ISSN: 0067-2904

Computational Studies of Rhubarb's Anthraquinone Glycosides as Anti-Diabetic Drug Candidates through Sodium-Glucose Cotransporter-2 Inhibition

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Received: 24/1/2023 Accepted: 14/7/2023 Published: 30/8/2024

Abstract

Type 2 diabetes mellitus continues to pose a significant global health concern, warranting increased attention. This metabolic disorder is influenced by various factors, such as lifestyle, diet, environment, and genetics. While several approaches have been developed to address the incidence of type 2 diabetes mellitus, current treatments remain inadequate and necessitate further improvement. In this study, our objective was to assess the potential antidiabetic efficacy of anthraquinone glycosides present in rhubarb (specifically physcion diglucoside and aloe-emodin-8-glucoside) as potential inhibitors of the sodium-glucose cotransporter-2. We employed an in silico study to evaluate their inhibitory properties. The computational prediction was performed included the target protein, ligands retrieval, and preparation. Furthermore, the molecular docking process was conducted to assess the interaction between the target protein and ligands. Subsequently, data visualization and analysis were performed. Various indicators were evaluated, including the characteristics of the target molecule and drug candidates, binding affinity scores, interaction positions, types of chemical interactions, amino acid residues involved, hydrophobicity, formation of hydrogen bonds, interpolated charge, and ionizability. These comprehensive evaluations provided valuable insights into the molecular interactions and potential efficacy of the tested compounds. In this computational study, we demonstrated that the both rhubarb anthraquinones, physcion diglucoside and aloe-emodin-8-glucoside, have a great potential as antidiabetic agent by inhibiting the action of the sodium-glucose cotransporter-2. Thus, the inactive the sodium-glucose cotransporter-2 can prevent the excessive number of glucose plasma level in type two diabetes mellitus people.

Keywords: Anti-diabetic, inhibitor, *in silico*, rhubarb, Sodium-Glucose Cotransporter-2

1. Introduction

Type 2 diabetes mellitus (T2DM) is a prevalent global health issue [1, 2]. The incidence of T2DM continues to be risen annually due to factors such as population growth, aging, and

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changes in lifestyle [3, 4]. According to the World Health Organization, the number of individuals affected by T2DM has reached approximately 415 million and is projected to reach around 642 million by 2040 [5, 6]. Elevated blood glucose levels are a key factor in the development of T2DM, with several associated complications such as cardiovascular disease, end-stage renal disease, retinopathy, and neuropathy [5, 9]. Blood glucose levels are influenced by both extrinsic and intrinsic factors. Extrinsic factors include unhealthy lifestyle choices and uncontrolled dietary habits, while intrinsic factors pertain to dysfunctions within biological systems, such as the gastrointestinal, endocrine, or cardiac systems [10, 11].

When it comes to tackling the profound effects of T2DM, a wide array of modalities spanning traditional and modern approaches have emerged. The current emphasis lies in the adoption of a holistic and health-conscious lifestyle, entailing regular physical activity and the cultivation of improved dietary patterns, as the cornerstone for warding off the onset of T2DM [12, 13]. Complementing these lifestyle measures are an array of pharmacological interventions that have gained prominence in the management of T2DM. Synthetic insulin, alpha-glucosidase inhibitors, and PPAR-γ agonists are among the pharmacotherapeutic agents extensively employed, leveraging their ability to activate targeted biological mechanisms aimed at curtailing elevated blood glucose levels [14, 15]. The significance of these medications in combatting the incidence of T2DM cannot be overstated, as their utilization has become widespread in clinical practice [14-16].

The sodium-glucose cotransporter-2 (SGLT2) is known as the transporter protein responsible for glucose reabsorption in renal [17]. This system is highly efficient in re-collecting glucose in normal conditions, which can be used or stored for energy sources [18]. However, in T2DM people, the regular biological activity of SGLT2 needs to reduce to minimize the plasma glucose level. As a result, the SGLT2 inhibitor has been utilized in T2DM therapy to reduce SGLT2 function, causing filtered glucose in the kidneys to fail to reabsorb [19]. Despite the fact that current SGLT2 inhibitors such as canagliflozin, dapagliflozin, empagliflozin, and ertugliflozin have a promising future as antidiabetic drugs [20], these drugs require further evaluation or the identification of new drug candidates due to adverse effects such as acute kidney injury, bone fracture, infection, ketoacidosis, osmotic diuresis, pyelonephritis, and urosepsis [21, 22].

The use of natural products for medicinal purposes has increased dramatically [23, 24]. It has been known that medicinal plants contain bioactive compounds which exert their therapeutic effect [24]. Rhubarb, a group of plants widely distributed in tropical areas, has been used for many years, especially as the source of traditional medicine. Uniquely, anthraquinones are the major components of rhubarb, including aloe emodin, aloe-emodin-8-glucoside, chrysophanol chrysophanol-8-glucoside, emodin, isoemodin, laccaic acid, physcion, physcion diglucoside, physcion-8-glucoside, and rhein [25, 26]. Rhubarb plants, renowned for their therapeutic prowess encompassing antiviral, antitumor, anti-inflammatory, antioxidant, acute pancreatitis, acute ischemic stroke, chronic kidney disease, and neuroprotective properties, owe their efficacy to the presence of bioactive compounds [25-27]. While extensive research has delved into exploring the biological activity of free anthraquinones, the investigation specifically targeting anthraquinone glycosides remains limited in number, failing to provide a comprehensive understanding [28]. In light of this knowledge gap, the prime objective of this endeavor is to undertake an in-depth exploration of the antidiabetic potential harbored within rhubarb's anthraquinone glycosides, specifically physcion diglucoside and aloe-emodin-8-glucoside. This investigation will employ an in

silico study approach, enabling us to assess their efficacy as candidate inhibitors of the sodium-glucose cotransporter 2 (SGLT2).

2. Materials and Methods

The preparatory phase involved gathering the requisite materials, which encompassed the acquisition of ligand structures such as physcion diglucoside (CID: 442762), aloe-emodin-8-glucoside (CID: 126456371), and dapagliflozin (CID: 9887712) as the control drug for this particular investigation. The chemical structures were procured from the esteemed PubChem database (https://pubchem.ncbi.nlm.nih.gov/), providing a reliable and comprehensive source of information. Furthermore, to facilitate a thorough comparison of chemical structures, visualization, properties, and the prediction of target proteins between physcion diglucoside and aloe-emodin-8-glucoside (as depicted in Figure 1), pertinent data was extracted from the Swiss Institute of Bioinformatics (https://www.sib.swiss/) [29, 30]. The utilization of these reputable resources ensures the robustness and reliability of the data employed in the study.

In the subsequent steps, the homology modeling of the target protein was accomplished using the SWISS-MODEL platform (https://swissmodel.expasy.org/). To initiate this process, the protein sequence of SGLT-2 (ID: P31639) was retrieved from UniProt (https://www.uniprot.org), ensuring the utilization of accurate and up-to-date information. The obtained data from this study encompasses a comprehensive analysis of the chemical properties, target protein prediction, binding affinity scores, chemical interactions, amino acid residues, hydrophobicity, hydrogen bonds, interpolated charge, and ionizability of the protein-ligand complex. To carry out these analyses, additional procedures such as ligand and target protein structure optimization were performed. Subsequently, molecular docking was conducted using PyRx software (https://pyrx.sourceforge.io/), following similar protocols as described in our previous study [31, 32]. These standardized procedures ensure the reliability and consistency of the methodology employed in the investigation.

3. Results and Discussion

In the current study, a computational investigation was conducted to evaluate the antidiabetic activity of two anthraquinone compounds found in rhubarb, namely physcion diglucoside and aloe-emodin-8-glucoside. The findings revealed that these compounds exhibited a more favorable binding affinity score compared to the control drug, dapagliflozin (as shown in Table 1). These results highlight the potential of physcion diglucoside and aloe-emodin-8-glucoside as promising candidates for antidiabetic therapy. The binding affinity scores of rhubarb compounds showed more negative value. This negativity of the binding scores indicate that the physcion diglucoside and aloe-emodin-8-glucoside have favorable and more stable binding interaction to the target protein compared to the control drug [33, 34]. Consistent with that result, the binding coordinate of both physcion diglucoside and aloe-emodin-8-glucoside were in the same binding area with the control drug (Figure 2). Specifically, the data also showed that the three ligands share similar amino acid residues on van der Waals interaction cluster such as SER A:74, ASP A:201, ILE A:208, SER A:392, and ILE A:397. According to the binding position, physcion diglucoside and aloe-emodin-8-glucoside are comparable and suitable for subsequent anti-diabetic drug candidates.

Table 1: The properties of target protein and ligand complexes which define the binding affinity scores, chemical interactions, and amino acid residues

Complex	Binding Affinity	Chemical Interaction	Amino Acid Residue
SGLT2 - Physcion diglucoside	-10.4 kcal/mol	Van der Waals	PHE A:56, SER A:70, ALA A:73, SER A:74, GLY A:77, SER A:78, LYS A:154, MET A:198, ASP A:201, THR A:202, GLN A:204, THR A:205, ILE A:208, ILE A:297, CYS A:301, SER A:392, SER A:396, ILE A:397
		Conventional Hydrogen Bond	GLY A:79, ALA A:389, SER A:393
		Carbon Hydrogen Bond	ILE A:76
		Pi-Anion	ASP A:158
		Pi-Pi Stacked	HIS A:80
		Alkyl	VAL A:157, ILE A:456
SGLT2 - Aloe-emodin-8-glucoside	-9.6 kcal/mol	Van der Waals	SER A:74, SER A:78, GLY A:79, VAL A:157, ASP A:201, ILE A:208, ALA A:389, SER A:396, ILE A:397, ILE A:456
		Conventional Hydrogen Bond	LYS A:154, GLN A:204, THR A:205, SER A:393
		Carbon Hydrogen Bond	ALA A:73, ILE A:76, GLY A:77
		Pi – Anion	ASP A:158
		Pi – Pi Stacked	HIS A:80
SGLT2 - Dapagliflozin	-7.7 kcal/mol	Van der Waals	SER A:74, ASN A:75, GLY A:77, GLY A:79, GLY A:83, LYS A:154, VAL A:157, ASP A:201, GLN A:204, THR A:205, ILE A:208, TYR A:290, SER A:392, SER A:393, ILE A:397, PHE A:453, ILE A:456
		Conventional Hydrogen Bond	ALA A:73
		Pi – Anion	ASP A:158
		Pi – Pi Stacked	HIS A:80

Furthermore, each complex of protein – ligand interaction has certain chemical bond types namely; SGLT2 – physcion diglucoside complex (van der Waals, conventional hydrogen bond, carbon hydrogen bond, pi – anion, pi – pi stacked, and alkyl interaction); SGLT2 – aloe-emodin-8-glucoside complex (van der Waals, conventional hydrogen bond, carbon hydrogen bond, pi – anion, and pi – pi stacked interaction); SGLT2 – dapagliflozin complex (van der Waals, conventional hydrogen bond, pi – anion, and pi – pi stacked interaction) (Table 1). On the other hand, we also showed additional properties of SGLT2 and ligand complexes such as hydrophobicity, H-bonds, interpolated charge, and ionizability (Figure 3).

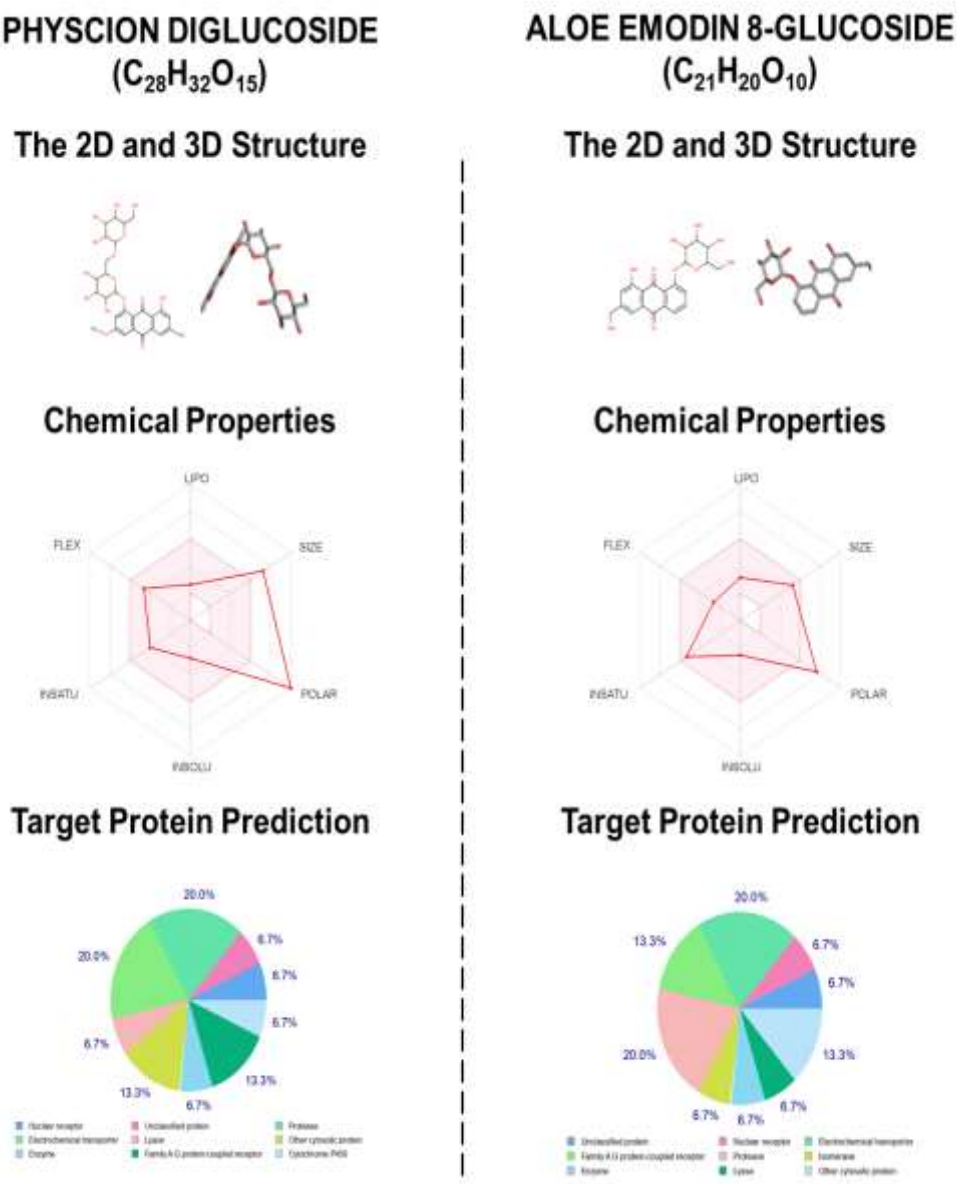

Figure 1: Chemicals structure visualization, properties, and target protein prediction physcion diglucoside and aloe-emodin-8-glucoside (https://www.sib.swiss/).

Within rhubarb, an assortment of chemical compounds can be found, encompassing stilbenes, tannins, polysaccharides, anthraquinones, and anthrones. Extensive investigations have underscored the diverse therapeutic benefits offered by these chemical constituents, including hepatoprotective, anticancer, anti-inflammatory, antibacterial, cardiovascular, gastrointestinal, and cerebrovascular protection. Notably, the rhubarb anthraquinones emerge as a pivotal bioactive component, housing various derivative compounds such as emodin, aloe-emodin, rhein, physcion, and more [26, 28]. The comprehensive array of bioactive compounds within rhubarb highlights its potential for multifaceted therapeutic applications, fostering a broad scope for further exploration and research endeavors. Furthermore, a different kind of constituent called anthraquinones glycosides are formed as the combination of free antraquinones and glycosyl, including aloe-emodin-8-glucoside, emodin-6-glucoside, emodin-8-glucoside, physcion diglucoside, and rhein-8-glucoside.

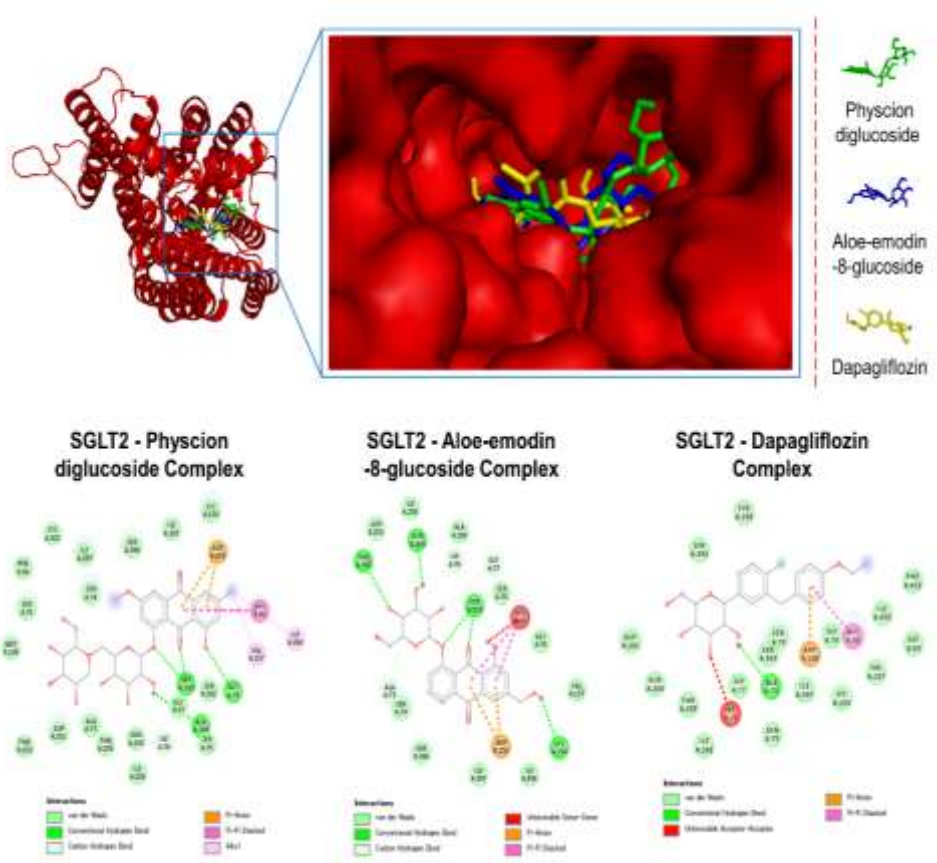

Figure 2: The natural compounds are perfectly bind in the same binding area with the drug control, dapagliflozin. Each target protein and ligand complex has the certain chemical interaction which also considered as the binding properties (https://discover.3ds.com/).

Importantly, multiple studies have found that antraquinone glycosides have therapeutic characteristics as antioxidants, anticancer agents, antiinflammatory agents, and protection against cerebral injury [26, 35]. Based on the study conducted by Cheng *et al*., the purified anthraquinone-glycoside isolated from rhubarb has the antidiabetic potency. Those isolated compounds reduce the oxidative stress in diabetic rat model by promoting the blood lipid metabolism and improving the antioxidant level [36]. Interestingly, another study conducted by Cui *et al*., has shown that the anthraquinone glycoside preparation from rhubarb (RAGP) ameliorated the T2DM rats model by modulating the gut microbiota and reducing the inflammation. The above study showed that the RAGP treatment combined with metformin during six weeks could significantly lower the gasting blood glucose, glycated serum protein, and insulin concentration [37]. Those above evidences indicate that the importance to evaluate and transform the computational study results into the experimental evaluation in order to confirm the role of physcion diglucoside and aloe-emodin-8-glucoside as anti-diabetic agent by blocking the SGLT2 activation.

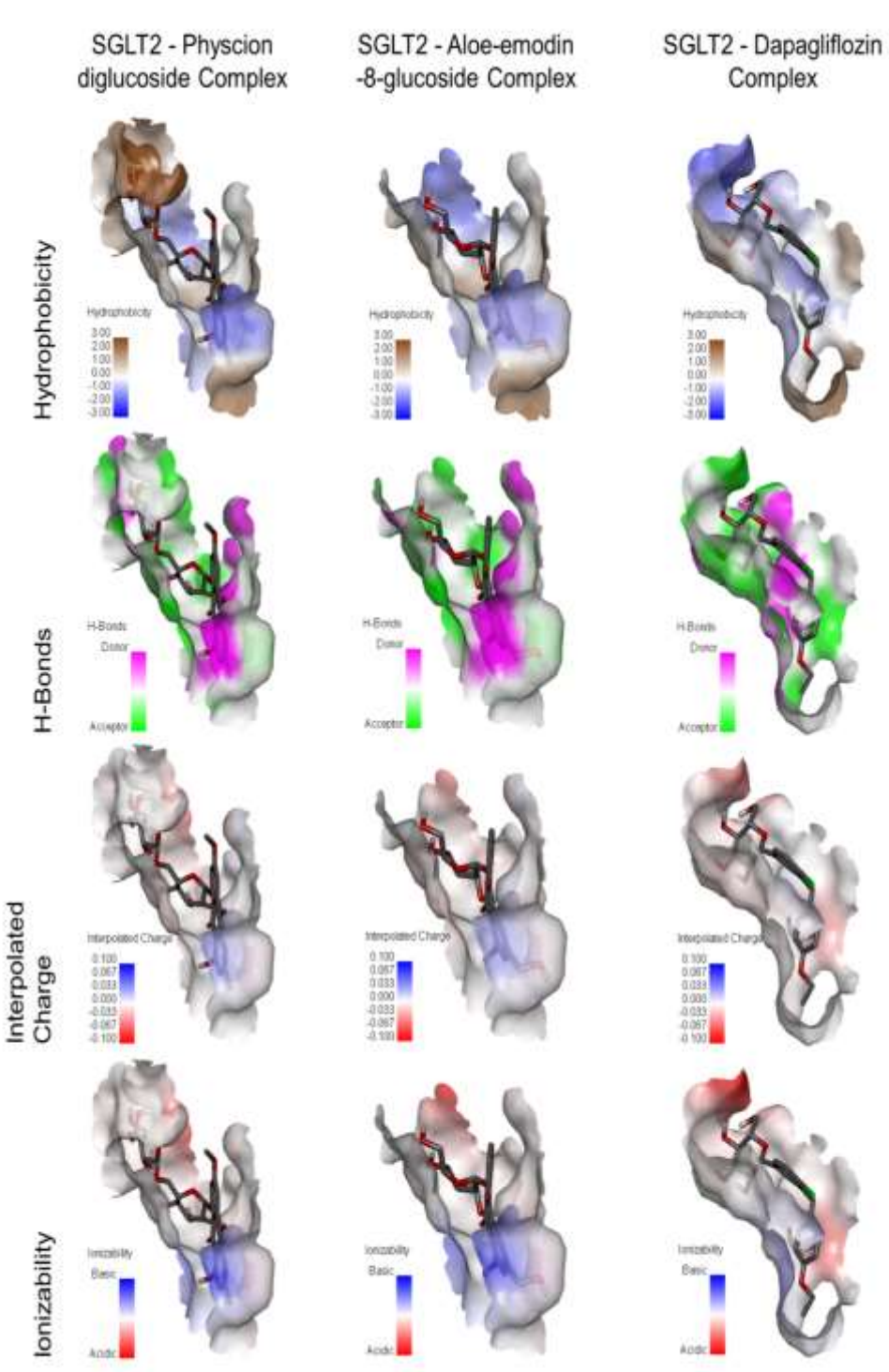

Figure 3: The additional physicochemical properties of target protein and ligand complexes including hydrophobicity, H-bonds, interpolated charge, and ionizability (https://discover.3ds.com/).

The investigation of drug discovery inherently revolves around the intricate interplay between specific targeted proteins and drug candidates [38-42]. Furthermore, certain fundamental aspects necessitate thorough validation, including the psychophysical properties such as the nature of the target molecule and drug candidate, binding affinity scores, interaction positions, types of chemical interactions, amino acid residues, hydrophobicity, hydrogen bonds, interpolated charge, and ionizability of the complex [31, 43-45].

Accordingly, the present study endeavors to assess the interactions between the target protein and the compounds physcion diglucoside, aloe-emodin-8-glucoside, as well as the control drug dapagliflozin. This evaluation is conducted through the prism of several essential indicators aforementioned, shedding light on the intricate dynamics of these interactions and their significance in the context of drug discovery.

SGLT2 proteins are widely expressed in the proximal convoluted tubule of the renals. In the normal condition, the SGLT2 has important role in optimizing the reabsorption of 90% filtered glucose in renal [46]. But, if this condition still work as usual, then it cause adverse condition on T2DM people. The reducing quantity of plasma glucose level was postulated as one strategy to ameliorate the T2DM [47]. Developing SGLT2 inhibitor may become one of the concern in treating T2DM [48, 49]. The inhibitor works to reduce and stop the glucose reabsorption, thus minimizing the glucose level on the blood [48, 50]. SGLT2 inhibition, on the other hand, induces glucosuria as well as other metabolic changes such as decreased blood pressure, reduced insulin production, increased ketogenesis, and increased lipolysis [51].

Conclusion

Through this computational study, compelling findings emerged concerning the rhubarb anthraquinone glycosides, namely physcion diglucoside and aloe-emodin-8-glucoside, showcasing their lower binding affinity scores compared to the control drug. This discovery underscores their immense potential as antidiabetic agents, operating through the inhibition of SGLT2 activity. Notably, the binding positions of physcion diglucoside and aloe-emodin-8-glucoside exhibit comparability, rendering them suitable candidates for subsequent development as anti-diabetic drugs. The inactivation of SGLT2 can effectively counteract the excessive elevation of plasma glucose levels in individuals with type 2 diabetes mellitus. To substantiate these findings and unveil the full extent of their biological activity as anti-diabetic agents, further research encompassing in vitro or in vivo experiments is imperative. Such investigations will provide deeper insights into the potential therapeutic benefits of physcion diglucoside and aloe-emodin-8-glucoside in managing diabetes mellitus.

Conflict of Interest

No conflict of interest

Acknowledgement

The authors thank Biocomputation Laboratory, Brawijaya University and Universitas Negeri Malang for support this study.

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