

Indonesian Herbal SGLT2 Inhibitor Discovery through Pharmacophore-Based Virtual Screening

Rezwendy, Rezi Riadhi Syahdi, Arry Yanuar*

ABSTRACT

Objective: Sodium-glucose cotransporter 2 (SGLT2) inhibitor had been evaluated in clinical trials as the basic strategy of hyperglycemia handling in diabetes. However, because of SGLT2 inhibitors is the new class of oral antidiabetic, it is rare to be found in Indonesia, and it is costly. This study was intended to find compounds from Indonesian herbal database that show capability to be used as SGLT2 inhibitors through a pharmacophore-based virtual screening approach. **Methods:** The SGLT2 inhibitor pharmacophore models were made from 10 training sets of SGLT2 ligand inhibitors using the Ligand Scout 4.1.5. Ten pharmacophore models which had been made were validated using test set and decoy set methods to know how the performance of pharmacophore model worked. Virtual screening were then applied to the best pharmacophore model. **Results:** The model-1 pharmacophore was the best model, with values of 0.9080, EF1% = 56.5, EF5% = 56.5 and AUC100% = 0.87 which served as model for virtual screening. Model-1 consisted of one hydrophobic interaction, one aromatic ring, four hydrogen bond donors and five hydrogen bond acceptors. Virtual screening showed three compounds (Hits) with best pharmacophore fit scores according to model-1 among 1377 compounds, they were vitexin = 113.62; cucumerin A = 112.62; and cucumerin B = 113.51. **Conclusion:** These results showed that vitexin, cucumerin A, and cucumerin B potentially have activity as an SGLT2 inhibitor. **Key words:** Diabetes, Pharmacophore, SGLT2 Inhibitor, Virtual Screening.

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History

- Submission Date: 30-11-2017;
- Review completed: 18-01-2018;
- Accepted Date: 14-02-2018

DOI : 10.5530/pj.2018.4.136

Article Available online

<http://www.phcogj.com/v10/i4>

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INTRODUCTION

According to the International Diabetes Federation, Indonesia had 10 million cases of diabetes in 2015 and is predicted to continue to rise until 2040. Diabetes mellitus (DM) is a significant risk factor in the progression of microvascular complications (retinopathy, nephropathy, and neuropathy) and macrovascular (coronary heart disease, cerebrovascular, and peripheral blood vessels).¹ Diabetes mellitus type-2 is one of the most common types of diabetes, i.e., more than 90-95% of patients with type-2 diabetes mellitus. A study from United Kingdom Prospective Diabetes (UKPDS) showed that every 1% decrease in hemoglobin (HbA1c) was related with a 37% decrease in the risk of microvascular complications and 21% risk of diabetes-related complications or death. The consensus of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) recommends the target of HbA1c in the treatment of diabetes is less than 7%, but most patients fail to achieve it. Therefore, an ideal pharmacological compound for lowering blood glucose is required so that the target HbA1c <7% can be fulfilled.²

A new class of drugs, the sodium-glucose cotransporter 2 inhibitor (SGLT2) had been evaluated in randomized-controlled clinical trials for its role as a

treatment for hyperglycemia in diabetes. SGLT2 is an efficient transport system for glucose reabsorption of glomerular filtration. SGLT2 has 90% role in inhibiting glucose reabsorption in the renal tubules. The elevated levels of glucose can be inhibited by SGLT2 by transferring glucose from the blood to the urine, where glucose is mainly drawn from the endothelium and organs having harmful effects on the urine system.³ So, this SGLT2 inhibitor raised a new perspective on glycosuria, especially in patients with diabetes mellitus. The clinical effect of SGLT2 inhibitors, including lowering HbA1c levels, lead to weight loss when accompanied by healthy lifestyle and diet, lowers systolic blood pressure, lowers fasting blood glucose levels and decreases uric acid which indicates the decrease of cardiovascular events.⁴ Some drugs that had been approved by the FDA are Canagliflozin, Dapagliflozin, and Empagliflozin.⁵ However, the SGLT2 inhibitor is a new class of drugs that are expensive and still rare to be found in Indonesia. There are lots of herbs in Indonesia which can use for alternative diseases therapy, one of them is diabetes mellitus. Some herbs that can be used as an alternative treatment for patients with diabetes mellitus are *Camellia sinensis*, *Pometia pinnata*, *Syzy-*

Cite this article: Rezwendy, Syahdi RR, Yanuar A. Indonesian Herbal SGLT2 Inhibitor Discovery through Pharmacophore-Based Virtual Screening. *Pharmacogn J.* 2018;10(4):803-7.

gium polyanthum, *Artocarpus heteropyllus*, *Lagerstroemia speciosa* and *Persea Americana*.⁶ Based on that, the idea to look for a potential new SGLT2 inhibitor of Indonesian herbs appears.

The research for new compounds from potential herbal plants as SGLT2 inhibitors can be done by virtual screening. It is a cheap and fast method for searching for new lead compounds in various targets.⁷ Universitas Indonesia had developed a database contains data of herbal compounds and species of plant in Indonesia. This database can be used as a reference source for virtual screening which can be accessed through herbaldb.farmasi.ui.ac.id. The pharmacophore modeling method is the most commonly used in virtual screening to find molecules that trigger the desired biological effects.⁸ In 2014, a researcher had found eight candidate compounds having activity as the HDAC2 inhibitor by using pharmacophore-based virtual screening method. This has proved that virtual screening could be used as method for exploring candidate compounds and also, the method is efficient because it is very suitable for virtual screening on a large scale.⁹

Based on above, so, the study of pharmacophore-based virtual screening on SGLT2 inhibitor from Herbal Indonesia databases was conducted and expected to find herbal compounds that can be used for further research of diabetes drugs with a faster time and lower costs.

MATERIALS AND METHODS

Materials

The instrument which was used were Terminal, LigandScout 4.1.5 (InteLigand®, Austria), MarvinSketch (ChemAxon).

The materials used in this study were a three-dimensional structure of SGLT2 ligand inhibitor from several kinds of literature. The ligands can be downloaded from PubChem or drawn with MarvinSketch.¹⁰⁻¹³ Ligands from Indonesian herbal database (herbaldb.farmasi.ui.ac.id) were also used in this work.¹⁴ A decoy structures were made using the software on website A Directory of Useful Decoys - Enhanced (DUD-E) (<http://www.dude.docking.org>) using the known ligand structures of SGLT2 inhibitor structure.

Inhibitor SGLT2 Pharmacophore Modelling

Pharmacophore modeling of the SGLT2 inhibitor by using LigandScout 4.1.5 program which began with selecting training set. The first step was to download active ligands of SGLT2 inhibitors from PubChem site (<https://pubchem.ncbi.nlm.nih.gov/>) and made the structure of the compound by using MarvinSketch application. Then the active ligands were inserted into the “ligand-based perspective” in the LigandScout application. The ligand conformation was generated from the ligand set. Then the ligands were set and clustered according to pharmacophore similarity and the distance. Furthermore, the active compounds that had been clustered were selected and chosen to serve as a training set, and the rest was used as a test set. Then the pharmacophore model was created from the ligand training set.

Validation of Pharmacophore Model

The decoy structure files that had been obtained from [dude.docking.org](http://www.dude.docking.org) in “picked” format were converted to “smiles/sdf” format with MarvinSketch. The formation of the test set and decoy set was conducted by using LigandScout by clicking on the “create screening database” icon. Then, at the input part, the decoy file was inserted and in the output part, filled the desired name with the format “ldb”, then click next. The desired conformation generator was selected from conformation settings menu. After it had been done, the step of inserting the decoy file on the input section, for other decoy files, with the same output file as the update and keep old settings to add the new file without deleting the old files in the database. The formation of the test set database was conducted like a

decoy set formation with a different “ldb” output file name. The test set and decoy databases which were created in “ldb” format then entered in the database screening by clicking “Load screening databases” for validating the established model of pharmacophore. Mark the test set in green as the active molecule and red for the inactive molecule. A model of pharmacophore was selected, and the screening process was run by clicking “perform screening” and “ROC curve plot.” After the screening process was completed, the value of ROC curve (Receiver Operating Characteristics) which consisted of AUC (Area Under Curve) and EF (Enrichment Factor) were checked.

Virtual Screening Based on Pharmacophore 3D Model

Virtual screening was conducted in a virtual screening perspective, then several stages performed, namely the herbal database of Indonesian herbal which has been downloaded through the website www.herbaldb.farmasi.ui.ac.id in “ldb” format, then put into the database of virtual screening by clicking on the “Load screening databases” icon. The herbal database that has been entered was marked in green. Then model of pharmacophore was selected, and the screening process (click “perform screening icon”) was conducted to the selected model, this process ran for some time. This process was then carried out on another preferred pharmacophore model. The candidate compound (hits) of herbal database screening results were sorted according to the best pharmacophore-fit score. Visualization of pharmacophore features of candidate compounds (hits) included HBA (Hydrogen Bond Acceptors), HBD (Hydrogen Bond Donor), AR (Aromatic Ring), and hydrophobic interactions (H).

RESULTS AND DISCUSSION

Inhibitor SGLT2 Pharmacophore Modelling

A total of ten ligands were selected for training set and the remainder were served as a test set for validation of the established pharmacophore model from 108 ligands. The chemical structure of the selected training set is shown in Figure 1.

Ten training set compounds were selected on each cluster that had been made before. Selection of each class was also based on variations of IC₅₀ data and some FDA-approved ligands as well as canagliflozin, dapagliflozin, and empagliflozin to provide a good pharmacophore model.

Furthermore, based on the results of the pharmacophore modeling, produced ten models of pharmacophore ligand-based 3D mapping and served as a model best pharmacophore virtual screening. The results of model mapping and the score of the formed pharmacophore were shown in Table 1.

Based on the results of the mapping Table pharmacophore models, the model-1 had the best scoring pharmacophore model which was 0.9080. Furthermore, each pharmacophore models formed was validated by using test sets and decoy sets that have been made using LigandScout 4.1.5 application.

Validation of Pharmacophore Model

Pharmacophore model validation was performed to obtain the ROC curve, the value of Area Under Curve (AUC), and Enrichment Factor (EF), aimed to help evaluate the quality of the pharmacophore model is made from the training set before the virtual screening process. It also tests the performance of the test, how well the test can recognize the active and inactive compounds during the screening process. Validation of the pharmacophore model was performed using test set and decoy set. Test sets were made of 98 ligands that were not included in the training set and described the active compound for validation of the pharmacophore, while the decoy set describing the inactive compound. Decoy sets were made from the SGLT2 inhibitor ligands that were incorporated into A Directory of Useful Decoys-Enhanced (DUD-E) website (<http://www.dude.docking.org>).

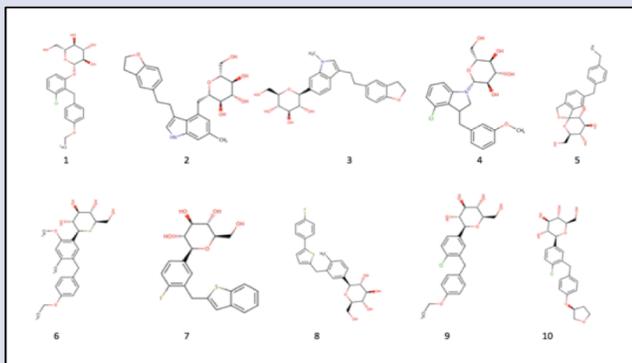


Figure 1: Training Set Ligands 1. Compound 2 (0.49 nM); 2. Compound 7 (24.0 nM); 3. Compound 69 (67 nM); 4. Compound 93 (893.0 nM); 5. Tofogliflozin (2.9 nM); 6. Luseogliflozin (2.26 nM); 7. Ipragliflozin (7.38 nM); 8. Canagliflozin (3.1 nM); 9. Dapagliflozin (1.2 nM); 10. Empagliflozin (2.7 nM).

Table 1: SGLT2 Inhibitor Pharmacophore Model Mapping.

Pharmacophore Model	Pharmacophore Features	Scoring of Pharmacophore Model
1	H, AR, 5 HBA, 4 HBD	0.9080
2	H, AR, 5 HBA, 4 HBD	0.8863
3	2 AR, 5 HBA, 4 HBD	0.8847
4	2H, 2 AR, 5 HBA, 4 HBD	0.8739
5	2H, 2 AR, 5 HBA, 4 HBD	0.8715
6	3H, 2 AR, 5 HBA, 4 HBD	0.8596
7	2H, 2 AR, 5 HBA, 4 HBD	0.8536
8	3H, 1 AR, 5 HBA, 4 HBD	0.8493
9	3H, 1 AR, 5 HBA, 4 HBD	0.8396
10	3H, 1 AR, 5 HBA, 4 HBD	0.8330

H: Hydrophobic

AR: Aromatic ring

HBA: Hydrogen Bond Acceptor

HBD: hydrogen Bond Donor

dude.docking.org). The result were 5,430 compounds in a decoy set. ROC analysis ten models of pharmacophores which had been conducted, then validated by assessing the sensitivity and specificity of SGLT2 inhibitors using test sets and decoy sets to generate ROC curves consisted of AUC (Area Under Curve) and EF (Enrichment Factor). Table 2 shows results from the pharmacophore models validation.

Based on the data, model-1 was considered the best model used for pharmacophore-based SGLT2 inhibitor virtual screening to obtain the active compound from Indonesia herbal databases. It was based on the scoring model of pharmacophore 0.9080, with EF1% and EF5% which got the highest and consistent value of 56.5 and AUC100% of 0.87 with a sensitivity of 0.7347 and TPR 0.0000. Besides, the value of ROC result which was shown to be very close to the upper left corner.¹⁵ Model-1 showed that the pharmacophore model could recognize and difference to the true positive, false positive, true negative and false negative hits with the highest accuracy.

Table 2: Result of pharmacophore model validation.

Model	Sensitivity	1-Specificity	AUC100%	EF1%	EF5%
1	0.7347	0	0.87	56.5	56.5
7	0.7959	0.000184162	0.90	56.4	55.7
3	0.7959	0.000736648	0.90	56.4	53.7
6	0.5510	0	0.78	56.4	56.4
4	0.5306	0	0.77	56.4	56.4
2	0.5408	0	0.77	56.4	56.4
10	0.8061	0.001289134	0.90	56.4	51.8
5	0.7857	0.000184162	0.89	55.4	55.7
9	0.7041	0.000184162	0.85	55.4	55.6
8	0.6836	0.000184162	0.84	55.4	55.6

Models sorted by EF1%, EF5% and AUC100%

Table 3: Hits of Virtual Screening.

No	Hits	Molecule Formula	Pharmacophore-Fit score
1	Vitexin	C ₂₁ H ₂₀ O ₁₀	113.62
2	Cucumerin B	C ₂₉ H ₂₈ O ₁₁	113.51
3	Cucumerin A	C ₂₉ H ₂₈ O ₁₁	112.62

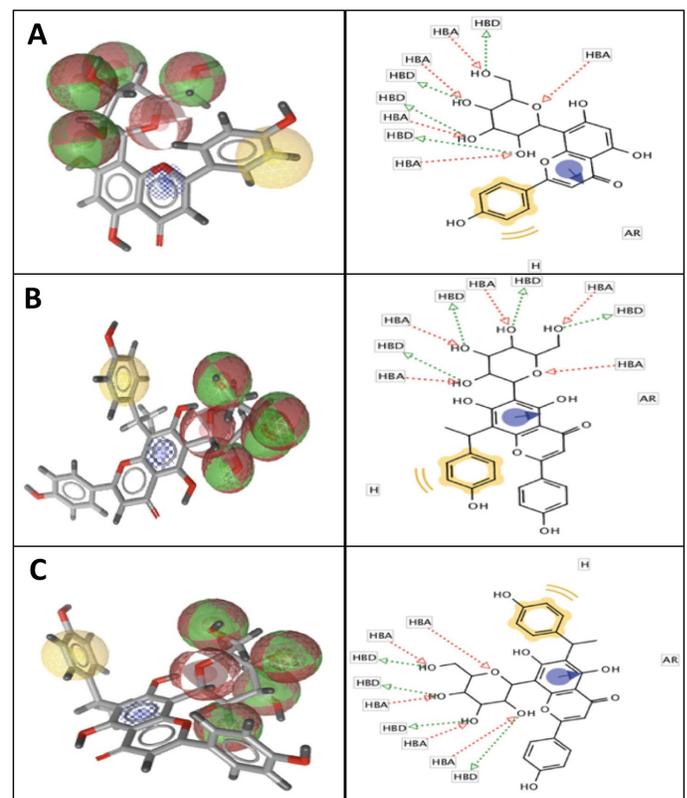


Figure 2: Pharmacophore model visualization with hits in 2D and 3D structure. A. Vitexin; B. Cucumerin B; C. Cucumerin A.

Virtual Screening Analysis

Indonesia's herbal databases which contain a total of 1377 active compounds were screened, in which the compounds with the same pharmacophore similar features to the model-1, will be used as hits or candidates that were predicted to have SGLT2 inhibitor activity. Virtual screening performed on model-1 yielded 58 candidate compounds (hits). A total of 58 candidate compounds had been produced were; three compounds had the best score and suitability based on the model-1 pharmacophore feature. The three compounds can be seen in the Table 3. Based on Figure 2, the three candidate compounds (Hits) comprising vitexin, cucumerin B, and cucumerin A, comply with all pharmacophore features of model-1 that made from the SGLT2 compound training set inhibitor. It was characterized by aromatic rings (purple), hydrophobic areas (yellow), hydrogen bonding acceptors (red color) and hydrogen bonding donors (green) each occupying each structure of vitexin, cucumerin B, and cucumerin A.

Vitexin

Vitexin was one of the flavonoid group compounds, namely apigenin flavon glycoside which has very low solubility in water.¹⁶ The results of a search through the database of Indonesian herbal showed that the compound vitexin derived from some plants. The plants containing vitexin are *Camellia sinensis* (tea), *Clinacanthus nutans* (Gendis), *Combretum quadrangulare* (gulp), *Crataegus laevigata* (Hawthorn), *Crataegus monogyna* (Hawthorn), *Desmodium triflorum* (Delilan), *Garcinia dulcis* (Kemenjing), *Garcinia hombroniana* (Manggis Utan), *Rhynchosia rufescens*, *Tamarindus indica* (Tamarind), *Theobroma cacao* (tree cacao), *Toona sureni* (suren), *Trigonella foenum-graecum* (fenugreek) and *Vitex littoralis* (Wood kula). Vitexin in these plants has hypoglycemia activity that can be used as an antidiabetic. This was evidenced by several studies have been done on these plants, including the results of dekokta leaves and unripe fruit of the plant *Crataegus* mice induced Streptozotocin diabetes to produce the effect of normalizing the value of plasma lipid peroxides and lowering blood glucose levels in diabetes.¹⁷

Cucumerin A and Cucumerin B

Result through Indonesian herbal databases showed that the cucumerin A and cucumerin B compounds were from the same plant that is *Cucumis sativus* (cucumber). No research shows that cucumerin A and cucumerin B compounds have antihyperglycemic activity, but according to Mukherjee *et al.*, cucumerin A and cucumerin B compounds contained in cucumbers, noted to have antidiabetic activity. Mukherjee *et al.* mentioned that the Cucumerin A and Cucumerin B compounds were two new major C-glycosyl flavonoid products found in cucumber leaf tissue.¹⁸

CONCLUSION

Based on the pharmacophore-based virtual screening analysis, it can be concluded that the candidate compounds (hits), i.e., vitexin, cucumerin A and cucumerin B, potentially having an activity of SGLT2 inhibitor according to suitable of pharmacophore features in the model created from the training set of SGLT2 inhibitor active compound.

ACKNOWLEDGMENT

This study was supported by PITTA 2017 Grant, Directorate of Research and Community Engagement, Universitas Indonesia.

CONFLICT OF INTEREST

The authors are declare that there is no conflict of interest.

ABBREVIATIONS

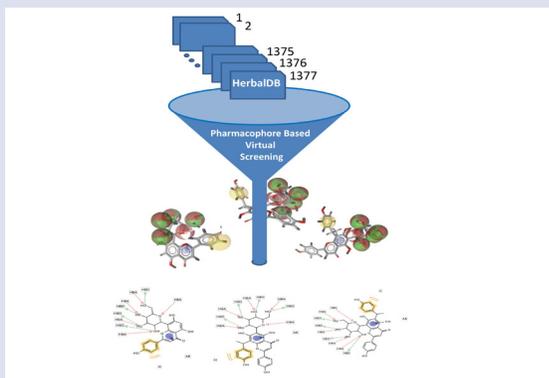
SGLT2: Sodium glucose cotransporter 2; **H:** Hydrophobic; **AR:** Aromatic ring; **HBA:** Hydrogen Bond Acceptor; **HBD:** hydrogen Bond Donor; **AUC:** Area Under Curve; **EF:** Enrichment Factor; **ROC:** Receiver Operating Characteristic.

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Cite this article: Rezwendy, Syahdi RR, Yanuar A. Indonesian Herbal SGLT2 Inhibitor Discovery through Pharmacophore-Based Virtual Screening. *Pharmacog J*. 2018;10(4):803-7.

GRAPHICAL ABSTRACT



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SUMMARY

- This paper examines about Indonesian Herbal SGLT2 Inhibitor Discovery through Pharmacophore-Based Virtual Screening. Sodium glucose cotransporter 2 (SGLT2) inhibitor had been evaluated in clinical trials as the basic strategy of hyperglycemia handling in diabetes. However, because of SGLT2 inhibitors is the new class of oral antidiabetic, it's rare to be found in Indonesia and it is costly. This study was intended to find compounds from Indonesian herbal database (herbaldb.farmasi.ui.ac.id) that capable to work as SGLT2 inhibitors database through a pharmacophore-based virtual screening approach. The methods of SGLT2 Inhibitor Discovery are Inhibitor SGLT2 Pharmacophore Modelling, Validation of Pharmacophore Model and Virtual Screening Based on Pharmacophore 3D Model using LigandScout 4.1.5 (InteLigand®, Austria), Terminal and MarvinSketch (ChemAxon). The SGLT2 inhibitor pharmacophore models were made from 10 training sets of SGLT2 ligand inhibitors, then they were validated. The best model served as model for virtual screening. Virtual screening showed three compounds (Hits) with best pharmacophore fit scores according to the best model among Indonesian herbal database structure, they were vitexin, cucumerin A and cucumerin B. Therefore, based on the pharmacophore based virtual screening analysis, it can be concluded that the candidate compounds (hits) i.e. vitexin, cucumerin A and cucumerin B, potentially having an activity of SGLT2 inhibitor according to suitable of pharmacophore features in the model created from the training set of SGLT2 inhibitor active compound.