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QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP STUDY OF ESTER-BASED FERULIC ACID DERIVATIVES AGAINST CERVICAL CANCER CELL (HELA)

Helda Wika Amini[1*] , Istiqomah Rahmawati[1], Rizki Fitria Darmayanti[1], and Boy Arief Fachri[1]

[1]Department of Chemical Engineering, Universitas Jember, Indonesia 68121

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Abstract. Quantitative structure-activity relationship (QSAR) has been studied for ferulic acid derivatives to determine the QSAR model able to predict anticancer. As the subject of this research was a set of experimentally calculated IC_{50} value data of 6 ferulic acid derivatives against cervical cancer cells (HELA). QSAR analysis was based on multilinear regression calculation on fitting subset using log ($1/IC_{50}$) as the dependent variable, and dipole moment, partition coefficient in the n-octanol/water, and atomic net charges of the aromatic carbons as independent variables. The values of the descriptors were obtained from semiempirical PM3 quantum mechanic calculation. The relationship between log ($1/IC_{50}$) and the descriptors was described by the result in the QSAR model. The QSAR model for ferulic acid derivatives against HELA cell lines was developed with the statistical parameters of R=0.998; R^2=0.999; SE=0.00857; and F=394. The calculated log ($1/IC_{50}$) using QSAR Hansch Model for ferulic acid derivatives have excellent agreement with experimental data of Log ($1/IC_{50}$).

Keywords: *QSAR Study, Ferulic Acid Derivatives, Descriptors*

1. Introduction

Indonesia is the world's fourth-largest coffee producer, in which the production reached up to 668.70 thousand tons in 2017 [1]. The processing of coffee cherry into coffee beans generates by-products such as pulp coffee, which is the primary residue of this process (40%-45%). Recently, several studies focused on the composition of pulp coffee and their secondary application. The main constituents of coffee pulp are fiber with 80% composition, followed by lignin 12%, crude protein 9.9%, ash 1.5%, and moisture 7.6% [2]. Besides, highly valuable bioactive compounds are existing in the coffee pulp such as caffeic acid, ferulic acid, and chlorogenic acid [2–3].

Phenolic acids have been reported widely of the activity as antioxidants [4–6]. Other application of phenolic acids includes anticancer [7], antiviral [8], antitumor [9], antidiabetic [10], antihypertensive [11]. Hydroxycinnamic acid-like ferulic acid, caffeic acid, and chlorogenic acid are natural compounds in coffee pulp [12]. Approximately 10% weight of the dry coffee pulp contain these bioactive compound [2]. These available contents are promising for the reuse of coffee pulp for various purposes.

Ferulic acid has shown to regulate the key enzymes which were responsible for free radical-induced cell damage [13]. Ferulic acid is biosynthesized from amino acid phenylalanine

*corresponding author: heldawikaamini@unej.ac.id

through a shikimic acid pathway. Ferulic acid has an anticancer activity that possesses free carboxylic acid with gastric irritation as an inevitable adverse effect when consumed orally. Thus, ester derivatives of ferulic acid are favorable in used as drugs, as reported by Serafim et. al (2011) cafeic and ferulic acid ester derivatives exerted cytotoxicity to the breast cancer cells compared with the parent compound as well as the study for HELA cells [13]. Modification of the carboxylic group with a different alkyl group of ferulic acid including methyl, ethyl, n-propyl, n-butyl, bromo ethyl, and chloroethyl affected the anticancer activity [13, 15].

The value of an anticancer activity is an important parameter in drug design. Anticancer activity might measure both experimental and theoretical. The properties of the predicted derivative compounds can be predicted using the QSAR technique using the built molecular descriptors. This consists of the similarities between molecules in a large database of existing molecules with known properties [16]. This technique has been used in various procedures for various applications such as anticancer [16–17], antibacterial [18], antifungus [19], and hormone controlling [20]. The result of the QSAR prediction model shows a high correlation with the experimental data [16, 18].

Cervical cancer is the second most happening cancer in women in the world [21]. HELA is one type of cervical cancer cell that has been widely studied for the elimination using natural anticancer agents including flavonoids and polyphenols [22]. As far as our knowledge, the QSAR study has not been reported specifically for the active compound in HELA cells treatment. This study is expected to build the model to design the compound with optimized anticancer activity using QSAR.

In this research, we study about QSAR of six ferulic acid derivatives to establish the relationship between structural characteristics of the ferulic acid derivatives molecules and their activities using the semi-empirical method of PM3 (Parametric Method 3).

2. Methods

2.1. Studied compounds

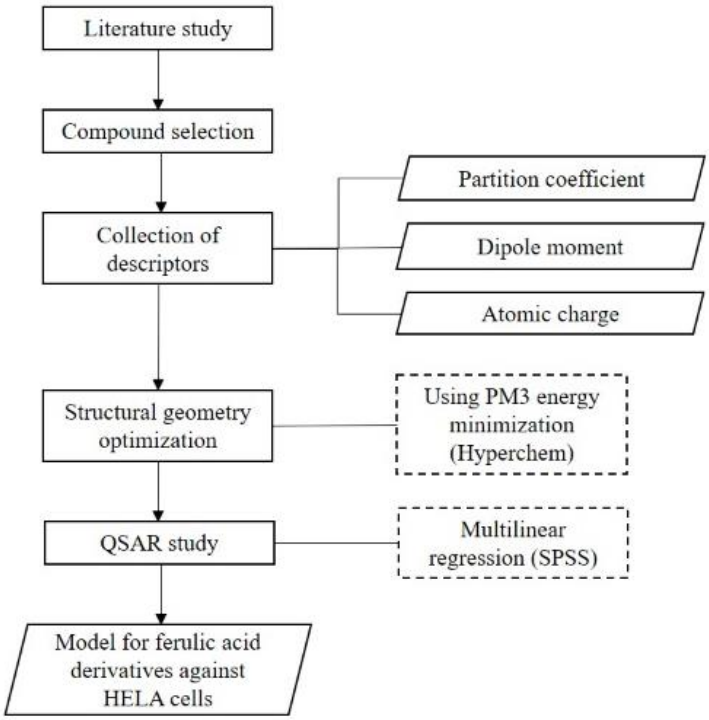

Figure 1. Experimental methods

The methods is summarized in Figure 1. In this study, we calculated properties of 6 known ferulic acid derivatives compounds, as shown in Table 1. The structure of ferulic acid derivatives including the ones with methyl, ethyl, n-propyl, n-butyl, bromo-ethyl, and chloro ethyl, is shown in Figure 2. These compounds were observed for their activities against HELA cancer lines as being studied by Kiran et al (2015).

Table 1. The experimental anticancer activity of ferulic acid derivatives against HELA cell lines [13]

Compound	R'	Anticancer Activity	
		IC_{50} (µg/mL)	Log ($1/IC_{50}$)
FE1	Methyl	92	-1.96
FE2	Ethyl	70	-1.85
FE3	n-propyl	64	-1.81
FE4	n-butyl	61	-1.79
FE5	chloroethyl	32	-1.51
FE6	bromoethyl	55	-1.74

2.2. Equipment

Intel (R) Celeron (C) processor was used with 204 MB RAM as computer hardware. The computational chemical calculation was conducted using Hyperchem 7.02 computational chemistry software. The statistical analysis was performed with SPSS 16.020. Two dimensional molecular structure was created using ChemDraw Ultra 8.0.

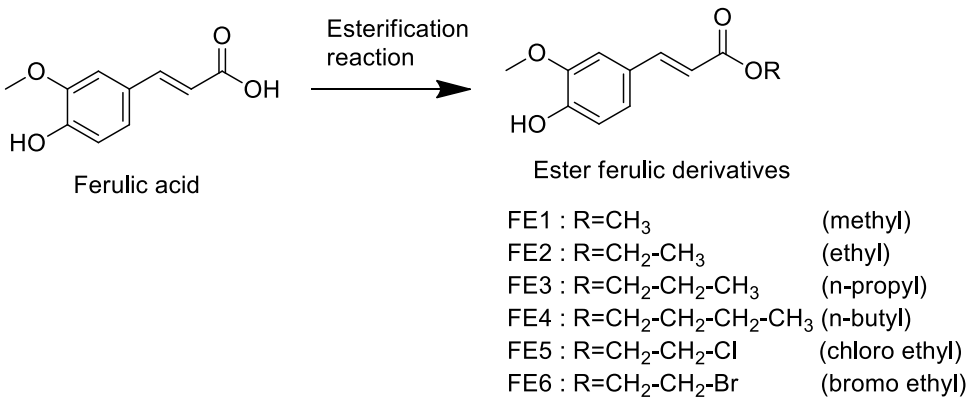

Figure 2. Ferulic derivatives [13]

2.3. Procedures

2.3.1.Collection of descriptors

QSAR equation was built by the relationship between activity and descriptors. The logarithm of partition coefficient in the n-octanol/water (log P), the atomic charge, and the dipole moment (µ) were selected as the descriptors. Table 2 shows the descriptors and the calculation methods. The calculations for electronic descriptors are performed using computational chemistry modeling with the geometry optimization procedure of each compound structure. E as described previously [23]. Each compound was created into a 2D structure model using the ChemDraw Ultra 8.0 and equipped with hydrogen atoms to form a 3D structure.

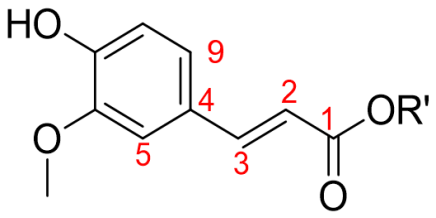

Figure 3. The label of selected carbon charge in ferulic acid derivatives

We analyze the net atomic charge of carbon number 1, 2, 3, 4, 5, 9, which have a high probability affect in the different attachment of alkyl group for ferulic acid derivatives. Figure 3 shows the position of the carbon. The net atomic charge, the dipole moment, and the partition coefficient were determined as described in the previous report [23]. Then the most stable molecular geometry was optimized by minimizing the molecular energy using PM3 method, the convergence limit was 0.001 kcal / Å.mol for ferulic acid derivatives, according to Polak-Ribiere algorithm.

Table 2. List of descriptors and how to optimize them

No	Symbol	Descriptor	Unit	Calculation Method
1	qC_1, qC_2, qC_3, qC_4, qC_5, qC_{12}	The atomic charge of C_1, C_2, C_3, C_4, and C_{12}	Coulomb	Semiempirical method of PM3, Hyperchem, compound optimization
2	μ	dipole moment	Debye	Semiempirical method of PM3, Hyperchem, compound optimization
3	Log P	The partition coefficient of n-octanol/water	-	QSAR Properties, Semiempirical method of PM3, Hyperchem, compound optimization

2.3.2.QSAR study

The most representative QSAR equation to predict IC_{50} was determined by multilinear regression statistical analysis with backward method using SPSS. The QSAR equation was optimized by fitting six ferulic acid derivatives and the influence of the dependent and independent variables to the QSAR equation. The variables were determined as described previously in Rahmawati et al. (2020). The following regression equation was expressed as the resul of QSAR approach:

$$\text{Log } (1/IC_{50}) = k_1 \log P + k_2 qC_1 + k_3 qC_2 + k_4 qC_3 + k_5 qC_4 + k_6 qC_5 + k_7 qC_9 + k_8 \mu + k_9 \quad (1)$$

Statistical analysis was performed as explained in the previous report [23].

3. Results and Discussions

3.1. The Result of descriptors calculation on ferulic acid derivatives

This research was performed by PM3 semi-empirical method for optimizing the structure of six ferulic acid derivatives. Semi-empirical methods are more reliable than *ab-initio* methods for QSAR study and produce the best model for the QSAR model [24–25]. The six compounds are FE1 which attached methyl group on carboxylic moiety in ferulic structure. FE2 has an ethyl group attached in carboxylic moiety and FE3 has n-propyl group bind to a carboxylic moiety in ferulic structure. Besides, incorporating an n-butyl group on carboxylic moiety in ferulic structure named as FE4. FE5 and FE6 have chloroethyl and bromoethyl substituent, respectively, which are shown in Figure 2. These compounds resulted in the relatively higher anticancer activity against HELA cell lines compared with other ferulic acid derivatives with longer alkyl chain studied [8, 13].

In this research, we study about QSAR between dependent variable anticancer activity (see Table 1) and eight independent descriptors. The anticancer activity of ferulic acid derivatives was expressed as IC_{50} (μg/ml) which is the concentration of the compound that inhibited proliferation rate of HELA cell lines by 50% as compared to the control untreated cells which were reported on the previous research [13]. Table 1 shows that all the synthesized of ferulic acid derivatives are having higher anticancer activity than the parent compound. Longing the alkyl chain from methyl to n-butyl (FE1-FE4) was observed, generating higher anticancer activity. Furthermore, FE5 having chloroethyl is most favorable, which produces the lowest IC_{50} value against HELA cell lines.

Table 3. Descriptor data as independent variables for ferulic acid derivatives

No	Compound	Net Atomic Charge (Coulomb)						Dipol μ (deybe)	LogP
		C_1	C_2	C_3	C_4	C_5	C_{12}		
1	FE1	0.4105	-0.2007	0.004779	-0.08484	-0.09780	-0.07153	4.334	-0.60
2	FE2	0.4149	-0.2003	0.003293	-0.08413	-0.09809	-0.07191	4.354	-0.25
3	FE3	0.4150	-0.2004	0.003415	-0.08422	-0.09804	-0.07189	4.353	0.21
4	FE4	0.4149	-0.2005	0.003572	-0.08425	-0.09805	-0.07185	4.356	0.61
5	FE5	0.05136	0.08998	-0.1772	-0.07007	-0.09100	0.4191	1.836	0.12
6	FE6	0.05136	0.08983	-0.1771	-0.07018	-0.09058	0.4118	1.479	0.42
7	Ferulic acid	0.4199	-0.1986	0.01114	-0.08772	-0.09657	-0.06980	4.491	-0.63

The result of the calculation of atomic net charge, dipole, and log P of six ester ferulic derivatives are shown in Table 3. The substitution of a different chain of alkyl groups influenced the carbon net charge. The c of C_1 in ferulic acid derivatives slightly decreased relative to that of the atomic charge of C_1 in ferulic acid. This tendency is similar to the net atomic charge of C_2, C_3, C_5, and C_{12}. This indicates incorporating different alkyl chain can induce atoms in the adjacent position. The longer carbon chain establishes more non-polar compounds, so the solubility of the compound in lipid is greater and the anticancer activity enhances, respectively (See Table 1). The solubility in water and lipid of the compound enhanced its bioavailability in the cells, so the anticancer activity was performed more [26–27]. Ferulic acid has lower log P of -0.63. Furthermore, the dipole of ferulic ester declined to compare to the dipole of ferulic acid. Substitution of alkyl enhanced the value of log P. Higher hydrophobicity of a compound resulted in a better interaction with the binding site of the protein during the metastasis phase

of cancer cell development. This was reported in research using MMP-9 as a target protein using molecular docking [28]. On the other hand, the substituent chloroethyl in FE5 and bromoethyl in FE6 bear significantly lower dipole than other studied compounds which increase anticancer activity. The molecule with smaller dipole value demonstrated higher polarity, affecting the binding interaction with oxidative species. The addition of the ferulic compound provided the ability to reduce reactive oxygen species (ROS) which stimulated cancer cell proliferation [29]. High polarity molecule bound more comfortably with ROS leading to the prevention of cell proliferation by activating caspases, as well as associated protein which changes the chemical and morphological properties of the cells causing cell elimination by apoptosis [30].

3.2. Analysis of QSAR on ferulic acid derivatives

The relationship between chemical structure and biological activity (anticancer activity) was conducted by statistical calculation using the SPSS program. The best correlation between descriptors and anticancer activity for ferulic acid derivatives is shown in Table 4. It is seen that the descriptors which have a strong correlation with anticancer activity are log P, dipole (μ), and the net charge in C_4 and C_5. The prolongation of the alkyl group might increase nonpolar properties followed by enhancing of log P-value. Also, it shows that the importance of the halogen atom for enhancing anticancer activity. SE is the standard error of the estimated which explains error value about the calculation. It is seen that the SE value of this calculation is small (0.00857). Besides, the R^2 for the calculation is excellent at 0.998. This value represents that the calculation result can significantly explain the descriptors of the response data around its mean. It is seen that the equation is excluded from several descriptors, which are qC_1, qC_2, qC_3, and qC_9. This result indicated that those descriptors didn't influence the anticancer activity significantly. The activities of the ferulic acid derivatives were determined by log P, dipole, qC_4, qC_5. Atomic charges of C_4 and C_5 were the most affecting descriptors followed by dipole and log P. The prolongation of the alkyl chain of ferulic acid derivatives and incorporation halogen atom in ethyl moiety showed higher log P followed by a higher anticancer activity. This work suggests esterification with long alkyl chain and incorporation halogen atom in ferulic acid is favorable to give the higher anticancer activity of ferulic acid derivatives.

Table 4. The result of the best correlation between descriptors and anticancer activity for ferulic acid derivatives against HELA cell lines

Equation	$11.0 + 129C_4 + 53.8C_5 + 0.085\log P + 0.751\mu$
R	1
R^2	0.999
SE	0.00857
Sig	0.038
F	394

We were employed PRESS (predicted residual error sum of square) as cross-validation of this calculation. PRESS statistic is calculated as the sum of the squares of all the resulting prediction errors. The calculated Log ($1/IC_{50}$) has a low PRESS value of 7.50E-05 (see Table 5) which indicates the calculation of calculated log ($1/IC_{50}$) using the QSAR Hansch Model for ferulic acid derivatives has excellent agreement with experimental data of Log ($1/IC_{50}$).

Table 5. Experimental log ($1/IC_{50}$), calculated log ($1/IC_{50}$), and PRESS value for ferulic acid derivatives against HELA cell lines

Compound	Experimental Log ($1/IC_{50}$)	Calculated Log ($1/IC_{50}$)	Residual error	[Residual error]^2
FE1	-1.964	-1.963	-1.17E-03	1.38E-06
FE2	-1.845	-1.842	-3.26E-03	1.06E-05
FE3	-1.806	-1.813	6.49E-03	4.22E-05
FE4	-1.785	-1.781	-4.55E-03	2.07E-05
FE5	-1.505	-1.505	-2.54E-04	6.43E-08
FE6	-1.740	-1.740	-1.02E-05	1.03E-10
		PRESS		7.50E-05

4. Conclusion

Based on that best QSAR model, the chemical descriptors that strongly influence anticancer activity are partition coefficient of n-octanol/water (Log P), dipole moment (μ), and atom charge in C_4 and C_5 on ferulic acid derivatives. In the future study, this research suggests to design the new compound with higher Log P and lower dipole moment for giving excellent bio-activity. The result of this study could be used to develop the structure of new ferulic acid derivatives to be produced in the lab-scale to confirm their actual performance.

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