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Original Article

MOLECULAR DOCKING STUDY OF THE MAJOR COMPOUNDS FROM *GARCINIA ATROVIRIDIS* ON HUMAN SGLT-2 PROTEIN TRANSPORT USING STRUCTURE-BASED DRUG DESIGN METHOD

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ABSTRACT

Objective: The objective of this work was to study the molecular interactions of phytochemicals in *Garcinia atroviridis* with SGLT-2 protein transport.

Methods: Molecular docking simulation using Autodock 4.2 was performed to explore the binding affinity of phytochemicals in *Garcinia atroviridis* against SGLT-2 protein transport. The structure-based pharmacophore model was derived using LigandScout 4.4 Advanced to investigate the important chemical interactions of the ligands and protein target. The evaluation was conducted based on the free energy binding and visualization *in silico*.

Results: From this study, Myricetin is the most effective compound having similarity of interaction with the amino acid residue, 4 of 5 are hydrogen bond interactions between the amino acid; HIS80, ASN75, TRP291, and LYS321 amino acid interacted with the oxygen as the proton acceptor from benzenes of the Myricetin structure, in addition, Myricetin also has the lower binding energy and inhibition constant (-9.54 kcal/mol and 101.93 nM, respectively) as compared to other compounds.

Conclusion: Hence, Myricetin could become the potential compound as an antidiabetic agent in the future with good activity and lower side effects.

Keywords: *Garcinia atroviridis*, SGLT-2, Molecular Docking, Pharmacophore Modeling

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INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder involving inappropriately elevated blood glucose levels [1, 2]. It may be due to impaired insulin secretion, resistance to peripheral actions of insulin, or both fields [3]. Diabetes is a worldwide epidemic; according to the International Diabetes Federation (IDF), the global prevalence of DM was 537 million adults (20-79 y). This number is predicted to rise to 643 million by 2030 and 783 million by 2045. In 2021, the number of people with diabetes mellitus in Indonesia is estimated at around 19,5 million people with a prevalence rate of 10,6%.

For non-pharmacological therapy, lifestyle changes and physical activity such as exercise is the most common strategy to manage DM therapy, while pharmacological therapy involves the administration of oral anti-diabetic medications, and insulin therapy is also available. Biguanide, Sulfonylurea, Thiazolidinedione, GLP-1 Receptor Agonist, and Sodium-Glucose co-Transporter 2 (SGLT 2) Inhibitors are some of the most often prescribed oral antidiabetics [4]. SGLT2 inhibitors are a new class of antidiabetic drugs approved to treat diabetes [5].

However, some oral diabetes medications have disadvantages in terms of adverse effects, solubility, permeability, and absorption. Metformin has a limited permeability, which causes it to absorb slowly. Sulfonylureas have several negative side effects, including the inhibition of liver regeneration and the development of obesity and osteoporosis, both of which increase the risk of fracture. Furthermore, OADs with short half-lives, such as Ripaglinide and Pioglitazone, have reduced bioavailability [6, 7]. Alternative therapies, such as herbal medicine, are needed in the treatment of diabetes mellitus due to the limits of some OAD.

G. atroviridis, often known as gelugur acid, is a common cooking ingredient among Indonesians. Citric acid, tartaric acid, malic acid, and ascorbic acid are among the acids found in *G. atroviridis* fruit. *G. atroviridis* also includes Hydroxycitric Acid (HCA), which is found in the fruit of Garcinia cambogia, Garcinia indica, Garcinia cowa, and Garcinia atroviridis. HCA extracted from Garcinia cambogia is currently being sold as a weight-loss supplement. This acid aids in weight loss and appetite control by inhibiting the formation of the enzyme ATP citrate lyase, which can turn excess carbs into fat. Furthermore, this enzyme has the potential to stimulate the production of glycogen from glucose [8].

Studies related to the activity of chemicals found in gelugur acid, particularly assays targeting the SGLT-2 transporter protein, which is a transporter in the proximal renal tubule that reabsorbs glucose from the tubular lumen, are still limited. Drug development is time-consuming and expensive; hence *in silico* testing is an excellent choice to determine the effectiveness of the molecules in gelugur acid are as antidiabetics agents targeting the SGLT-2 transport protein by *in silico* structure-based drug design study.

MATERIALS AND METHODS

Identification of target receptors and the lead compound

The identification of targets in this study was carried out based on a common receptor used for studying antidiabetic effects, particularly on protein transport SGLT-2. Initial screening on the receptor targets and the lead compound were assessed from several parameters, namely based on the method used to extract the receptor as well as the amino acid composition and based on the source of the organism and the resolution of each receptor [9].

Validation using molecular docking method

The validation using the molecular docking method was carried out on the Structure of a human SGLT2-MAP17 complex bound with empagliflozin [10]. The receptor was downloaded from the Protein Data Bank database (https://www.rcsb.org/) in (pdb) format [11, 12]. The preparation was then carried out on the receptor, starting by separating the receptor with the complex lead compound in the receptor using the Discovery Studio Visualizer software. The water molecule at the receptor was removed to minimize the deviation of the formation of the hydrogen bonding interaction. The next preparation was further carried out using Autodock 4.0.1 software,

where each receptor and ligand were added Kollman Charge and Compute Gasteiger Charge. The step was continued with the addition of polar hydrogen to the protein molecule and non-polar merged-hydrogen to the ligand molecule. Each of the ligand and receptor molecules was then saved into the Protein Data Bank Partial Charge (Q) and Atom Type (T) (pdbqt) format. The Grid Parameter File (gpf) and the Docking Parameter File (dpf) were prepared by combining the data of the ligand (pdbqt) and the receptor (pdbt) and then setting other docking parameters (set GA Runs to 100 and energy to 2500000). In the final stage, redocking was carried out using the command prompt (CMD) to interpret the data obtained from the validation results of the docking method [12].

Virtual screening on test compounds

In this study, the antidiabetic activity test of molecular compounds from *Garcinia atroviridis* was carried out using the Structure-based drug design (SBDD) method. The SGLT-2 receptor (Protein Transport) was used as a test target, while the Empaglifozin was used as the lead compound [13].

The molecular test compounds were modeled using Chem Draw 2D and then the energy minimization of the model was performed using MM2 0.01 in Chem Draw 3D. The results of the structure obtained after the energy minimization stage were then saved into (pdb) format. The preparation was then continued using Autodock 4.0.1 to add the Compute Gasteiger Charge and non-polar merged hydrogen to both structures and compounds. In the final step, a Grid Parameter File (gpf) and a Docking Parameter File (dpf) were created by combining each test compound with the target receptor for the docking process [12].

Pharmacophore modeling

A Structure Based-pharmacophore model has been derived automatically from the X-ray derived structure of SGLT-2 protein transport in complex with Empaglifozin (PDB code: 7VSI) using Ligandscout 4.4 Advanced [14]. All of the phytochemical compounds from *Garcinia atroviridis* were screened virtually using the Structure-Based pharmacophore model and the LigandScout 4.4 Advanced.

RESULTS

Table 1: Identification of receptors and the lead compound

PDB ID	Receptor	Classification	Complexed ligand	Method	Organism	Resolution
7VSI	SGLT2-MAP17	Transport Protein	Empaglifozin	Electron Microscopy	Homo Sapiens	2.95 A

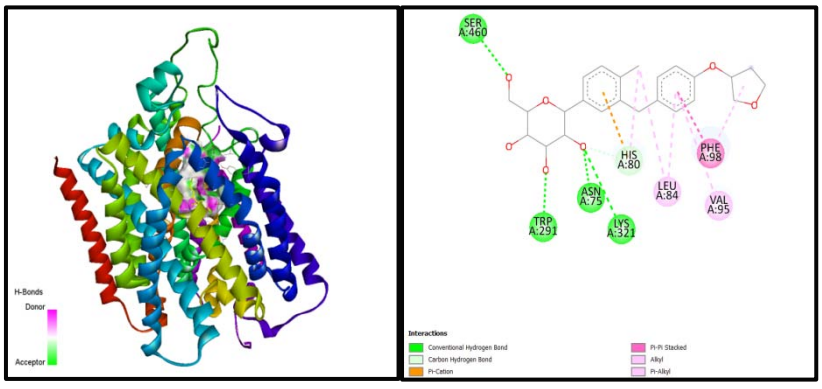
Table 1 shows the profile of the SGLT2 receptor as obtained from the PDB database, where this receptor is classified as a transport protein (involves the movement of a protein from one cellular or extracellular compartment to another) [15]; the receptor has a resolution value of 2.95 Å (is a measure of the level of detail present in the diffraction pattern and the level of detail that will be seen when the electron density map is calculated). High-resolution structures, with resolution values of 1 Å or so, are recommended for a clear visual of every atom in the electron density map. Lower resolution structures, with the resolution of 3 Å or higher, show only the basic contours of the protein chain, and atomic structure must be inferred) [16]. Empagliflozin (SGLT2 Inhibitor) complexed with the receptor, which will be employed as the lead drug in this trial [17]. Empagliflozin is a newer class of antihyperglycemic agent that its inhibitor-related with weight loss and blood pressure reductions and provide a low inherent risk of hypoglycemia [18, 19].

Table 2: Validation using molecular docking method

Receptor	Amino acid interaction	Free energy (ΔG Gibs)	Inhibition constant	RMSD
7VSI	SER460, LYS321, ASN75, TRP291, HIS80, PHE98, VAL95, LEU84	-11.58 kcal/mol	3.27 nM	0.91 A

Table 2 shows the values of the validation method's parameter values, where the major parameter is the value of the Root Mean Standard Deviation (RMSD) and the cluster of population. RMSD is a value that represents the difference in the position of the native ligands before docking and after redocking (with a requirement of<2 Armstrong) [20]. The data from the test results show that the SGLT-2 receptors have results that meet the requirements with RMSD values of 0.91. These clusters showed the distribution of data from 100 docking conformations thus, they are called the best clusters and the best molecular docking [21]. Clustering of docked conformations is determined by the rms tolerance parameterized by "rmstol" in the docking parameter file (dpf). The more number of clusters, the more favorable because of the probability value of the preferred conformation to be docked to the protein target [17].

From the validation results of the docking method, the free energy binding value for the Empaglifozin was-11.58 kcal/mol with an inhibition constant of 3.27nM. These findings serve as primary data for the first validation test as for this receptor; as a result, we are unable to make a comparison with previous studies, which are still limited after our review, given that this receptor comprises a receptor that was first released in 2022. However, when considering the same lead compound and target category, the results of this investigation tend to be similar to those of Nair *et al.*, in terms of energy binding values [22].



1A **1B**

Fig. 1: A (3D Visualization); B (2D Visualization) of molecular interaction between SGLT-2 and empaglifozin

Fig. 1 Represent the molecularly interaction between Emphaglifozin with SGLT-2. Conventional hydrogen bonding interaction on the SER460, LYS321, ASN75, TRP291 as well as the non-hydrogen bonding interaction on HIS 80, PHE98, VAL95 and LEU84 amino acids. These values were used as standards to assess the antidiabetic activity of the test compounds on molecular docking screening. The type of amino acid that interacts with the receptor, as previously indicated, is comparable to that found in previous studies. This data is used as a reference to see if the test compounds have the same pattern, especially on LYS321, ASN75, PHE98, HIS80, VAL95, and LEU84, which will eventually indicate if the compound has activity or not [17].

Table 3: Virtual screening result

No	Lead and test compounds	Amino acid residue	Free energy (ΔG Gibs)	Inhibition constant (IC)
1	Empaglifozin (Lead compound)	SER460, LYS321, ASN75, TRP291, HIS80, PHE98, VAL95, LEU84.	-11.58 kcal/mol	3.27 nM
2	Hydroxycitric Acid	GLN457, ASN75, LYS321, GLU99, TRP291, PHE98, TYR290.	-4.56 kcal/mol	214.73 µM
3	Citric Acid	TRP291, HIS80, ASN75, LYS321, GLU99, GLN45, TYR290.	-4.74 kcal/mol	335.67 µM
4	Malic Acid	LYS321, GLU99, HIS80, ASN75, GLN457, TRP29.	-4.30 kcal/mol	708.29 µM
5	Tartaric Acid	GLN457, LYS321, GLU99, TRP291, ASN75, HIS80, TYR290	-4.35 kcal/mol	651.93 µM
6	Luteolin	GLY79, LYS321, TRP291, HIS80, GLY83, LEU283, PHE98, TYR290.	-9.38 kcal/mol	113.912.19 nM
7	Myricetin	HIS80, ASN75, GLU99, LYS321, TRP291, TYR290, LEU84, ALA102.	-9.54 kcal/mol	101.93 nM
8	Quercetin	GLY79, HIS80, TRP291, LYS321, GLU99,TYR290, PHE98, LEU84	-9.26 kcal/mol	161.91 nM

Table 3 Virtual screening result of compounds test on SGLT-2 protein transport compared with Emphaglifozin, using structure-based drug design method, with the value of GA runs 100 and medium energy of 250.000. The data includes amino acid residue (parameters used to assess the similarity of activity between the test compound and the lead compound based on the type of interaction and amino acids), Free energy (parameter value used to assess the strength of the interaction formed, where the lower the energy, the stronger the bond and the spontaneous bond formed) [23], and the value of the Inhibition constant are among the information provided (a parameter that describes the potency of a drug based on the value of the inhibition constant, where the smaller the value, the higher the biological activity) [24].

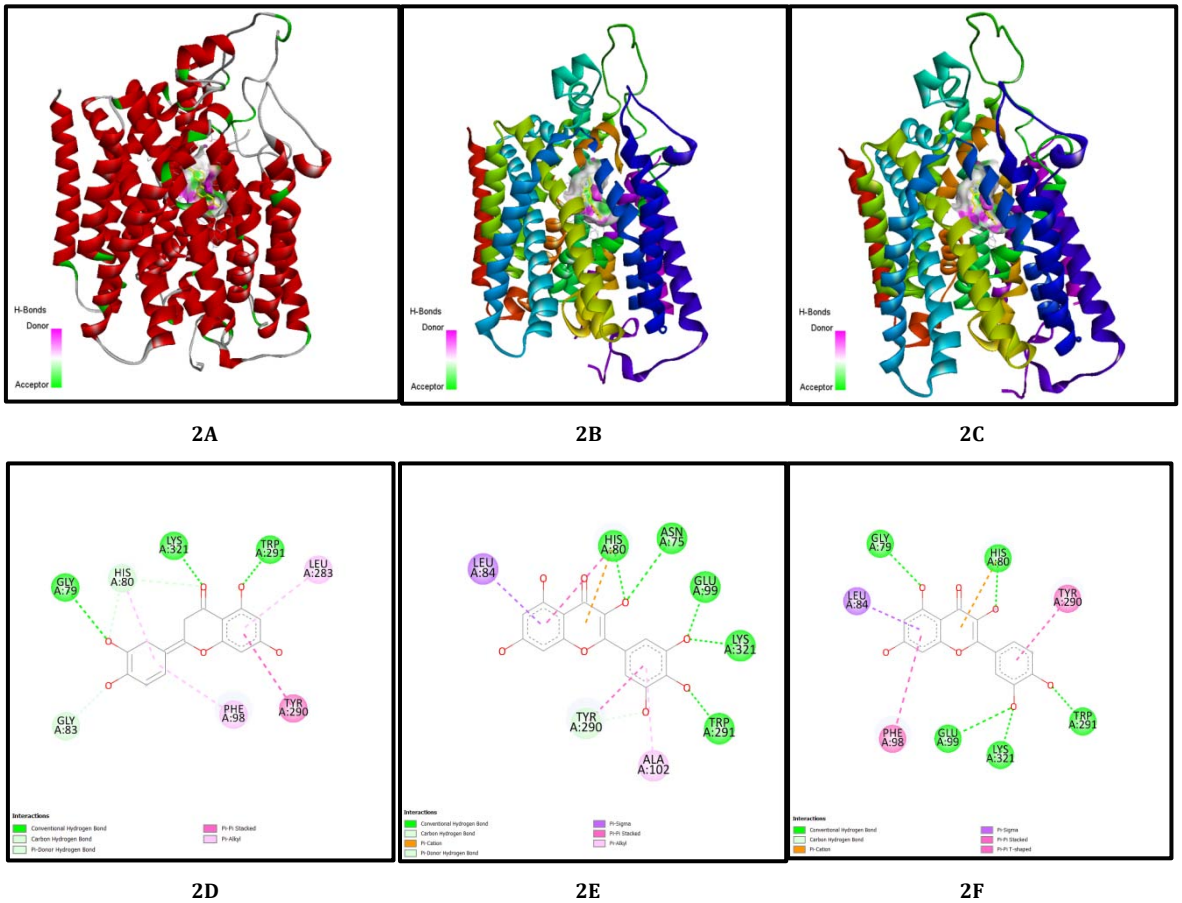


Fig. 2: 3D Interaction of Luteoin (A), Myricetin (B), and Quercetin (C) against SGLT-2 and 2D-Interaction of Luteoin (A), Myricetin (B), and Quercetin (C)) Interaction against SGLT-2

Fig. 2. 3D visualization shows the binding site of the test compounds at the SGLT-2 receptor, which can be used to determine whether the compound has competitive or non-competitive inhibitory activity. Based on these findings, all potent compounds bind to the same active site in the receptors, similar to as the lead compound (Empaglifozin), whereas for 2D Visualization, it refers to the type of

amino acid residue interaction carried by each test compound, as described in table 2. We discovered that the amino acid residue of the three compounds had the same interaction pattern with the lead compound (LYS321, TRP291, HIS80, and PHE98 for Luteolin; TRP291, ASN75, LYS321, HIS80, and LEU84 for Myricetin; and TRP291, LYS321, HIS80, LEU84, and PHE98 for Quercetin. This amino acid is a key amino acid that can represent the similarity of activity between the test compound and the lead compound [17, 25].

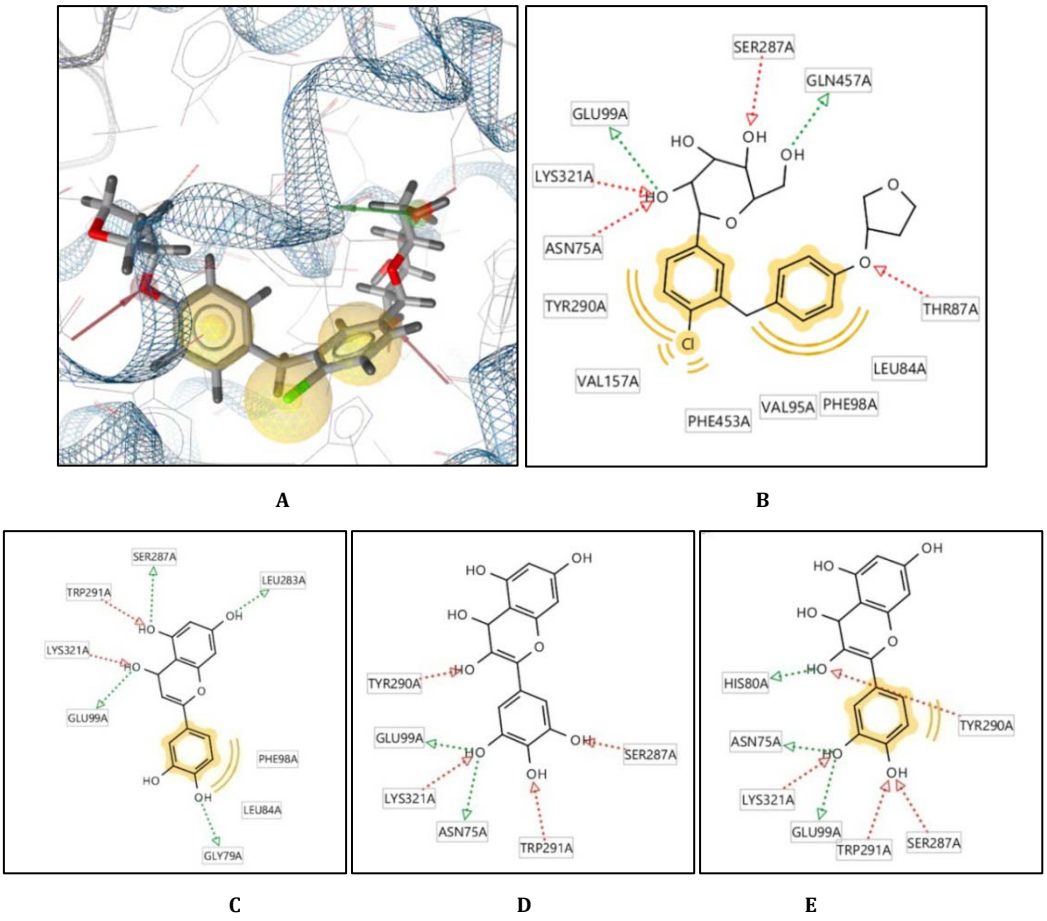


Fig. 3: Pharmacophore model of (A-B): Empagliflozin (3D and 2D Visualisation) and pharmacophore model of luteolin (C), Myricetin (D), and Quercetin (E)

Fig. 3 represents the pharmacophore model of Emphaglifozin and the best compounds (Luteolin, Myricetin, and Quercetin). Pharmacophore modeling was carried out to investigate the active functional groups responsible for interacting with the target, as well as the potential parts of the structure that could be modified to improve the effectiveness and/or recover the chemical structures' physicochemical limitations [14, 26]. Empagliflozin was used as a lead compound or a comparator for the subjects' test. The result shows that hydroxyl is the major functional group that corresponds to bond with the important amino acid residue, while trihydroxybenzopiran becomes the potential part that can be modified in future drug development due to its un present interaction with the amino acid residue. We discovered the same results after doing a literature review on the activity of Luteolin, Myricetin, and Quercetin on a variety of disease targets, which revealed that the hydroxy group plays a key role in the interaction with amino acids at the receptor [27-32].

DISCUSSION

Based on the analysis results from the Protein Data Bank database, the SGLT-2 receptor was obtained using electron microcopy X-ray crystallography, NMR spectroscopy, and electron microscopy, which are among the current methods in determining a protein's structure. Each method has its own set of benefits and drawbacks. For each approach, numerous pieces of information were employed to develop the final atomic model, The molecule's structure was in the X-ray diffraction pattern in X-ray crystallography which contains information on the conformation and distance between atoms that are close to one another for NMR spectroscopy. The SGLT-2 receptor was also obtained from human which is good as a representative to gain similar results in the human body. The SGLT-2 receptor has a resolution value of 2.95 which is considered to be the most fulfilling receptor towards the standard due to its resolution value that is closed to 2 Armstrong [8]. The resolution value parameter represents the similarity of the structure obtained with the original receptor structure.

Molecular docking methods were carried out on the SGLT-2 receptor and the results were emphasized on the value of *Root Mean Standard Deviation* (RMSD). RMSD is a value that represents the difference in the position of the native ligands before docking and after redocking (with a requirement of<2 Armstrong) [20]. The results show that the SGLT-2 receptors have RMSD values of 0.91. The free energy binding for Empaglifozin was-11.58 kcal/mol with an inhibition constant of 3.27nM and having hydrogen bonding interaction on the SER460, LYS321, ASN75, TRP291 as well as the non-hydrogen bonding interaction on HIS80, PHE98, VAL95, and LEU84 amino acids. These values were used as standards to assess the antidiabetic activity of the test compounds in the molecular docking screening [31].

Based on the results, 3 of 6 compounds (luteoin, myricetin, and quercetin) have the highest free energy binding of-9.38, 9.58, and-9.26 kcal/mol respectively as compared to the other compounds.

Lower free energy binding indicates lower activation energy. Therefore, the potential for the interaction between the compound and the receptor is accelerated (Spontaneous Reaction) [33] and assessed from the constant inhibition data. Three of the compounds have a lower value of inhibition constant (113.99 nM, 101.93 nM, and 161.91 nM, respectively). This value represents the capacity of the compound to inhibit receptors or enzymes, whereas a relatively low value is considered to have great power because at low concentrations, a compound has a large inhibitory capacity [34]. In addition to considering the value of energy and inhibition constants, one of the parameters determining the activity of a compound is assessed from the interaction between the structure of the test compound and the amino acids at the receptors. Myricetin is a good candidate because it has a similar interaction with the amino acid residue, 4 of 5 are hydrogen bonding interactions with the HIS80, ASN75, TRP291, and LYS321 amino acid interaction with the oxygen as the proton acceptor from benzenes of the Myricetin structure. The hydrogen bonding interaction has a reversible interaction and is relatively stronger than other types of interactions [35, 36]. The similar interaction between the test compounds and the lead compound illustrates the same activity in binding to the receptor. The interaction that occurs between these compounds in the active pocket of the receptor is competitively able to prevent activation of the SGLT-2 receptor [37]. This mechanism of action will inhibit the Sodium-Glucose Co-Transport in the human body, which leads to reduce the glucose reabsorption in the renal tubule. The results show that Myricetin is the most potent compound as an antidiabetic agent than other compounds due to its low free energy binding, inhibition constant, and a high number of specific hydrogen-bonding-interaction.

In pharmacophore modeling studies, Emphaglifozin's hydroxyl and ether functional groups act as hydrogen bonding donors and acceptors, interacting with amino acids GLN457A, GLU99A, ASN75A, LYS321A, SER287A, and THR87A. (fig. 5). Luteolin has a functional group that is comparable to Emphaglifozin in that the hydroxyl groups are the major portion of the molecule that interacts with the receptor's amino acid. GLU99A, LYS321A, and SER287A are amino acid residues that are comparable to the lead molecule. While for Myricetin and Quercetin have ASN75, GLU999A, SER287A, and LYS321A are the amino acid residue which is similar to the lead compound. Both molecules also has hydroxyl as the primary functional group that interacts with the receptor. The key difference between these compounds is that on the pharmacophore model of myricetin, there is no pi-pi interaction, in addition, the trihydroxy and oxygen groups from benzopyran exhibit no interaction with the receptor. This indicates that it could be a potential choice for drug future development, to gain full effectivity, excellent physicochemical properties, and low adverse effects.

CONCLUSION

Luteolin, Myricetin, and Quercetin have antidiabetic activity by competitively inhibiting the Protein Transport SGLT-2 receptor. Among the compounds tested, Myricetin shows a good hydrogen bonding interaction, inhibition constants, and type of amino acid interaction binding as compared to Luteolin and Quercetin. From the pharmacophore modeling, hydroxyl is the major functional group exhibiting bonding with important amino acid residue. In addition, trihydroxy benzopyran has the potential to be a powerful functional group in future drug development.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

We declare that there is no conflict of interest in this study.

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