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Article

In silico Prediction of Sodium-Glucose Co-Transporter-2 (SGLT2) Inhibition Activity by *Allium Fistulosum* Compound Based on SkelSpheres Molecular Descriptor

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Abstract: The Sodium-Glucose co-transporter-2 (SGLT2) inhibitor represents a novel agent for the treatment of type 2 diabetes. Drugs of this class function by inhibiting glucose reabsorption in the kidneys, thereby controlling blood glucose levels. It is known that SGLT2 inhibitors activate the AMPK signaling pathway by increasing the expression and activity of AMP-activated protein kinase (AMPK). In vivo tests have demonstrated that ethanolic and aqueous extracts of Welsh onion leaves (*Allium fistulosum* L) can reduce body weight, liver weight, adipocyte size, and enhance AMP-activated protein kinase (AMPK) expression. In this study, the inhibitory activity (IC_{50}) of compounds within *Allium fistulosum* against SGLT2 was predicted using the Support Vector Regression (SVR) predictive model and the SkelSpheres descriptor. The results of the predicted IC_{50} measurements for compounds present in the 70% ethanol extract of *Allium fistulosum* in silico indicate that 4 tyramine derivatives and 1 decursidate compound exhibit Excellent or Potent inhibitor activity criteria (IC_{50} < 1 μM). Among these, the four tyramine group compounds are the isomers N-trans-feruloyltyramine and N-cis-feruloyltyramine, as well as the isomers N-trans-feruloyl-3'-methoxytyramine and N-cis-feruloyl-3'-methoxytyramine. The findings of this study suggest that the ability of *Allium fistulosum* to enhance AMPK expression is possibly achieved through the inhibition of SGLT2.

Keywords: *Allium fistulosum*; SGLT2 Inhibitor; IC_{50}; Support Vector Regression (SVR); SkelSpheres

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1. Introduction

Diabetes mellitus has become a significant global health problem. Besides disrupting blood glucose metabolism, diabetes also has the potential to affect various organs and systems within the body. One of the components that has been a focus of research in diabetes treatment is the Sodium-Glucose co-transporter-2 (SGLT2) protein transport. The use of SGLT2 inhibitors has proven effective in controlling blood sugar in type 2 diabetes patients. Additionally, the use of SGLT2 inhibitors has been associated with additional benefits such as weight reduction, blood pressure control, and cardiovascular risk reduction [1-3]. Several drugs of this class have been approved by regulatory agencies in various countries and have become an integral part of diabetes management. These drugs include canagliflozin, dapagliflozin, empagliflozin, and ertugliflozin, all of which have

been approved by the Food and Drug Administration (FDA) for the treatment of type 2 diabetes [4].

Sodium-glucose co-transporter 2 (SGLT2) is a protein found in the kidney tubules that plays a crucial role in glucose reabsorption from the urine back into the bloodstream. SGLT2 inhibitors are a class of medications designed to treat type 2 diabetes by targeting this specific protein. These inhibitors work through the following mechanisms: [5,6]. They inhibit the function of the SGLT2 protein in the kidney tubules, reducing the kidney's ability to reabsorb glucose. This leads to more glucose remaining in the urine and being excreted from the body. This process is called glucosuria, which is the excretion of glucose in the urine. As a result, blood glucose levels can be better controlled because glucose is eliminated from the body through urine, thereby reducing the circulating glucose levels. By reducing the reabsorption of glucose by the kidneys, SGLT2 inhibitors help lower blood glucose levels in type 2 diabetes patients. This condition helps improve glycemic control and reduces the risk of long-term diabetes complications such as organ damage, vascular disorders, and neuropathy.

Welsh onion (*Allium fistulosum L*) is a commonly used plant in Indonesia as a culinary ingredient and is usually consumed fresh (stalks and leaves). *A. fistulosum* in water and ethanol extracts is known to reduce body weight, liver weight, adipocyte size, and increase the expression of AMP-activated protein kinase (AMPK) in the livers of rats on a high-fat diet [7,8]. In a series of experimental studies, SGLT2 inhibitors were found to activate the AMPK signaling pathway, manifested by increased AMPK expression and activity [9]. AMPK is known to enhance glucose uptake and insulin sensitivity, a process that is reduced in obese individuals and contributes to insulin resistance development [10].

Figure 1. Welsh onion (*Allium fistulosum* L) [11]

Based on the above explanations, there is a possible connection between the ability of *A. fistulosum* to increase AMPK expression and its role as an SGLT2 inhibitor. In this study, the activity of compounds in *A. fistulosum* as SGLT2 inhibitors will be predicted using an in-silico approach employing a Machine Learning model based on their chemical structure similarity descriptors (including stereochemical similarity) with SGLT2 inhibitor compounds present in the ChEMBL database [12]. The chemical structure similarity descriptor used in this research is SkelSpheres. This descriptor works by assessing the similarity of size and the number of matching ring fragments between two molecules compared to the total number of ring fragments in each molecule [13]. These fragments overlap with each other, requiring alignment of stereo centers within these fragments. Thus, molecules with high similarity in size and the number of ring fragments will match (resemble) the 3D shape in most molecules. Compounds commonly found in *A. fistulosum* include allicin, phenolic acids such as p-coumaric, ferulic acid, and

flavonoids like rutin and quercitrin. There are also 12 new compounds that are more stable and have been successfully extracted using 70% ethanol, making them suitable for quality standardization and industrialization of *A. fistulosum* as an herbal medicine [14]. These compounds will be predicted for their activity as SGLT2 inhibitors, expressed as the half maximal inhibitory concentration (IC_{50}) value.

2. Results

The SGLT2 inhibitor data (CHEMBL3884; UniProt P31639) retrieved through Datawarrior were filtered to include only data with Type IC_{50} activity, resulting in 1558 compounds. Out of these 1558 compounds, 17 compounds with IC_{50} values equal to 0 were removed, resulting in a total of 1541 compounds. Clustering the compounds using the SkelSpheres descriptor with a highest similarity of 0.9 yielded 349 representative compounds that were used as training data. The remaining 1192 compounds (non-representative) were used as testing data. The results of the predictive model validation against the predicted Log (IC_{50}) values of the compounds in the testing data, compared with the actual Log (IC_{50}) values, are shown in Figure 2 and Table 1.

In Figure 2, it can be observed that the predictive model for Log (IC_{50}) values using SVR on the testing data compounds resulted in an R2 value of 0.6995 and a Pearson correlation coefficient of 0.8360. According to Chin (1998), an R-Square (R2) value above 0.67 is considered strong [17]. Additionally, following Hinkel et al. (2003), correlation coefficients ranging from 0.70 to 0.90 are categorized as high positive correlation [18]. Therefore, in comparison to the other two predictive models (KNN regression and PLS), SVR is the best-performing model for predicting Log (IC_{50}) values of *A. fistulosum* compounds as SGLT2 inhibitors.

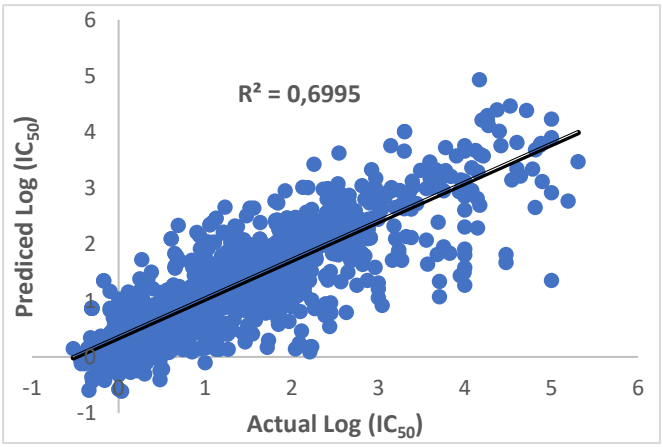


Figure 2. Predicted Log (IC_{50}) vs Actual Log (IC_{50}) regression of SGLT2 inhibitor using SVR

Table 1. Log (IC_{50}) prediction model validation of SGLT2 inhibitor

Prediction Models	R-square (R2)	Koefisien Korelasi Pearson
SVR	0.6995	0.8360
PLS	0.6492	0.8060
KNN	0.6213	0.7880

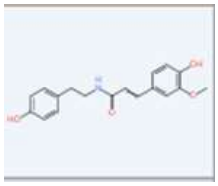
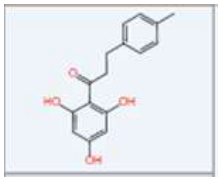
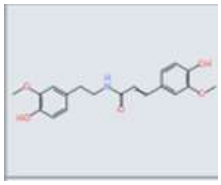
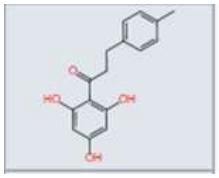
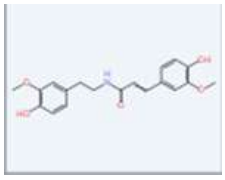
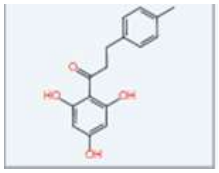
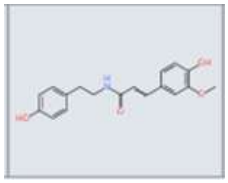
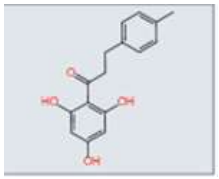
The predicted IC50 values of A. fistulosum compounds as SGLT2 inhibitors (from Table 4) using the SVR prediction model are presented in Table 2. In Table 2, it can be observed that the range of predicted IC_{50} values falls within the range of 0.25 – 48.94 μM. According to the activity criteria established by Indrayanto et al. (2020), [16] this IC_{50} range corresponds to moderate inhibitor activity up to excellent or potent inhibitor activity.

Table 2. Predicted IC_{50} values of *A. fistulosum* compounds as SGLT2 inhibitors using the SVR prediction model.

COMPOUND	CID	IC_{50} (μM)	Criteria
coumaran	10329	33.33	Moderate inhibitor activity
4-hydroxyphenylglycol	3081980	48.45	Moderate inhibitor activity
N-trans-feruloyltyramine	5280537	0.78	Excellent or potent inhibitor activity
N-cis-feruloyl-3′-methoxytyramine	5322166	0.42	Excellent or potent inhibitor activity
N-trans-feruloyl-3′-methoxytyramine	5352115	0.37	Excellent or potent inhibitor activity
N-trans-coumaroyltyramine	5372945	5.72	Good inhibitor activity
N-cis-feruloyltyramine	6440659	0.90	Excellent or potent inhibitor activity
2-hydroxy-1-(2-hydroxyphenyl) ethanone	13625346	48.94	Moderate inhibitor activity
3-methoxy-coumaran	45090856	34.79	Moderate inhibitor activity
(1-(2-ethoxyphenyl)-1,2-ethanediol)	82712982	35.17	Moderate inhibitor activity
decursidate	102004630	0.25	Excellent or potent inhibitor activity

Out of the 5 compounds with excellent or potent inhibitor activity criteria, 4 of them belong to the tyramine group, while the remaining 1 compound is decursidate. The four tyramine group compounds are the isomers N-trans-feruloyltyramine and N-cis-feruloyltyramine, as well as the isomers N-trans-feruloyl-3'-methoxytyramine and N-cis-feruloyl-3'-methoxytyramine. Therefore, it can be inferred that the two tyramine groups of compounds and decursidate are the most potent candidates as SGLT2 inhibitors. If the four compounds in the 'excellent' category are examined for their similarity using the SkelSpheres descriptor with one of the compounds in the training data (CID 58178431) with an IC_{50} value of 1.8 μM (excellent), the highest similarity obtained is 0.6775, as shown in Table 3.

Table 3. Similarity analysis of four compounds with 'excellent' criteria against compound CID 558178431 using the SkelSpheres descriptor

CID	IUPAC	Structure of SMILES	Highest Similiarity (SkelSpheres)	Most Similar Structure
5280537	N-trans-feruloyltyramine		0.67775	
5322166	N-cis-feruloyl-3?-methoxytyramine		0.60662	
5352115	N-trans-feruloyl-3?-methoxytyramine		0.60662	
6440659	N-cis-feruloyltyramine		0.67775	

3. Discussion

The sodium-glucose cotransporter-2 (SGLT2) inhibitor has been proven effective in enhancing glycemic control and weight reduction in patients with type 2 diabetes mellitus (T2DM). Additionally, SGLT2 inhibitors can also be utilized in certain overweight and non-diabetic obese adults [19]. Obesity itself is recognized as a significant risk factor for the development of type 2 diabetes (T2DM) [20]. SGLT2 inhibitors play a role in improving glucose uptake and insulin sensitivity by increasing the expression and activity of AMP-activated protein kinase (AMPK) [9,10]. Based on the findings of this study, the ability of *A. fistulosum* to enhance AMPK7 expression appears to be linked to the potential of its compounds as SGLT2 inhibitors. The mechanism of action of SGLT2 inhibitors can be shown in Figure 3.

In this research, two compound groups within *A. fistulosum*, namely tyramine and decursidate, were predicted in silico to act as Excellent or Potent SGLT2 inhibitors. Tyramine is known to stimulate glucose uptake and glucose transport in insulin-sensitive tissues both in vitro and in vivo [21,22]. Tyramine has also demonstrated hypolipidemic and anti-obesity effects in rats with hypercholesterolemia and obesity [23]. Tyramine derivatives have been shown to inhibit α-glucosidase in vitro [24]. Moreover, in studies on alloxan-induced diabetic rats, tyramine was found to reduce blood glucose levels, triglycerides, total cholesterol, and glycosylated hemoglobin concentration [25]. Further exploration of tyramine and decursidate derivatives within *A. fistulosum* is needed to evaluate their potential as SGLT2 inhibitors and their ability to enhance AMPK expression.

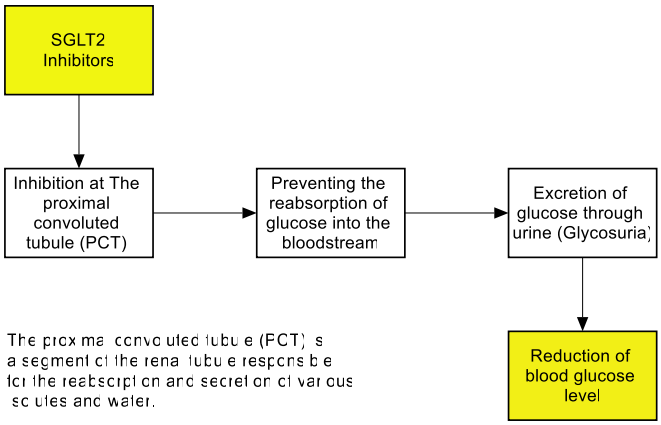


Figure 3. The mechanism of action of SGLT2 inhibitors

The SVR predictive model using the SkelSpheres descriptor is the most appropriate regression model based on the R-square value for predicting SGLT2 inhibition (in terms of IC_{50}) compared to the KNN regression and PLS models. This is because SVR adapts the best regression line around the threshold value. The threshold value represents the distance between the hyperplane and the decision boundary. This is different from other regression models that aim to minimize the error between actual and predicted values. In this way, the SVR model selects points within the decision boundary line that have the smallest error rate, or are within tolerance limits [26]. Based on the validation results, the SVR predictive model with the SkelSpheres descriptor can be used for screening compounds as SGLT2 inhibitors. Nevertheless, to improve the R2 (accuracy) value generated by the predictive model, other machine learning models can be utilized. DataWarrior only provides three types of predictive models: SVR, KNN regression, and PLS, which becomes a limitation in this study. However, it is essential to measure the actual IC_{50} values of compounds within *A. fistulosum* as SGLT2 inhibitors in vitro to compare them with the predicted IC_{50} values obtained in this study.

4. Materials and Methods

4.1 Dataset Preparation

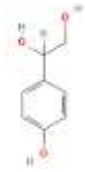
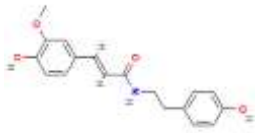
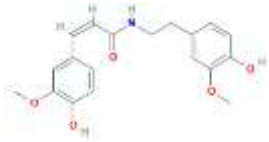
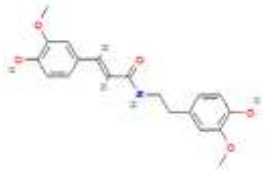
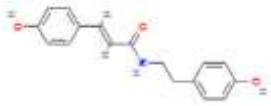
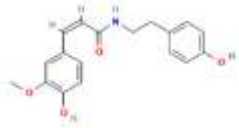
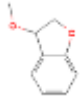
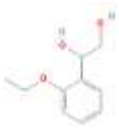
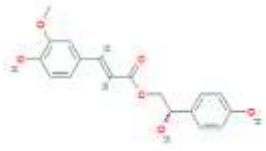
SGLPT2 inhibitor compounds were obtained from the ChEMBL database (CHEMBL3884; UniProt P31639) through Datawarrior [15]. ChEMBL is a database of bioactive chemical molecules with drug-like properties that are manually curated [11]. In Datawarrior, SGLPT2 inhibitor compound data containing half maximal inhibitory concentration (IC_{50}) values were extracted and separated. The clustering of compounds was performed using the SkelSpheres descriptor with a highest similarity threshold of 0.9. The results of compound clustering broadly consisted of two groups: 1) compounds that represent the molecules within their group (as training data) and 2) other compounds outside these representative compounds (non-representative). Compounds within the representation group were then used as training data, while compounds in the non-representation group were used as testing data. For the purpose of predictive model validation, IC_{50} values in both training and testing data were transformed into Log (IC_{50}).

4.2 Predictive Model Validation/Machine Learning

The Log (IC_{50}) values in the testing data were considered the 'actual Log (IC_{50})' values, and the values predicted by the predictive model/machine learning were the 'predicted Log (IC_{50})' values. The 'predicted Log (IC_{50})' values were obtained using the 'predict missing value' feature in the machine learning tools available in the 'chemistry' menu of Datawarrior. The validation of various Log (IC_{50}) prediction models such as Partial Least Square (PLS), K-Nearest Neighbor (KNN) regression, and Support Vector Regression (SVR) was performed by measuring the R-square (R2) values and Pearson correlation

coefficients based on the 'predicted Log (IC_{50})' values and the 'actual Log (IC_{50})' values of the SGLT2 inhibitor compounds present in the testing data.

Table 4. Compounds of *A. fistulosum* in 70% ethanol extract [14]

COMPOUND	STRUCTURE	CID
coumaran		10329
4-hydroxyphenylglycol		3081980
N-trans-feruloyltyramine		5280537
N-cis-feruloyl-3′-methoxytyramine		5322166
N-trans-feruloyl-3′-methoxytyramine		5352115
N-trans-coumaroyltyramine		5372945
N-cis-feruloyltyramine		6440659
2-hydroxy-1-(2-hydroxyphenyl)-ethanone		13625346
3-methoxy-coumaran		45090856
(1-(2-ethoxyphenyl)-1,2-ethanediol		82712982
decursidate		102004630

4.3 Prediction of IC_{50} Values for Compounds in *A. fistulosum*

The compounds within *A. fistulosum* for which 'predicted Log (IC_{50})' values against SGLT2 will be determined can be seen in Table 4. The 'Predicted Log (IC_{50})' values for the compounds in Table 1 were determined using the best prediction model (based on the highest R2 and Pearson correlation coefficients) from the validation results. The obtained 'Predicted Log (IC_{50})' values were then transformed into IC_{50} values, and the inhibitory activity criteria were determined based on the criteria established by Indrayanto et al. (2020) [16].

5. Conclusions

The SkelSpheres Molecular Descriptor, used to create a predictive model for the activity of compounds as SGLT2 inhibitors, yielded an R-squared (R2) value of 0.6995 with an SVR model. This SVR model was then utilized to predict the IC_{50} value for SGLT2 inhibitors in compounds from *Allium fistulosum*. The IC_{50} prediction results indicate that there are four derivatives of the tyramine and decursidate in *Allium fistulosum* that show excellent potential as SGLT2 inhibitors.

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