, … $a_n$ are regression coefficient of the descriptors.

*C. Statistical analysis*

The statistical evaluation of the data was performed using EXCEL. To test the quality of the regression equations, correlation coefficient (R2) and adjusted correlation coefficient ($R^2$adj.) were used.

## RESULTS AND DISCUSSION

A main goal of this investigation was a finding correlation between the experimental VCEAC values obtained from (Dae-Ok & Lee, 2004) (Table 2) and several characteristic calculated descriptors (Table 3) for set of selected flavones.

### 1. *VCEAC values*

The flavone used as reference did not show any antioxidant capacity. The remaining flavones are arranged in the following descending order:

*luteolin > baicalein > 7,8-dihydroxyflavone > apigenin > diosmin > chrysin > 6-hydroxyflavone > 5-hydroxyflavone > 7-hydroxyflavone > rhoifolin.*

The VCEAC values indicate the following facts:

- *Flavones with no substitution in B ring*
  (i) The level of antioxidant capacity of those increased as follows:
  7-hydroxyflavone < 5-hydroxyflavone < 6-hydroxyflavone < chrysin < < 7,8-dihydroxyflavone < baicalein

- *Flavones with the OH or $CH_3O$ group on the ring B*
  (i) luteolin had the highest VCEAC.
  (ii) rhoifolin showed lower VCEAC.
  (iii) The addition of hydroxy group into 4 position of chrysin increased the VCEAC in apigenin implying some contribution of antioxidant capacity by monohydroxylation in B ring.
  (iii) VCEAC in apigenin is higher than VCE AC in chrysin (without OH group in position 4), indicating some improvement of antioxidant capacity by B ring monohydroxylation.

- *Hydroxylation on A ring*
  (i) compared to the flavone itself, the introduction of an OH group into the A ring leads to an improvement in antioxidant activity
  (iii) presence of three hydroxyl groups in aromatic A ring as in flavone baicalein gave higher VCEAC compared with other flavones with two OH group in A ring and without substituent in B ring (7,8-dihydroxyflavone, chrysin, 6-, 5- and 7-hydroxyflavone).

### 2. *QSAR study*

The structural features of flavones (characterized by various types of physicochemical descriptors) important for antioxidant activities can be used to build appropriate QSAR models in order to predict the activities of many other untested flavonoids, and to direct the synthesis of flavonoid compounds with higher potency for potential further application. Determination of correlation matrix is first step in QSAR analysis (Table 4).

Table 4. Correlation matrix for the chosen parameters

| Variables | MR | MV | Pr | n | ST | D | Pol | RDBE | VCEAC |
|---|---|---|---|---|---|---|---|---|---|
| **MR** | **1.000** | | | | | | | | |
| **MV** | 0.996 | **1.000** | | | | | | | |
| **Pr** | 1.000 | 0.997 | **1.000** | | | | | | |
| **n** | 0.246 | 0.162 | 0.242 | **1.000** | | | | | |
| **ST** | 0.812 | 0.759 | 0.810 | 0.764 | **1.000** | | | | |
| **D** | 0.723 | 0.661 | 0.720 | 0.847 | 0.988 | **1.000** | | | |
| **Pol** | 1.000 | 0.996 | 1.000 | 0.246 | 0.813 | 0.723 | **1.000** | | |
| **RDBE** | 0.997 | 0.997 | 0.997 | 0.190 | 0.778 | 0.680 | 0.997 | **1.000** | |
| **VCEAC** | -0.302 | -0.372 | -0.305 | 0.749 | 0.246 | 0.375 | -0.302 | -0.362 | **1.000** |

All physicochemical descriptors (Table 3) were selected as independent variables and VCEAC values as dependent values. Following QSAR model is statistically reliable model for antioxidant capacity prediction of other natural and synthetic flavones.

**$QSAR_{flavones}$: VCEAC = -1694 + 13.5011*Molar Volume + 1401*Density - 98.1331*Polarizability**

**$R^2$ = 0.866 $R^2$adj. = 0.799**

$QSAR_{flavones}$ is three parametric model consist by molar volume, density and polarizability. According the coefficient sings, molar volume and density have positive impact, but polarizability have negative impact on VCEAC value. A flavone with a higher density value should be an effective antioxidant.

In order to confirm our findings, VCEAC values predicted by QSAR model 1, with the corresponding VCEAC values (Table 2), were compared. A plot between the experimental and calculated VCEAC values is shown in Figure 2.

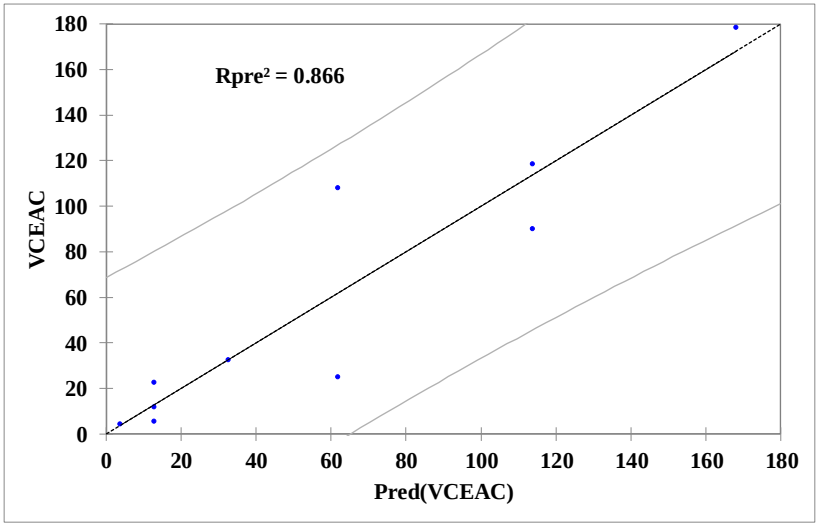



Figure 2. Plot of the experimental and calculated VCEAC values for selected flavones

The predictive correlation coefficient ($R_{pre}^2$) was calculated, by correlating the estimated VCEAC values with the experimental once. The obtained predictive correlation coefficient ($R_{pre}^2$ = 0.866) confirms our findings.

## CONCLUSIONS

VCEAC values for ten flavones: luteolin, baicalein, 7,8-dihydroxyflavone, apigenin, diosmin, chrysin, 6-hydroxyflavone, 5-hydroxyflavone, 7-hydroxyflavone and rhoifolin, were selected for QSAR modeling. Physicochemical descriptors such as: molar refractivity, molar volume, parachor, refractive index, surface tension, density, polarizability and rings plus double bonds equivalent were independent variables and VCEAC values as dependent values in this QSAR study. Three-parametric models consist by molar volume, density and polarizability, has been selected as statistically significant, according the values of correlation coefficient and adjusted correlation coefficient. Predicted VCEAC value compared with the corresponding observed values VCEAC gave predictive correlation coefficient $R_{pre}^2$=0.866, confirming our conclusion.

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