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Molecular Docking Study of Standardized Lime (*Citrus aurantiifolia*) Peel Decoction as an Antidiabetic Herbal

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ABSTRACT

Diabetes mellitus (DM) is a chronic endocrine, metabolic disease characterized by elevated blood glucose levels that will affect more than 500 million adults in 2021. *Citrus aurantiifolia* peels are discarded by-products that contain flavonoid compounds, offering potential as an antidiabetic herb. This study aims to evaluate *C. aurantiifolia* water extract (CPWE) compounds responsible for antidiabetic properties through *α*-glucosidase, *α*-amylase, and sodium-glucose transporter 2 (SGLT-2) inhibitor using molecular docking simulation. CPWE was extracted using ultrasonic-assisted extraction (UAE) with distilled water, and its chemical profile was then analyzed using LC-MS/MS. Compounds detected in LC-MS/MS were tested for antidiabetic activity using molecular docking simulation carried out using Molegro Virtual Docker into *α*-glucosidase (PDB ID: 3A4A), *α*-amylase (PDB ID: 1OSE), SGLT-2 (PDB ID: 7VSI), and dipeptidyl peptidase 4 (DPP-4) (PBD ID: 3G0B). The major compounds found in CPWE were hesperidin, limonin, and scoparone, along with other compounds, including hesperetin, naringin, naringenin, bergaptol, citric acid, quercetin, and rutin. Molecular docking simulation demonstrates that rutin inhibited *α*-glucosidase, *α*-amylase, and DPP-4, hesperidin inhibited *α*-amylase, SGLT-2, and DPP-4, and naringin inhibited SGLT-2. Absorption, distribution, metabolism, excretion, and toxicity (ADMET) prediction of CPWE compounds showed that active compounds have limited absorption and permeability, but none of the CPWE compounds are toxic. Molecular docking predictions of 10 compounds from CPWE revealed that flavonoid compounds have antidiabetic potential.

Keywords: *Citrus aurantiifolia* Peels, Water Extract, Chemical Profile, Antidiabetic, Molecular Docking, Pharmacokinetics.

Introduction

Diabetes mellitus (DM) is a chronic endocrine metabolic disease characterized by elevated blood glucose levels caused by insulin deficiency, as seen in type 1 DM or insulin resistance in type 2 DM. Insulin resistance and pancreatic beta cell failure are two pathophysiological pathways in type 2 diabetes. Type 2 diabetes affects 536.6 million adults aged 20-79 globally in 2021, and by 2045, that figure is predicted to increase to 783.2 million.[1] Uncontrolled DM can lead to several complications, such as kidney failure, liver disease, cardiovascular disease, diabetic retinopathy, and diabetic neuropathy.[2] FDA classifies medications for diabetic management into several classes, such as metformin, sulfonylureas, thiazolidinediones, sodium-glucose cotransporter 2 (SGLT-2) inhibitors, *α*-glucosidase inhibitors, dipeptidyl peptidase 4 (DPP-4) inhibitors.[3] *Citrus aurantiifolia*, or lime, is one of the horticultural plants that plays a vital role in various aspects of Indonesian people's lives. *C. aurantiifolia* is widely used as an ingredient in drinks, cooking spices, food, cosmetics, and even herbal concoctions.[4,5]

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In addition, the lime fruit processing industry in Indonesia is also growing rapidly, products such as juice, syrup, essential oils, and other herbal products. However, only fruit juice is commonly consumed, leaving the dehydrated flesh, seed, and fruit peel as by-products, accounting for 52% of its total weight.[6] *C. aurantiifolia* peels are discarded by-products, even though they contain a lot of chemical content that is good for health, including essential oils, saponin, triterpenoid, coumarin, flavonoids, phenolic, and alkaloid.[7,8] So, utilizing *C. aurantiifolia* peels as a medicinal herb will help promote sustainability and increase its commercial value.

C. aurantiifolia peel contains more flavonoids than seed, fruit, and juice.[8] Its major flavonoids are narirutin, eriocitrin, and hesperidin.[9] Flavonoids are antidiabetic compounds that exert hypoglycemic activity through various mechanisms, including *α*-glucosidase, *α*-amylase, SGLT-2 inhibitor, and DPP-4 inhibitor.[10] This study investigates bioactive compounds in *C. aurantiifolia* water extract (CPWE) and identifies the compounds responsible for antidiabetic activity through a computational approach. The computational method is a practical approach to predict CPWE potential as an antidiabetic agent. Furthermore, it can also predict potential toxicity to ensure the safety of CPWE.[11,12] Antidiabetic activity was assessed through molecular docking by *α*-glucosidase, *α*-amylase, SGLT-2, and DPP-4 inhibitor. Four proteins were identified as prospective therapeutic targets through a molecular docking approach, each associated with a distinct physiological response. The physicochemical and ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties of CPWE compounds were also predicted. Previous studies have shown the antidiabetic potential of *C. aurantiifolia* polyflavonoids through expression of the PI3K/P-AKT/FOXO-1 genes, in-vivo hypoglicemic effects of essential oils and methanolic

extract, and in-vitro α-glucosidase and α-amylase, but none have investigated the molecular enzyme inhbition and safety profile of CPWE.[13] Hence, this study aims to valorize CPWE to develop into standardized antidiabetic herbal products, ensuring safety and efficacy.

Material and Methods

Instrument

LC-MS/MS (Xevo G2-S Qtof, USA), Food dehydrator (IRASTAR SS-20H, Indonesia), Ultrasonic Assisted Extraction (Hielscher UP200St-T), Karl-Fischer Moisture Titrator (Metrohm 870 KF Titrino Plus, Switzerland), analytical balance (AND HR-120, Japan), balance (Sartorius Entris 3202-1S), hardware with following specification: Processor: AMD Ryzen 3 4300U/2.7 GHz with Radeon Graphics; GPU: AMD Radeon Graphics; RAM: 8 GB.

Plant Collection and Identification

Lime (*Citrus aurantiifolia*) at eight months of age was obtained from Ciapus, Bogor, Indonesia (6° 21' 28" South, 106° 30' 12" East), in September 2023. *C. aurantiifolia* was identified by Anom Bowolaksono, PhD and deposited at Herbarium Depokenesis, Department of Biology, University of Indonesia with specimen voucher J123-P-126.

Extraction

C. aurantiifolia peels were weighed, cut into small pieces, dried in a food dehydrator at 45°C, then ground into powder. Fifty grams of *C. aurantiifolia* peel powder was extracted using ultrasonic-assisted extraction (UAE) with 500 mL distilled water. The extract was filtered and dried using a food dehydrator at 45°C.

Extract Standardization

CPWE standardization was carried out according to the second edition of the Indonesian Herbal Pharmacopeia (IHP).[14] Extract standardization included yield, organoleptic, water content, total ash content, and acid insoluble ash content.

Yield

The dried extract was weighed, and the yield was calculated using the following formula (equation 1):

$$\text{Equation 1: Yield (\%)} = \frac{\text{Extract weight}}{\text{Simplicia weight}} \times 100\%$$

Water content

Water content was determined using the Karl-Fischer Moisture Titrator.[14] The dried extract was weighed and put in a Karl-Fisher titrate chamber that contained CombiMethanol-5. The Sample was titrated automatically using the CombiTitran-5 reagent. The weight value was entered before and after the test, then the water content of the extract was recorded.

Ash content

Two grams of extract were weighed and put in a crucible that had previously been weighed. The extract is heated in a furnace until constant weight is achieved, then cooled in a desiccator and weighed.[14] The total ash content expressed in % (w/w) was calculated using the following formula (equation 2):

$$\text{Equation 2: Ash content (\%)} = \frac{\text{Ash weight}}{\text{Extract weight}} \times 100\%$$

Acid insoluble ash content

The ash obtained from determining total ash content is boiled with 25 mL of dilute sulfuric acid for 5 minutes. The acid-insoluble part is collected and filtered with ash-free filter paper, and the residue is rinsed with hot water. The filtered ash and the filter paper are returned to the initial crucible. The ash was heated in a furnace until constant weight was achieved, then cooled in a desiccator and weighed.[14] The acid insoluble ash content expressed in % (w/w) was calculated using the following formula (equation 3):

$$\text{Equation 3: Acid insoluble Ash content (\%)} = \frac{\text{Acid insoluble ash weight}}{\text{Extract weight}} \times 100\%$$

LC-MS/MS

LC-MS/MS performed on Ultra Performance Liquid Chromatography (UPLC) (ACQUITY UPLC®H-Class System (waters, USA), with C18 (1.8 µm 2x100 mm) HSS stationary phase, and combination of water + 5 mM Ammonium Formic (A) and acetonitrile + 0.05 % Formic acid (B) for gradient mobile phase presented at Table 1. Column temperature set at 50°C with flow rate 0.2 mL/min.

Molecular Docking Simulation

Detected compounds from LC-MS/MS were tested for activity to various antidiabetic proteins through molecular docking simulation. Molecular docking simulation is carried out on Molegro Virtual Docker (MVD) version 5.0, 2011 (invisible). Ligands are prepared by minimizing energy in chem3D 16.0, 2020 (PerkinElmer) and then stored in mol2 format. Protein is prepared by removing water molecules and cofactors, and then internal validation is carried out. Internal validation was carried out by redocking native ligands against selected target proteins using 12 combinations of algorithms and scoring functions to obtain an RMSD value of less than 2.[15] The algorithm and scoring function that generated the smallest RMSD value of each protein were used for docking simulation. The prepared ligands were docked to the proteins α-glucosidase (PDB ID: 3A4A), α-amylase (PDB ID: 1OSE), and SGLT-2 (PDB ID: 7vsi). α-glucosidase inhibitor.

α-glucosidase inhibitor

Molecular docking simulation against α-glucosidase (PDB ID: 3A4A) with acarbose as positive control was performed 10 times independently with a scoring system. The algorithm used is MolDock score and MolDock se at x, y, z coordinates = 14.01; -10.98; -18.01 with a radius of 15 Å.

α-amylase inhibitor

Molecular docking simulation against α-amylase (PDB ID: 1OSE) with acarbose as a positive control was performed 10 times independently with a scoring system. The algorithm used is Plant score and MolDock se at x, y, z coordinates = 35.58; 36.70; 1.52 with a radius of 15 Å.

SGLT-2 inhibitor

Molecular docking simulation against SGLT-2 (PDB ID: 7VSI) with empagliflozin as a positive control was performed 10 times independent runs with the Scoring system. The algorithm used is the Plant score grid, and iterated simplex at x, y, z coordinates = 41.86; 59.54; 52.54 with a radius of 18 Å.

DPP-4 inhibitor

Molecular docking simulation against DPP-4 (PDB ID: 3G0B) with alogliptin as positive control was performed 10 times independent runs with Scoring system and the algorithm used is Plant score and iterated simplex at x, y, z coordinates = -10.34; 30.45; 17.98 with a radius of 25 Å.

Physicochemical properties and ADMET (adsorption, distribution, metabolism, toxicity) prediction

Physicochemical properties and ADMET were predicted by inputting compounds in smiles format to pkcsm online (https://biosig.lab.uq.edu.au/pkcsm/), which was accessed on November 30, 2024.

Results and Discussion

Extract Standardization

CPWE standardization was carried out according to IHP guidelines, as shown in**Error! Reference source not found.**. The results of this study indicate that CPWE fulfills all of the IHP specifications. The water content was carried out to determine the water content in the extract, as high water content stimulates microbial growth that leads to extract decomposition.[16] Ash content indicates physiological inorganic elements such as magnesium, sodium, and calcium, as well as non-physiological ash from the environment and pollution such as Hg, As, Pb, and Si. High acid insoluble ash content indicates high contamination from external factors such as silicate content originating from the soil.[17]

LC-MS/MS

Evaluation of CPWE metabolite content carried out using LC-MS/MS. LC-MS/MS has advantages like high sensitivity and specificity. CPWE contains secondary metabolites in the form of flavonoids, coumarins, limonoids, and citric acids, similar to ethanol extract in the previous study (Figure 1 and **Error! Reference source not found.**).[18-20] LC-MS/MS chromatogram showed the major compounds were hesperitin, limonin, and scoparone.

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Hesperetin is a flavonoid aglycon found abundantly in citrus species, which has pharmacological activity such as antioxidant, antiinflammation, anticancer, antiaging, neuroprotective,

Table 1: Mobile phase of LC-MS/MS

Time	Flow (mL/min)	%A	%B
2.00	0.200	75.0	25.0
3.00	0.200	75.0	25.0
14.00	0.200	0.0	100.0
15.00	0.200	0.0	100.0
19.00	0.200	95.0	5.0
23.00	0.200	95.0	5.0

Table 2: Extract standardization results

No	Determination	Results	IHP Specification
1	Yield	16.71%	Not less than 15.0%
2	Organoleptic	Dried extract, green to brown in color, characteristic odor, and bitter taste	Viscous extract, green to yellow in color, characteristic odor, and bitter taste
3	Water content	6.25%	Not more than 10%
4	Total ash content	6.07%	Not more than 6.6%
5	Acid insoluble ash content	0.08%	Not more than 0.1%

IHP (Indonesian Herbal Pharmacopoeia)

Table 3: Identified compounds of C. aurantiifolia peel water extract using LC-MS/MS

RT (min)	Formula	Compound	Mass (m/z)	Fit Conf (%)
6.379	$C_{28}H_{34}O_{15}$	Hesperidin	611.1976	100%
6.329	$C_{16}H_{14}O_6$	Hesperetin	303.0869	99.48%
5.872	$C_{27}H_{32}O_{14}$	Naringin	581.1870	98.78%
4.571	$C_{15}H_{12}O_5$	Naringenin	273.0763	99.14%
5.098	$C_{26}H_{30}O_8$	Limonin	471.2019	99.65%
14.175	$C_{11}H_6O_4$	Bergaptol	203.0344	99.92%
14.357	$C_6H_8O_7$	Citric Acid	193.0348	99.97%
6.400	$C_{15}H_{10}O_7$	Quercetin	303.0505	100%
6.329	$C_{27}H_{30}O_{16}$	Rutin	611,1612	99.58%
9.212	$C_{11}H_{10}O_4$	Scoparone	207.0657	100%

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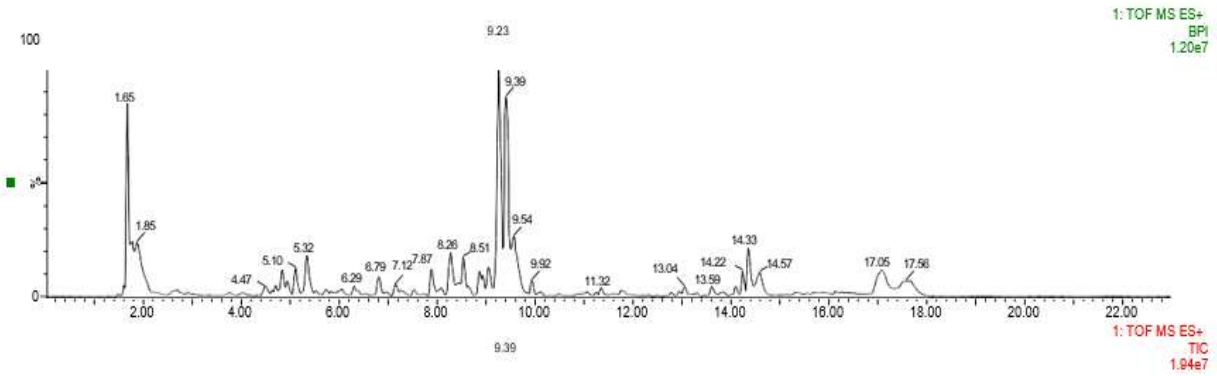

Figure 1: LC-MS/MS chromatogram of C. aurantiifolia peel water extract

Table 4: Docking score of C. aurantiifolia peel water extract compounds to antidiabetic proteins

No	Compounds	Docking score (kcal/mol)			
		3A4A	1OSE	7VSI	3G0B
1	Hesperidin	-129.074	-113.3	-127.458	-111.455
2	Hesperetin	-91.6945	-68.8263	-94.0877	-60.1642
3	Naringin	-117.666	-90.7863	-141.885	-84.9061
4	Narigenin	-83.8504	-63.5591	-86.927	-66.2288
5	Limonin	-100.672	-86.2942	-71.59	-87.0844
6	Bergaptol	-66.753	-58.0328	-75.7436	-65.9375
7	Citric Acid	-71.9971	-51.0406	-70.1375	-62.5216
8	Quercetin	-84.1689	-79.6184	-100.765	-89.2847
9	Rutin	-132.549	-103.334	-119.901	-118.397
10	Scoparone	-69.5115	-47.3967	-65.5816	-69.422
11	Acarbose	-130.381	-99.4492		
12	Empaglifozin			-120.187	
13	Alogliptin				-86.8207

Table 5: Absorption profile of C. aurantiifolia peel water extract compounds

Compounds	Water solubility (log mol/L)	Caco-2 permeability (log Papp in 10^{-6} cm/s)	Intestinal absorption (human) %	Skin permeability (log Kp)	P-glycoprotein substrate	P-glycoprotein I inhibitor	P-glycoprotein II inhibitor
Hesperidin	-3.014	0.505	31.481	-2.735	Yes	No	No
Hesperetin	-3.407	0.294	70.277	-2.737	Yes	No	No
Naringin	-2.919	-0.658	26.796	-2.735	Yes	No	No
Naringenin	-3.224	1.029	91.31	-2.742	Yes	No	No
Limonin	-4.379	0.952	100	-2.852	No	Yes	No
Bergaptol	-3.043	0.954	93.911	-2.83	Yes	No	No
Citric acid	-1.423	-0.24	0	-2.735	No	No	No
Quercetin	-2.925	-0.229	77.207	-2.735	Yes	No	No
Rutin	-2.892	-0.949	23.446	-2.735	Yes	No	No
Scoparone	-1.976	1.298	97.879	-2.346	No	No	No

antidiabetic, and antiapoptotic activity.[21] Limonin is a limonoid compound found in *C. aurantiifolia* peel ethanol extract with values of 3.353 ± 0.121 mg/g.[6] Limonin has various pharmacological activities, such as anticancer, antiinflammation, analgesic, antibacterial, antiviral, antioxidant, hepatoprotective, neuroprotective, antiosteoporosis, antiobesity, and antiallergy.[22] Scoparone is a coumarin found in *Artemisia capillaris* and known for treating hepatic disease.[23,24] Scoparone also has the function of treating diabetic nephropathy by inhibiting the ERK1/2 signaling pathway.[25]

Molecular Docking Simulation

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Internal validation was carried out by carrying out a redocking process on selected target proteins, namely *α*-glucosidase inhibitor (PDB ID: 3A4A), *α*-amylase (PDB ID: 1OSE), and SGLT-2 (PDB ID: 7VSI) with 12 combinations of scoring function and algorithm presented in Figure 2.

Molecular docking simulation of 10 compounds from *C. aurantiifolia* is shown in Table 4. Rutin showed *α*-glucosidase and *α*-amylase inhibitory activity with docking score of -132.549 and -103.334, respectively, stronger than acarbose with the docking score of -130.381 and -99.4492. Additionally, rutin exhibited the highest DPP-4 inhibition with a docking score of -118.397, stronger than alogliptin with a docking score of -86.8207. This result aligns with the previous study that showed rutin exhibited the highest DPP-4

inhibition compared to other citrus flavonoids.[26] Hesperidin shows *α*-amylase, SGLT-2, and DPP-4 inhibitory activity with docking scores of -113.3, -127.458, and -111.455, respectively, stronger than acarbose with a docking score of -99.4492, empagliflozin with a docking score of -120.187, and alogliptin with a docking score of -86.8207. Naringin only exhibited SGLT-2 inhibition with a docking score of -141.885. From Figure 3, hydroxyl and carbonyl groups are responsible for hydrogen bonds between ligands and proteins, while the ring A aromatic group possesses hydrophobic interactions with *α*-glucosidase and *α*-amylase, and ring B aromatic group possesses hydrophobic interactions with SGLT-2 and DPP-4.

The gastrointestinal tract secretes highly specific enzymes for carbohydrate metabolism, *α*-glucosidase and *α*-amylase, which catabolize carbohydrates into glucose. *α*-amylase is responsible for catabolizing polysaccharides into oligosaccharides, which *α*-glucosidase further catabolizes into monosaccharides.[27] Inhibition of these enzymes leads to slower glucose absorption and reduced postprandial glucose level peak.[28,29] SGLT-2 facilitated 90% of glucose reabsorption in the kidney. SLGT-2 inhibition is another method to treat DM since it stops a significant quantity of glucose from being reabsorbed into the body, and the excess glucose in the kidney is expelled through the urine after the transfer of sugar molecules is blocked.[30] DPP-4 is an enzyme responsible for the degradation of incretin. Incretin lowers blood sugar levels by suppressing appetite, increasing insulin secretion and

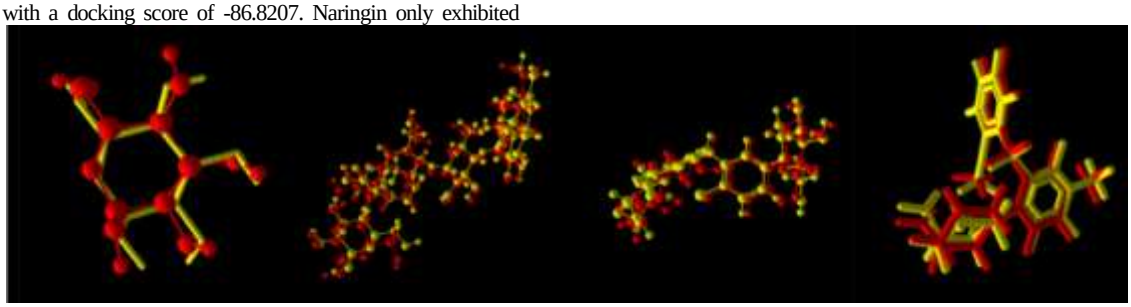

Figure 2: Docking validation left to right: α-glucosidase (PDB ID: 3A4A); αmylase (PDB ID: 1OSE); SGLT-2 (PDB ID: 7VSI); and DPP-4 (PDB ID: 3G0B). Native ligand before docking colored yellow and after docking colored red

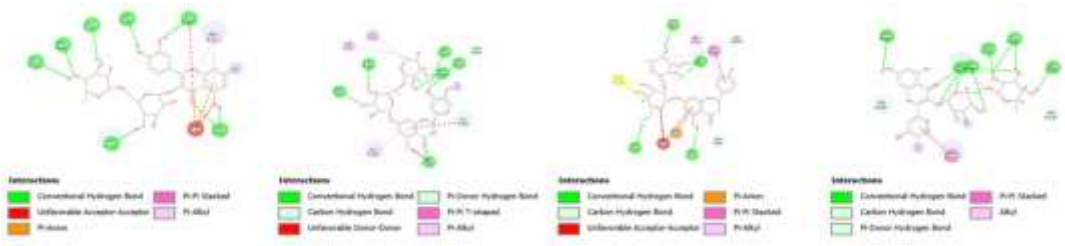

Figure 3:Docking visualization of the most active compounds. From left to right α-glucosidase and rutin complex; α-amylase hesperidin and α-amylase complex; SGLT-2-naringin complex.

Table 6: Distribution profile of C. aurantiifolia peel water extract compounds

Compounds	VDss human (log L/kg)	Fraction unbound (Fu)	BBB permeability (log BB)	CNS permeability (log PS)
Hesperidin	0.996	0.101	-1.715	-4.807
Hesperetin	0.746	0.118	-0.719	-2.976
Naringin	0.619	0.159	-1.6	-4.773
Naringenin	-0.016	0.064	-0.578	-2.215
Limonin	0.273	0.143	-0.841	-2.983
Bergaptol	0.197	0.332	0.39	-2.803
Citric acid	-0.418	0.51	-1.017	-3.61
Quercetin	1.559	0.206	-1.098	-3.065
Rutin	1.663	0.187	-1.899	-5.178
Scoparone	-0.344	0.298	0.177	-2.328

Vdss (Volume Distribution at steady state), BBB (Blood Brain Barrier), CNS (Central Nervous System)

glucagon secretion in the pancreas, decreasing glucagon uptake and storage in adipose cells, decreasing liver glucose production, accelerating gastric

emptying, and increasing glucose uptake in muscle cells. DPP-4 inhibition prevents the degradation of incretin, thereby increasing incretin blood and decreasing blood sugar levels.[31,32]

Flavonoids group is the active compound for *α*-glucosidase, *α*-amylase, and DPP-4 inhibitor recorded in previous studies, which in line with this molecular docking results where flavonoid is the compound responsible for antidiabetic activity in *C. aurantiifolia* peel.[27] Commercial SGLT-2 inhibitors are also derived from phlorizin, a dihydrochalcone flavonoid.[33] Based on the structure-activity relationship of hesperidin, sugar moiety and methoxy group at flavonoid C ring responsible for SGLT-2 inhibitor, as shown in Figure 3.[32] Rutin *α*-glucosidase, *α*-amylase, and DPP-4 inhibition due to presence of hydroxyl group at C3' and C4' at B ring, C4 carbonyl group, and double bond between C2 and C3 at C ring. Additionally, hydroxylation at C3 at C ring increases *α*-glucosidase inhibition but decreases *α*-amylase and DPP-4 inhibition.[27,35]

Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) Prediction

The (ADMET) profile can be predicted with the pkCSM web server by entering the SMILES of the test compound. ADMET prediction aims to predict the pharmacological and toxicological properties of drug candidates. In the absorption profile, the greater the % HIA (Human Intestinal Absorption) value, the higher the biological activity caused by the compound. The absorption of the compound in the intestine is considered poor if the %HIA is ≤ 30%, moderate if HIA is 30 – 79%, and high if HIA is ≥ 80%.[34] From Table 5, four compounds that have HIA value ≥ 80%, namely naringenin (81.31%), limonin (100%), bergaptol (93.911%), and Scoparone (87.879%). Another critical parameter described absorption profile is Caco-2 permeability. Caco-2 is a colorectal adenocarcinoma cell that possesses a similar structure to human intestinal cells. Compounds considered high permeability if Papp values > 0.9×10^{-6}.[35] From Table 5, naringenin, limonin, bergaptol, and scoparone showed high permeability. However, hesperidin, rutin, and naringin show limited absorption and permeability.

In the distribution profile seen from the VDss (Volume Distribution at steady state) value. The drug will be increasingly distributed into tissue rather than plasma if the VDss value is high. If the log value of volume distribution (VD) < - 0.15, then the test compound is considered to have a low VDss and considered a high VDss value if the log value of VD > 0.45.[36] Rutin showed the highest VDss value of 1.883 while naringenin showed the lowest VDss

Table 7: Metabolism and excretion profile of C. aurantiifolia peel water extract compounds

Compounds	Metabolism							Excretion	
	CYP2D6 substrate	CYP3A4 substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Total clearance (log ml/ min/kg)	Renal OCT2 Substrate
Hesperidin	No	No	No	No	No	No	No	0.211	No
Hesperetin	No	No	No	No	No	No	No	0.044	No
Naringin	No	No	No	No	No	No	No	0.318	No
Naringenin	No	No	Yes	No	No	No	No	0.06	No
Limonin	No	Yes	No	No	No	No	No	0.092	No
Bergaptol	No	No	Yes	No	No	No	No	0.71	No
Citric acid	No	No	No	No	No	No	No	0.895	No
Quercetin	No	No	Yes	No	No	No	No	0.407	No
Rutin	No	No	No	No	No	No	No	-0.369	No
Scoparone	No	No	Yes	No	No	No	No	0.793	No

CYP (Cytochrome P450), OCT2 (Organic Cation Transporter 2)

Table 8*:* Toxicity profile of C. aurantiifolia peel water extract compounds

Compounds	AMES Toxicity	Maximum Tolerated Dose (log mg/kg/day)	Oral Rat Acute Toxicity LD_{50} (mol/kg)	Hepatotoxicity	Skin Sensitisation	*T. pyriormis* Toxicity (log µg/L)	Minnow Toxicity (log mM)
Hesperidin	No	0.525	2.506	No	No	0.285	7.131
Hesperetin	No	0.25	2.042	No	No	0.39	2.305
Naringin	No	0.43	2.495	No	No	0.285	6.042
Naringenin	No	-0.176	1.791	No	No	0.369	2.136
Limonin	No	-0.457	3.454	No	No	0.286	0.805
Bergaptol	Yes	-0.317	2.07	No	No	0.599	1.777
Citric acid	No	0.749	2.148	No	No	0.285	4.251
Quercetin	No	0.499	2.471	No	No	0.288	3.721
Rutin	No	0.452	2.491	No	No	0.285	7.677
Scoparone	No	0.494	2.345	No	No	0.603	1.223

value of -0.016. Fraction unbound (Fu) calculates transported in unbound or bound states in the bloodstream. It is an essential parameter because only unbound compounds can bind to the target protein.[35] Compounds in CPWE showed Fu ranging from 0.064 to 0.51. Blood-brain barrier (BBB) permeability is a parameter that indicates the extent to which a compound can cross the blood-brain barrier. BBB permeability with a log BB value of more than 0.3 means the compounds can cross the BBB. In contrast, BBB permeability with a log BB value less than -0.1 means the compounds distributed to BBB are negligible. In contrast, for central nervous system (CNS) permeability, log PS more than -2 is considered capable of crossing CNS. Less than -3 is considered incapable of crossing CNS.[37] BBB Table **7**, only limonin is predicted to be metabolized by 3A4, and none of the CPWE compounds inhibit CYP2D6 or CYP3A4.

The total clearance value, a combination of renal and hepatic clearance, can determine the excretion profile of compounds. This value is related to the bioavailability of the drug and is useful for determining the dose level to achieve concentration. The total clearance value ranges from –0.338 to 0.895. Bergaptol, citric acid, and scoparone have the highest total clearance values for the 10 compounds, which are quickly excreted from the body. Organic Cation Transporter-2 (OCT2) is a renal transporter responsible for the uptake and clearing of cationic compounds from the liver and kidneys.[39] From **Error! Reference source not found.**, neither CPWE compound is an OCT2 substrate.

The toxicity value of the compound is predicted by AMES, maximum tolerated dose (MTD), oral rat acute toxicity (ORAT), hepatotoxicity, skin sensitization, *T. pyriormis*. AMES toxicity is used to determine the mutagenicity of the compounds using bacteria. From Table 8, bergaptol shows AMES toxicity, which means the test compound is mutagenic and possible to cause cancer. This result contradicted with a recent study that bergaptol did not demonstrate mutagenicity, unlike another furanocoumarin.[40] MTD of CPWE compounds ranging from -0.457 to 0.749. MTD value of ≤ 0.477 is considered low, and > 0.477 is considered high. From Table 8, hesperidin, citric acid, quercetin, and scoparone showed high MTD. Minnow toxicity is another parameter that describes LC_{50} to fathead minnow. It is considered toxic if log LC_{50} < -0.3 and none of CPWE compounds are considered toxic.[36]

Conclusion

Based on the results of molecular docking predictions of 10 compounds from CPWE, flavonoid compounds were found to have antidiabetic potential. Rutin inhibits *α*-glucosidase, *α*-amylase and DPP-4, hesperidin inhibits *α*-amylase and SGLT-2, and naringin inhibits SGLT-2. However, from ADMET prediction, these flavonoids show limited solubility and permeability. Therefore, CPWE has strong potential to be developed into an antidiabetic herbal product, as it demonstrated antidiabetic activity and no toxicity.

Conflict of interest

The author`s declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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