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Computational Screening of *Rheum palmatum* Phytochemicals as Potential Anti-Diabetes Agent via Sodium-Glucose Transport Protein 2 Inhibition

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ABSTRACT

Diabetes mellitus is a debilitating condition that affects people all over the world. Diabetes mellitus can be brought on by a number of different factors, the most common of which include being overweight and leading an unhealthy lifestyle. There have been a number of attempts made to cure diabetes, however these therapies are not precise and can cause adverse reactions. The quest for novel pharmaceuticals derived from plants with therapeutic properties is something that really has to be done in order to cut down on the use of synthetic drugs and the negative consequences that come along with them. In light of this, the purpose of this research was to investigate the potential of the bioactive molecules of *Rheum palmatum* to serve as an anti-diabetic therapeutic candidate by inhibiting Sodium-Glucose Transport Protein 2. Computational prediction consisting of protein and ligand optimization, molecular docking, and visualization was applied in this study. The generation of therapeutic candidates from the bioactive chemicals in *R. palmatum* showed promise based on computational analysis. In comparison to the control medication, the physcion-8-glucoside, laccaic acid, chrysophanol, rhein, and aloe emodin showed reduced binding affinity scores. When compared to the standard anti-diabetic medicine, the binding location and other

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physicochemical parameters following molecular docking produced excellent and competitive results.

Keywords: Diabetes mellitus; In silico; Inhibitor; *Rheum palmatum*; SGLT2

1. Introduction

Diabetes is a lifestyle illness with a growing global prevalence, affecting 415 million worldwide, with an annual incidence rate of 2.5 percent, according to the World Health Organization, indicating a significant risk of life-threatening complications [1, 2]. Diabetes mellitus is a condition characterized by high blood glucose levels due to insulin abnormalities [3]. Factors such as being overweight, poor eating habits, and low physical activity increase the risk of developing type 2 diabetes [4-6]. Treatment includes dietary modifications and increased physical exercise, with pharmacological treatments like blood glucose-lowering medicines or insulin therapy for advanced cases [7, 8]. Reduced physical activity and sedentary behavior contribute to chronic systemic inflammation, leading to type 2 diabetes [9, 10].

Diabetes treatment costs are 3.2 times higher than the average healthcare cost per capita, and 9.4 times higher in cases of complications [11]. Effective blood glucose and blood pressure management is challenging for many patients due to insufficient awareness about diabetes and health promotion [12-14]. A patient-centered strategy is needed for managing type 2 diabetes, focusing on lifestyle changes, and optimizing glycaemic control using available pharmaceutical alternatives. This helps maintain patient quality of life and reduces risk of complications [15, 16]. However, there is a demand for new agents that can be safely co-prescribed with existing treatment options, despite the high cost [17].

Sodium-Glucose Transport Protein 2 (SGLT-2) proteins, found in the kidney's proximal convoluted tubules, play a crucial role in reabsorbing filtered glucose. They are inhibited by SGLT-2 inhibitors, lowering the renal threshold for glucose, and increasing urine glucose excretion [18, 19]. This decreases SGLT-2-dependent glucose and sodium reabsorption, increasing sodium load in the distal tubule. This not only inhibits the renin-angiotensin-aldosterone system but also regulates physiological functions, such as lowering pressure within the renal intraglomerulus, promoting tubuloglomerular feedback, downregulating sympathetic activity, and lowering heart preload and afterload [20, 21].

Currently, numerous anti-diabetic medications are available for treating hyperglycemia, primarily by increasing insulin sensitivity, supplementing insulin, and boosting insulin secretion. However, these medications often have undesirable side effects like diarrhea, lactic acidosis, hepatic failure, weight gain, tachycardia, and hypothyroidism [7, 22]. Therefore, new safer anti-diabetic agents are needed to address these issues.

Plants have long been considered reliable sources of bioactive agents for treating cancer, illness, and improving immunity [23-26}. Antidiabetic agents derived from plants have been popular since ancient times due to their safety and affordability [7, 27]. Rhubarb, a member of the Polygonaceae family and genus Rheum, is a well-known medicinal plant used in traditional Chinese medicine and other countries to treat gastrointestinal problems, chronic renal failure inflammation, and liver ailments [28]. It has been shown that an anthraquinone-glycoside preparation from *R. palmatum* could lower fasting blood glucose levels, decrease total cholesterol and triglyceride levels, and improve pathological changes in liver, kidney, and pancreatic tissues in a rat model of type 2 diabetes caused by a high-fat diet [29, 30]. However, the mechanism of ameliorating T2DM is based on limited data. This study employed an in silico approach to evaluate *R. palmatum* phytochemicals, such as physcion-8-glucoside, laccaic acid, chrysophanol, rhein, and aloe emodin [30], which have abundant distribution as potential anti-diabetes agents via SGLT-2 inhibition.

2. Materials and Methods

2.1 Ligand and drug control retrieval

In this present study, physcion-8-glucoside (CID. 5319323), laccaic acid (CID. 5355255), chrysophanol (CID. 10208), rhein (CID. 10168), and aloe emodin (CID. 10207) were employed as some of the predominant components detected in *R. palmatum* plants. This study applied Dapagliflozin (CID: 9887712) as a control substance. Dapagliflozin is a regularly used anti-diabetic medication with a mode of action that inhibits SGLT-2 [31, 32]. The PubChem database (https://pubchem.ncbi.nlm.nih.gov/) provided the structures of all active substances and control medications. Each 2D structure was then stored as an sdf. file to ensure compatibility with the molecular docking program [33, 34].

2.2 Protein modelling and preparation

SGLT-2 was chosen to serve as the study's protein of interest. The SWISS-MODEL (https://swissmodel.expasy.org/) was utilized in order to create a model of the three-dimensional structure of the target protein. Protein sequences from SGLT-2 (ID: P31639) were taken from the UniProt database (https://www.uniprot.org) and used as the basis for the construction of the 3D protein modeling. In addition, the three-dimensional structure of the protein is stored in pdb. format, making it compatible with software used for protein docking and visualization. After that, protein optimization was carried out utilizing the PyMOL program (https://pymol.org/2/) and BIOVIA Discovery Studio (https://www.3ds.com/) [35, 36].

2.3 Molecular docking and visualization

Molecular docking is performed once the target protein has been optimized to its full potential. The target protein has been attached to both the active molecule and the control medication that has been obtained [37, 38]. PyRx is a software application that is used to do molecular docking (https://pyrx.source forge.io/). Docking coordinates (X: 5.2598, Y: -39.6208, and Z: -58.7245), as well as docking dimensions, are among the parameters that have been modified (X: 49.2452; Y: 30.2361; and Z: 70.0173). The results of the molecular docking were then visualized using BIOVIA Discovery Studio (https://www.3ds. com/) to examine the positioning of the ligand and the protein binding site, the different types of chemical bonds, amino acid residues, and the physicochemical properties of the complexes that were formed from molecular docking.

2.4 Biological activity prediction

Each molecule requires a SMILE to be examined for its chemical characteristics, protein target, and biological property prediction. SMILE representation for each molecule was retrieved from (https://pubchem.ncbi.nlm.nih.gov/). Additionally, the chemical characteristics of each active molecule as well as protein target predictions were collected from the Swiss Institute of Bioinformatics (https://www.sib. swiss/). During this time, the PASS online webserver (http://www.way2drug.com/passon line/) was utilized to predict the biological characteristics of each compound.

2.5. ADMET properties prediction

In a manner analogous to the analysis of protein targets and the prediction of biological characteristics, a SMILE is required for each molecule in order to get values for absorption, distribution, metabolism, excretion, and toxicity (ADMET) qualities. After that, an investigation of the pharmacokinetic characteristics was carried out on the pkCSM website (https://biosig.lab.uq.edu.au/pkcsm/ prediction) [39]. Several indicators were determined, including water solubility, Caco2 permeability, intestinal absorption, VDss, BBB permeability, CNS permeability, CYP2D6 substrate, CYP3A4 substrate, CYP2D6 inhibitor, CYP3A4 inhibitor, total clearance, renal OCT2 substrate, maximum tolerated dose, oral rat acute toxicity, and hepatotoxicity.

3. Results and Discussion

3.1. SGLT-2 inhibition activity of *R. palmatum* bioactive compounds

In the kidney's filtration system, SGLT-2 is crucial for the reabsorption of glucose. By avoiding and dramatically lowering blood sugar levels, SGLT-2 inhibitors can be used as an anti-diabetic approach in instances of diabetes mellitus (Fig. 1). According to the computational results, the five active substances utilized had a higher binding affinity value than the control medications (Fig. 2A).

The computational results revealed the active substance with the greatest binding affinity value was physcion-8-glucoside, with a value of -9.1 kcal/mol. Chrysophanol and laccaic acid came in second and third, respectively, with binding affinity values of -8.7 and -8.6 kcal/mol, respectively. Aloe emodin and rhein molecules, both of which had the same -8.0 kcal/mol value, were measured to be in last place. There is a greater chance of interaction between ligands and protein targets as the binding affinity score decreases to be more negative [40-42]. Surprisingly, the five active compounds' binding sites almost matched those of the control medicines (Fig. 2B). This demonstrates that the active substance from *R. palmatum* can function as a potential SGLT-2 inhibitor and contender to Dapagliflozin.

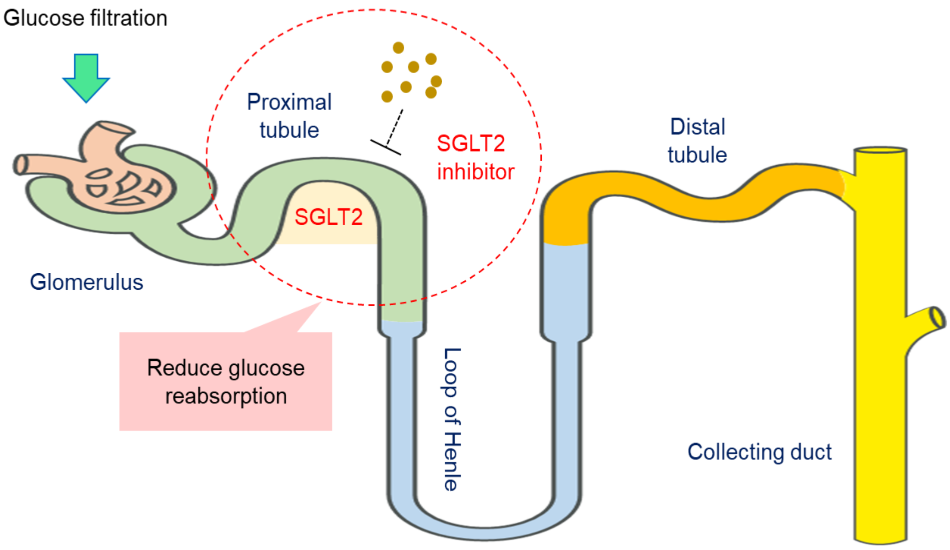

Fig. 1. The SGLT2 plays a pivotal role in the reabsorption of glucose filtered by the glomerulus in the kidneys. The presence of inhibitor can decrease glucose reabsorption.

Additionally, a few chemical interactions were created during the investigation of the protein-ligand complex interaction (Fig. 3). Each complex has both strong bonds, like hydrogen bonds, and weak bonds, like van der Waals bonds, as can be observed in common interactions. When it comes to the interaction that takes place between ligands and proteins, the significance of hydrogen bonds simply cannot be overstated. Additionally, the hydrogen bonds that exist within biomolecules help determine both their structure and function. They are also crucial for maintaining a ligand's location within a binding pocket throughout the catalytic process [43, 44]. Van der Waals forces, on the other hand, are a family of interactions that arise from the interaction of electron clouds surrounding two polar systems, either between atoms or inside particular molecules. This sequence of attractant and repellent interactions are created by the polarization fluctuation between nearby particles. Van der Waals forces are weaker than hydrogen bonds, hydrophobic attraction, and ionic contact [45]. However, this contact is necessary for molecules to adhere to inert surfaces, condense into larger molecules, cause friction, and adhere to one another [46]. More specifically, Table 1 demonstrates the interaction between the protein-ligand complex and the relevant amino acid residues.

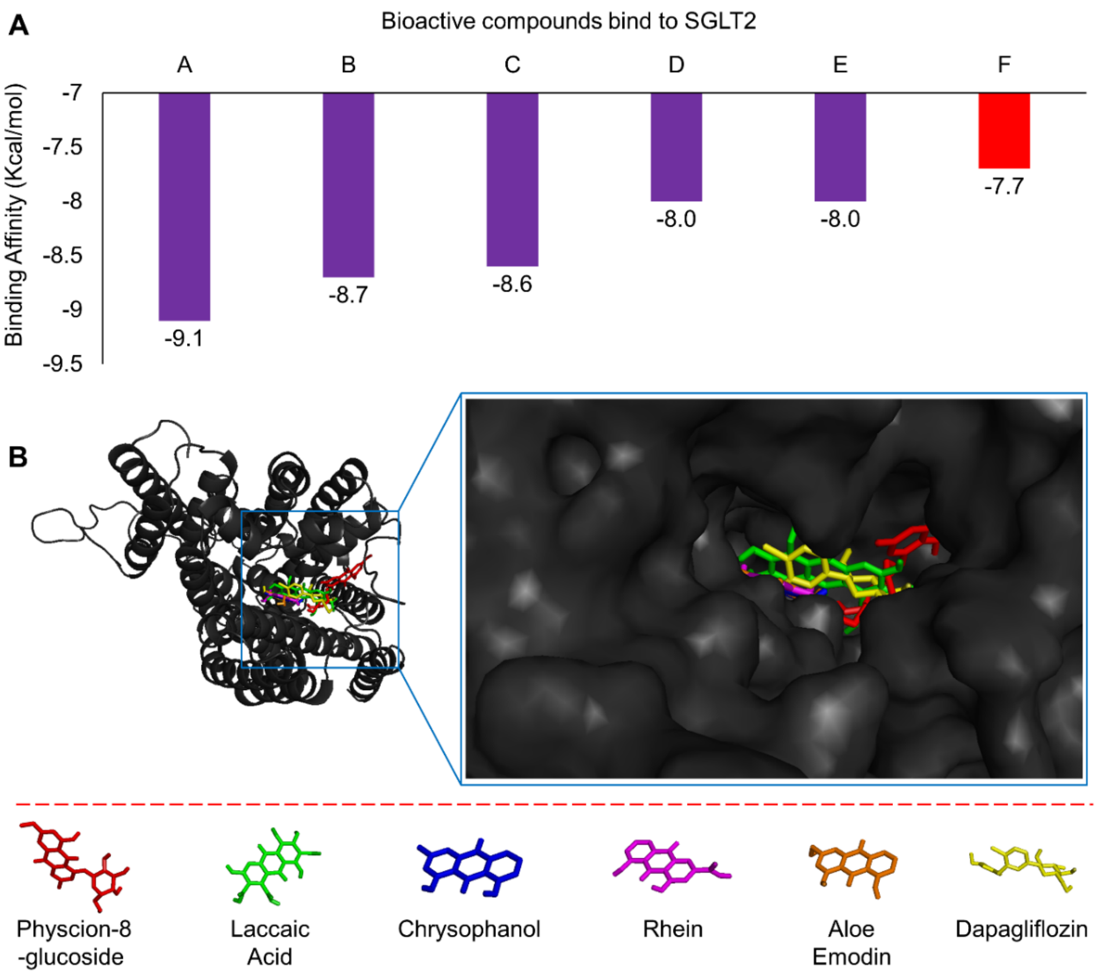

Fig. 2. (A). The binding affinity results of *R. palmatum* bioactive compounds and control drug towards SGLT2, A) Physcion-8-glucoside; B) Laccaic acid; C) Chrysophanol; D) Rhein; E) Aloe emodin; F) Dapagliflozin. (B). The 3D structure visualization of *R. palmatum* bioactive compounds and control drug toward SGLT2.

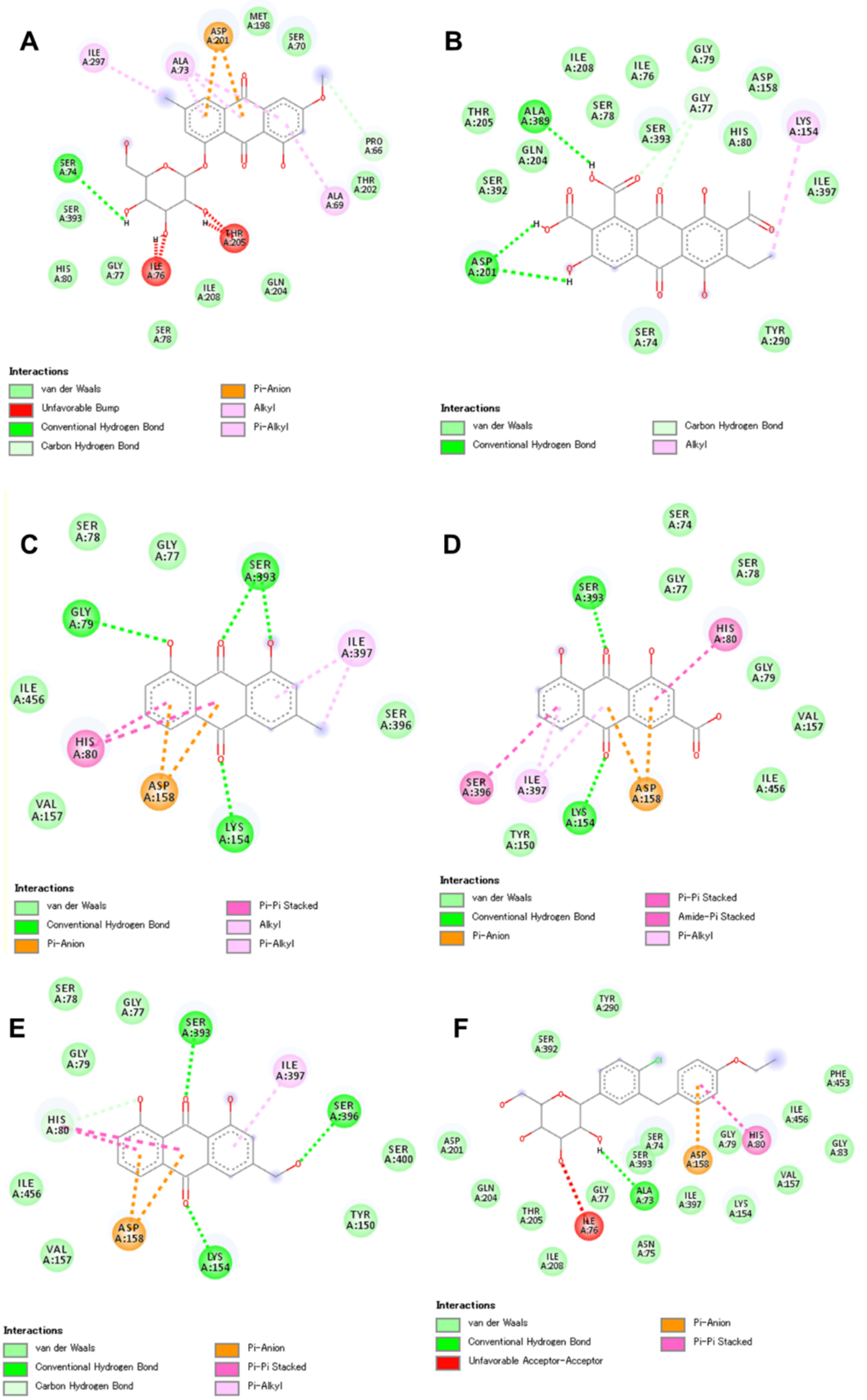

Fig. 3. The 2D structure visualization of *R. palmatum* bioactive compounds and control drug against SGLT2; A) Physcion-8-glucoside; B) Laccaic acid; C) Chrysophanol; D) Rhein; E) Aloe emodin; F) Dapagliflozin.

Table 1. List of chemical interactions and amino acid residue involvment of *R. palmatum* bioactive compounds and control drug against SGLT2.

No.	Compound	Chemical Interaction	Amino Acid Residues
1.	Physcion-8-glucoside	Van der Waals	PRO A:66, SER A:70, SER A:78, GLY A:77, HIS A:80, MET A:198, THR A:202, GLN A:204, ILE A:208, SER A:393
		Conventional Hydrogen Bond	SER A:74
2.	Laccaic acid	Van der Waals	SER A:74, ILE A:76, SER A:78, GLY A:79, HIS A:80, ASP A:158, GLN A:204, THR A:205, ILE A:208, TYR A:290, SER A:392, SER A:393, ILE A:397
		Conventional Hydrogen Bond	ASP A:201, ALA A:389
		Carbon Hydrogen Bond	GLY A:77
3.	Chrysophanol	Van der Waals	SER A:78, GLY A:77, VAL A:157, SER A:396, ILE A:456
		Conventional Hydrogen Bond	LYS A:154, SER A:393
4.	Rhein	Van der Waals	GLY A:77, SER A:78, GLY A:79, TYR A:150, VAL A:157, ILE A:456
		Conventional Hydrogen Bond	LYS A:154, SER A:393
5.	Aloe emodin	Van der Waals	GLY A:77, SER A:78, GLY A:79, TYR A:150, VAL A:157, SER A:400, ILE A:456
		Conventional Hydrogen Bond	LYS A:154, SER A:396
		Carbon Hydrogen Bond	HIS A:80
6.	Dapagliflozin	Van der Waals	ILE A:456, PHE A:453, ILA A:397, SER A:393, SER A:392, TYR A:290, ILE A:208, THR A:205, GLN A:204, ASP A:201, VAL A:157, LYS A:154, GLY A:83, GLY A:79, GLY A:77, ASN A:75, SER A:74.
		Conventional Hydrogen Bond	ALA A:73

In this study, we demonstrated several physicochemical properties of the protein – ligand complex surface after molecular docking including the aromatic, H-bond, charge (Fig. 4), hydrophobic, ionizability, and solvent accessible surface (SAS) properties (Fig. 5). Amino acid residues involved in the protein – ligand complex hints towards the ligand position in relation to the target protein. Furthermore, due to their characteristics, such as being hydrophobic, polar, aromatic, or aliphatic, the inclusion of amino acids in the complex may influence the effectiveness of the interaction between the protein and the ligand. Different numbers and intensities of covalent and non-covalent interactions are present in proteins with thermophilic, mesophilic, and psychrophilic origins. These interactions are essential for producing the observable properties of these proteins, such as their structure, stability, and dynamic nature, as well as their function. On the other hand, one cell can communicate with another through a different class of non-covalent interactions known as carbohydrate-protein interactions. A sizable amount of evidence suggests that proteins' carbohydrate-binding domains include aromatic amino acids [47].

3.2. Possible bioactive activity of *R. palmatum* bioactive compounds

To a greater extent, we examined the top three bioactive compounds with the greatest binding affinity score for its target protein and the biological properties prediction (Fig. 6). Interestingly, the data revealed that physcion-8-glucoside is the dominant target for enzymes and proteases, with a value of approximately 33.3% and 26.7%, respectively. Moreover, laccaic acid mostly targets kinases and phosphatases with similar percentages for both, at about 20%. Lastly, chrysophanol showed a tendency to target kinases, with a percentage around 33.3%.

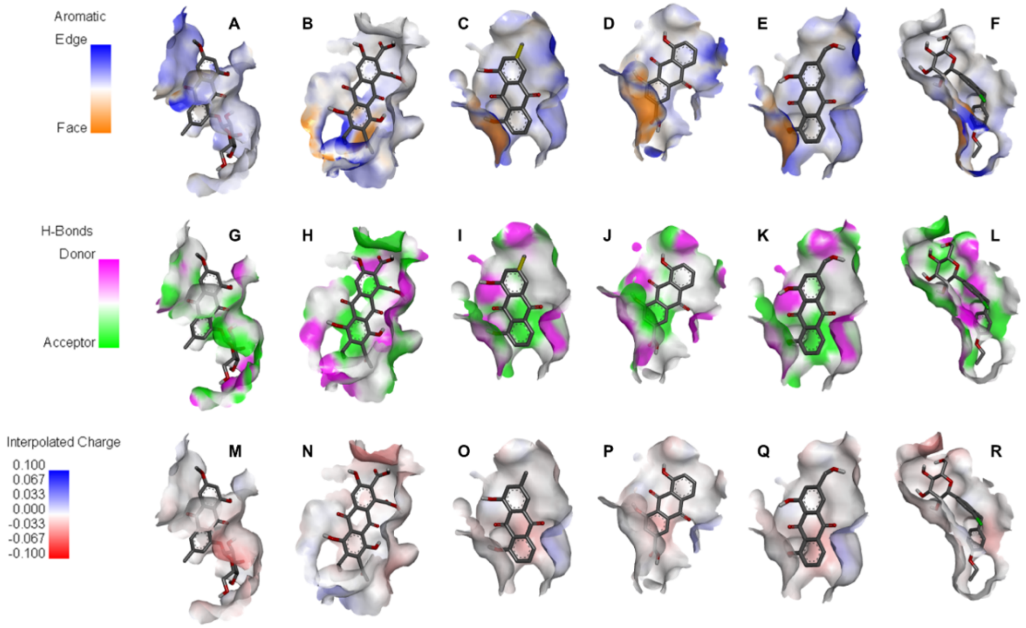

Fig. 4. The physicochemical properties of *R. palmatum* bioactive compounds and control drug toward SGLT2. Upper panel indicates aromatic properties; middle panel indicates H-bond properties, and lower panel indicates charge properties. A,G,M) Physcion-8-glucoside; B,H,N) Laccaic acid; C,I,O) Chrysophanol; D,J,P) Rhein; E,K,Q) Aloe emodin; F,L,R) Dapagliflozin.

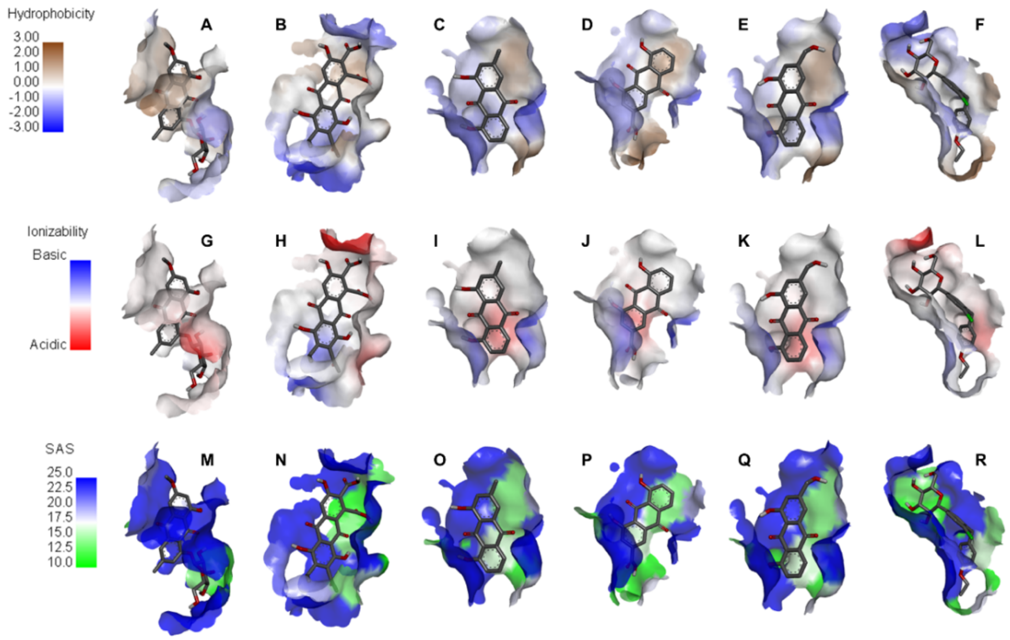

Fig. 5. The physicochemical properties of *R. palmatum* bioactive compounds and control drug toward SGLT2. Upper panel indicates hydrophobic properties; middle panel indicates ionizability properties, and lower panel indicates SAS properties. A,G,M) Physcion-8-glucoside; B,H,N) Laccaic acid; C,I,O) Chrysophanol; D,J,P) Rhein; E,K,Q) Aloe emodin; F,L,R) Dapagliflozin.

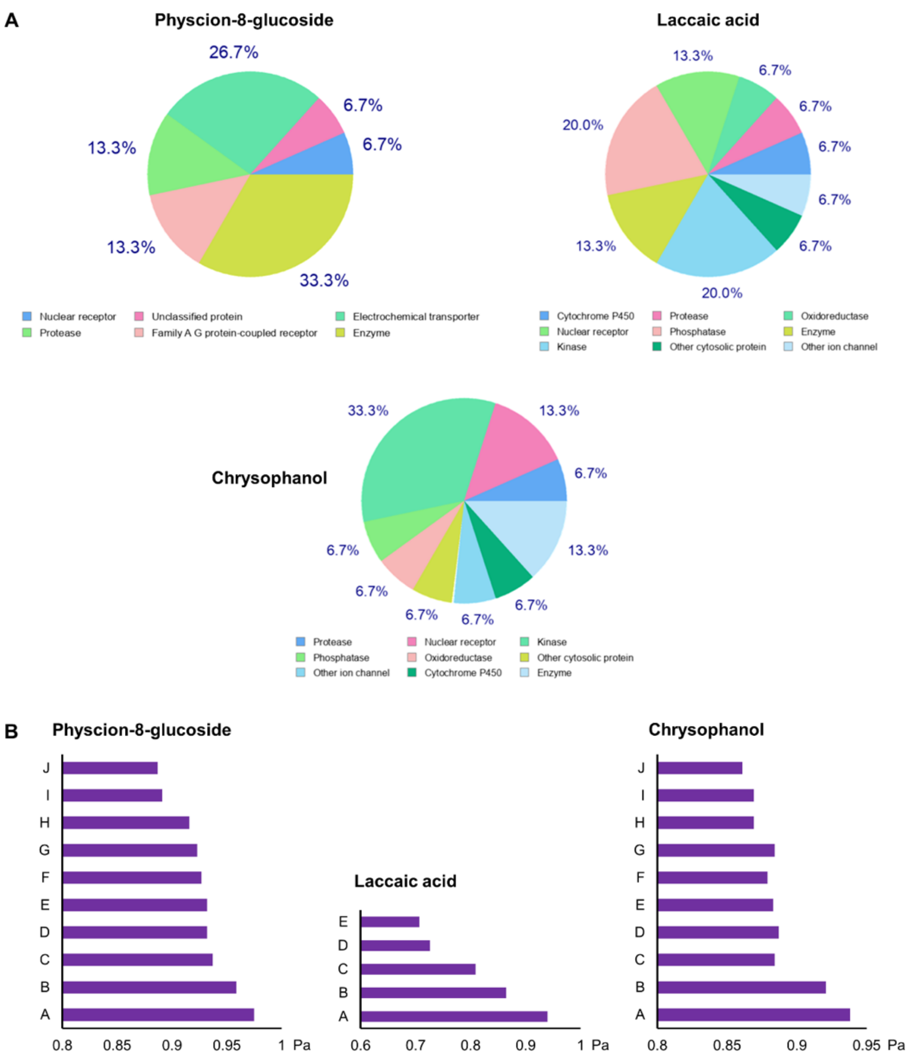

Fig. 6. The predicted target protein (A) and predicted biological properties (B) of *R. palmatum*'s top three bioactive compounds. The predicted biological properties of physcion-8-glucosidase including A) Laxative; B) Caspase 3 stimulant; C) Vasoprotector; D) Membrane permeability inhibitor; E) CDP-glycerol glycerophosphotransferase inhibitor; F) Anti-infective; G) Membrane integrity agonist; H) Monophenol monooxygenase inhibitor; I) Anticarcinogenic; J) NAD(P)+-arginine ADP-ribosyltransferase inhibitor. The predicted biological properties of laccaic acid including A) Histidine kinase inhibitor; B) Testosterone 17beta-dehydrogenase (NADP+) inhibitor; C) Chlordecone reductase inhibitor; D) Ubiquinol-cytochrome-c reductase inhibitor; E) Anti-inflammatory. The predicted biological properties of chrysophanol including A) CYP2C12 substrate; B) Alkane 1-monooxygenase inhibitor; C) Antiseborrheic; D) Aspulvinone dimethylallyltransferase inhibitor; E) Ubiquinol-cytochrome-c reductase inhibitor; F) Aldehyde oxidase inhibitor; G) Membrane integrity agonist; H) Histidine kinase inhibitor; I) NAD(P)+-arginine ADP-ribosyltransferase inhibitor; J) Antiseptic.

Interestingly, according to the biological property predictions, we demonstrated that physcion-8-glucosidase has several biological activities, including acting as a laxative, caspase 3 stimulant, vasoprotector, membrane permeability inhibitor, CDP-glycerol glycerophosphotransferase inhibitor, anti-infective, membrane integrity agonist, monophenol monooxygenase inhibitor, anticarcinogenic, and NAD(P)+-arginine ADP-ribosyltransferase inhibitor. Furthermore, laccaic acid acts as a histidine kinase inhibitor, testosterone 17beta-dehydrogenase (NADP+) inhibitor, chlordecone reductase inhibitor, ubiquinol-cytochrome-c reductase inhibitor, and anti-inflammatory agent. Additionally, chrysophanol has biological activities including acting as a CYP2C12 substrate, alkane 1-monooxygenase inhibitor, antiseborrheic, aspulvinone dimethylallyltransferase inhibitor, ubiquinol-cytochrome-c reductase inhibitor, aldehyde oxidase inhibitor, membrane integrity agonist, histidine kinase inhibitor, NAD(P)+-arginine ADP-ribosyltransferase inhibitor, and antiseptic. In addition, we investigated a number of ADMET indications for the three drugs that had the highest binding affinity values (Fig. 7). It is crucial to note that none of the three chemicals discussed above are hazardous to the liver. This demonstrates that these compounds do not create any adverse effects on the function of the liver; hence, the development of these compounds as anti-diabetic medications is something that has a very promising future ahead of it.

Property	Model Name	Physcion-8-Glucoside	Laccaic acid	Chrysophanol	Unit
		Predicted Value			
Absorption	Water solubility	-2.779	-2.891	-3.077	log mol/L
	Caco2 permeability	0.333	-0.962	1.298	log Papp in 10^{-6} cm/s
	Intestinal absorption	46.589	17.56	96.558	% Absorbed
Distribution	VDss (human)	0.212	-0.158	0.272	log L/kg
	BBB permeability	-1.262	-1.457	0.212	log BB
	CNS permeability	-4.024	-3.599	-2.111	log PS
Metabolism	CYP2D6 substrate	No	No	No	Yes/No
	CYP3A4 substrate	No	No	No	Yes/No
	CYP2D6 inhibitior	No	No	No	Yes/No
	CYP3A4 inhibitior	No	No	No	Yes/No
Excretion	Total Clearance	0.46	-0.217	0.02	log ml/min/kg
	Renal OCT2 substrate	No	No	No	Yes/No
Toxicity	Max. tolerated dose	0.087	0.504	-0.256	log mg/kg/day
	Oral Rat Acute Toxicity	2.661	2.427	2.275	mol/kg
	Hepatotoxicity	No	No	No	Yes/No

Fig. 7. ADMET properties of *R. palmatum*'s top three bioactive compounds.

4. Conclusion

This computational study showed promising drug candidates from the bioactive compounds found in *R. palmatum*. The physcion-8-glucoside, laccaic acid, chrysophanol, rhein, and aloe emodin demonstrated lower binding affinity scores to the control drug. The binding position and other physicochemical properties after molecular docking showed great and competitive result as compared to the control drug for anti-diabetes.

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References

[1] International Diabetes Federation. IDF diabetes atlas in International Diabetes Federation, 1–144, Brussels, Belgium, 7th edition; 2013: http://wwwdiabetesatlas.org.

[2] Kotwas, A, Karakiewicz B, Zabielska P, Wieder-Huszla S. Epidemiological factors for type 2 diabetes mellitus: evidence from the Global Burden of Disease. Arch Public Health. 2021; 79(1): 110.

[3] American Diabetes Association. Standards of medical care in diabetes 2013. Diabetes Care. 2013; 36(1): 11-66.

[4] Ladher N, Hinton R, Veitch E. Challenges of obesity and type 2 diabetes require more attention to food environment. BMJ. 2023; 9(383): 2269.

[5] Ojczyńska A. Use of aggregated parameters of lack of health in the assessment of the effect of selected diseases and disabilities on the health status of Polish population. Studia Ekonomiczne – Zeszyty Naukowe Uniwersytetu Ekonomicznego w Katowicach. 2017; 309: 169–78.

[6] Harikrishnan S, Jeemon P, Mini GK, Thankappan KR, Sylaja P. GBD 2017 Causes of Death Collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet. 2018; 392(10159); 1736-1788.

[7] Alam S, Sarker MMR, Sultana TN, Chowdhury MNR, Rashid MA. Antidiabetic phytochemicals from medicinal plants: Prospective candidates for new drug discovery and development. Front Endocrinol. 2022: 13; 800714.

[8] Esser N, Legrand-Poels S, Piette J. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes Res Clin Pract. 2014; 105(2): 141–50.

[9] Sindhu S, Ahmad R. Metabolic inflammation and cellular immunity. Cells. 2023; 12(12): 1615.

[10] Venkatasamy VV, Pericherla S, Manthuruthil S. Effect of physical activity on insulin resistance, inflammation and oxidative stress in diabetes mellitus. J Clin Diagn Res. 2013; 7(8): 1764.

[11] Al-Maskari F, El-Sadig M, Nagelkerke N. Assessment of the direct medical costs of diabetes mellitus and its complications in the United Arab Emirates. BMC Public Health. 2010; 10: 679.

[12] Al Slail FY, Abid O, Assiri AM, Memish ZA. Cardiovascular risk profiles of adults with type-2 diabetes treated at urban hospitals in Riyadh, Saudi Arabia. J Epidemiol Glob Health. 2016; 6: 29–36.

[13] Hashim MJ, Mustafa H, Ali H. Knowledge of diabetes among patients in the United Arab Emirates and trends since 2001: A study using the Michigan Diabetes Knowledge Test. East Mediterr Health J. 2017; 22: 742–8.

[14] Khan MAB, Hashim MJ, King JK, Govender RD. Epidemiology of type 2 diabetes - global burden of disease and forecasted trends. J Epidemiol Glob Health. 2020; 10(1): 107-111.

[15] Inzucchi SE, Bergenstal RM, Buse JB. Management of hyperglycemia in type 2 diabetes, 2015: A patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2015; 38: 140–149.

[16] Handelsman Y, Bloomgarden ZT, Grunberger G. American association of clinical endocrinologists and American

college of endocrinology – clinical practice guidelines for developing a diabetes mellitus comprehensive care plan – 2015. Endocr Pract, 2015; 21(1): 1–87.

[17] Shah N, Abdalla MA, Deshmukh H. Therapeutics for type-2 diabetes mellitus: A glance at the recent inclusions and novel agents under development for use in clinical practice. Ther Adv Endocrinol Metab. 2021; 12: 1-30.

[18] Sano R, Shinozaki Y, Ohta T. Sodium-glucose cotransporters: Functional properties and pharmaceutical potential. J Diabetes Investig. 2020; 11(4): 770-782.

[19] Plosker GL. Canagliflozin: A review of its use in patients with type 2 diabetes mellitus. Drugs. 2014; 74(7): 807-24.

[20] Lytvyn Y, Bjornstad P, Udell JA, Lovshin JA. Sodium glucose cotransporter-2 inhibition in heart failure: Potential mechanisms, clinical applications, and summary of clinical trials. Circulation. 2017: 136(17); 1643-1658.

[21] Padda IS, Mahtani AU, Parmar M. Sodium-glucose transport protein 2 (SGLT2) inhibitors. StatPearls Publishing, 2022.

[22] Li GQ, Kam A, Wong KH. Herbal medicines for the management of diabetes. In: Diabetes: An old disease, a new insight, advances in experimental medicine and biology. New York, Springer, 2013; 396–413.

[23] Putra WE, Rifa'i M. Hematopoiesis activity of Sambucus javanica on chloramphenicol-induced aplastic anemia mouse model. Nat Prod Sci. 2019; 25(1): 59-63.

[24] Putra WE, Rifa’i M. Assessing the immunomodulatory activity of ethanol extract of Sambucus javanica berries and leaves in chloramphenicol-induced aplastic anemia mouse model. Trop. Life Sci. Res. 2020; 31(2): 175–185.

[25] Putra WE, Agusinta AK, Ashar MSAA, Manullang VA, Rifa'i M. Immunomodulatory and ameliorative effect of Citrus limon extract on DMBA-induced breast cancer in mouse. Karbala Int. J. Mod. Sci. 2023; 9(2): 1-14.

[26] Rahayu S, Rifa'i M, Qosimah D, Widyarti D, Lestari ND, Jatmiko YD, Putra WE, Tsuboi H. Benefits of Coriandrum sativum L. seed extract in maintaining immunocompetent cell homeostasis. Sains Malays. 2022; 51(8): 2425-2434.

[27] Kim J, Giovannucci E. Healthful plant-based diet and incidence of type 2 diabetes in Asian Population. Nutrients. 2022; 14(15): 3078.

[28] Liudvytska O, Kolodziejczyk-Czepas J. A review on rhubarb-derived substances as modulators of cardiovascular risk factors-a special emphasis on anti-obesity action. Nutrients. 2022; 14(10): 2053.

[29] Cheng FR, Cui HX, Fang JL. Ameliorative effect and mechanism of the purified anthraquinone-glycoside preparation from Rheum Palmatum L. on type 2 diabetes mellitus. Molecules. 2019; 24: 1454.

[30] Cao YJ, Pu ZJ, Tang YP, Shen J, Chen YY, Kang A, Zhou GS, Duan JA. Advances in bio-active constituents, pharmacology and clinical applications of rhubarb. Chin Med. 2017; 12: 36.

[31] Dhillon S. Dapagliflozin: A review in type 2 diabetes. Drugs. 2019; 79(10): 1135-1146.

[32] Filippatos TD, Liberopoulos EN, Elisaf MS. Dapagliflozin in patients with type 2 diabetes mellitus. Ther Adv Endocrinol Metab. 2015; 6(1): 29-41.

[33] Putra WE. In silico study demonstrates multiple bioactive compounds of sambucus plant promote death cell signaling pathway via fas receptor. FTSTJ. 2018; 3(2): 682-685.

[34] Hidayatullah A, Putra WE, Sustiprijatno, Permatasari GW, Salma WO, Widiastuti D, Susanto H, Muchtaromah B, Sari DRT, Ningsih FN, Heikal MF, Yusuf AMR,

Arizona AS. In silico targeting DENV2's prefusion envelope protein by several natural products' bioactive compounds. CMUJ. Nat. Sci. 2021; 20(3): 1-20.

[35] Hidayatullah A, Putra WE, Sustiprijatno, Rifa'i M, Heikal MF, Widiastuti D. Determining the molecular interactions of natural inhibitors with the HPV-16 E6 oncoprotein by docking and dynamics simulation studies. Braz Arch Biol Technol. 2024; 67(1): 1-9.

[36] Hidayatullah A, Putra WE, Rifa'i M, Sustiprijatno, Heikal MF, Widiastuti D, Permatasari GW, Susanto H, Shuib AS. Molecular docking and molecular dynamic study of multiple medicinal plants' bioactive compounds as human papillomavirus type 16 E5 protein inhibitor. Kuwait J. Sci. 2023; 50: 1-14.

[37] Hidayatullah A, Putra WE, Rifa'i M, Sustiprijatno, Widiastuti D, Heikal MF, Susanto H, Salma WO. Molecular docking and dynamics simulation studies to predict multiple medicinal plants' bioactive compounds interaction and its behavior on the surface of DENV-2 E protein. Karbala Int. J. Mod. Sci. 2022; 8(3): 531-542.

[38] Hidayatullah A, Putra WE, Sustiprijatno, Rifa'i M, Widiastuti D, Heikal MF, Permatasari GW. Concatenation of molecular docking and dynamics simulation of human papillomavirus type 16 E7 oncoprotein targeted ligands: In quest of cervical cancer's treatment. An. Acad. Bras. Cienc. 2023; 95: 1-16.

[39] Pires DE, Blundell TL, Ascher DB. pkCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. J Med Chem. 2015; 58(9): 4066-72.

[40] Hidayatullah A, Putra WE, Sustiprijatno, Widiastuti D, Salma WO, Heikal MF. Molecular docking and molecular dynamics simulation - based identification of natural inhibitors against druggable human papilloma virus type 16 target. Trends Sci. 2023; 20(4): 1-12.

[41] Putra WE, Salma WO, Rifa'i M. Anti-inflammatory activity of Sambucus plant bioactive compounds against TNF-α and TRAIL as solution to overcome inflammation associated diseases: The insight from bioinformatics study. Nat Prod Sci. 2019; 25(3): 215-221.

[42] Gurung AB, Bhattacharjee A, Ali MA. Exploring the physicochemical profile and the binding patterns of selected novel anticancer Himalayan plant derived active compounds with macromolecular targets. Inform. Med. Unlocked. 2016; 5: 1-14.

[43] Kostal J. Chapter four - Computational chemistry in predictive toxicology: Status quo et quo vadis? Adv. Mol. Toxicol. 2016; 10: 139-186.

[44] Chen D, Oezguen N, Urvil P, Ferguson C, Dann SM, Savidge TC. Regulation of protein-ligand binding affinity by hydrogen bond pairing, Sci Adv. 2016; 2(3): e1501240.

[45] Cerda-Kipper AS. Radioimmunoassay and enzyme-linked immunosorbent assay. Reference Module in Chemistry, Molecular Sciences and Chemical Engineering. 2019; 2019: 55-75.

[46] Kawai S, Foster AS, Björkman T, Nowakowska S. Van der Waals interactions and the limits of isolated atom models at interfaces. Nat Commun. 2016; 7: 11559.

[47] Madhusudan MK, Mahalakshmi R. Implications of aromatic-aromatic interactions: From protein structures to peptide models. Protein Sci. 2015; 24(12): 1920-33.