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SCREENING OF DRUG LEAD CANDIDATES FROM *Eurycoma longifilia* AND *Crocus sativus* FOR DPP-4 INHIBITION VIA IN SILICO PHARMACOKINETIC AND MOLECULAR DOCKING ANALYSES

Hannah Zaihal Hazri[1], Nurfatini Zakaria[1], Muhajir Hamid[2], Mohd Badrin Hanizam Abdul Rahim[1] and Mohd Ezuan Khayat[1,*]

[1]*Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia.*
[2]*Department yof Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia*

*Corresponding Author: m_ezuan@upm.edu.my

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Abstract

Dipeptidyl peptidase 4 (DPP-4) is an enzyme responsible for the inactivation of incretion hormones, and its inhibition augments insulin release from the pancreas. Over the past years, the inhibition of DPP-4 has emerged as an important therapeutic route to treat type 2 diabetes (T2D) since it offers a lower risk of hypoglycemia. Currently, there are several DPP-4 inhibitors available in different molecular structures. The structural diversity of these inhibitors proves the flexibility of the enzyme to bind various shapes of inhibitors. This opens the opportunity for more discovery of novel DPP-4 inhibitors. In this study, 15 phytocompounds from *Eurycoma longifilia and Crocus sativus* were screened to obtain the candidate leads for DPP-4 inhibitor through in silico pharmacokinetics and molecular docking studies. In order to assess the drug-likeness of the phytocompounds, Lipinski rule of 5 was applied. Thirteen out of 15 phytochemicals were found to pass all the criteria set for this rule. Next, the absorption, distribution, metabolism, and excretion – toxicity (ADMET) profile of the 13 compounds were studied and the result showed only four of them (eurycomalactone, crocetin, picrocrocin and crocusatin D) possessed the characteristics of the good drug. Via molecular docking, it was found all four compounds bound to DPP-4, with the highest binding affinity was observed in eurycomalactone-DPP4 interaction. Eurycomalactone was found to bind to DPP-4 at His363, Ile407, Ile374, Arg356, as well as two amino acids that have previously shown to interact with DPP-4 inhibitor, Phe357 and Arg358. This study indicates that eurycomalactone from *Eurycoma longifilia* can potentially be developed into a new dpp-4 inhibitor.

INTRODUCTION

Diabetes mellitus (DM) is a chronic, non-communicable disease (NCD) that has emerged as one of the main worldwide health issues. Based on the National Health and Morbidity Survey 2019, diabetes accounts for around 3.9 million Malaysians. The prevalence rate has climbed from 13.4% in 2015 to 18.3% in 2019 [1]. This equates to one in every five adults in the country, earning Malaysia the moniker of "Sweetest Nation in Asia." The most common form of DM is T2DM. This type of diabetes originates from a condition termed as insulin resistance and an insufficient compensatory insulin secretory response of the pancreas, mainly caused by a sedentary lifestyle and unhealthy diets. In another hand, Type 1 diabetes (T1D) is characterized by the inability of the body to produce insulin due to the autoimmune destruction of the β-cells in the pancreas.

Incretins including glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are hormones that act on the pancreas to stimulate postprandial releases of insulin. These hormones have extremely short half-lives since they are readily degraded by DPP-4 [2]. Dipeptidyl peptidase-4 is a serine exopeptidase that cleaves proline and alanine dipeptides from N-terminus of peptides at the penultimate position [3]. The enzyme has attracted much attention as they are responsible for up to 70 % of postprandial insulin secretion [4]. The inhibition of DPP-4 prolongs the half-life of incretins thus allowing more insulin secretion. This strategy also seems to reduce the risk of hypoglycaemia since the stimulation of insulin secretion by incretin depends on the blood glucose level [5]. Currently, nine DPP-4 inhibitors are commercially sold, which are sitagliptin, vildagliptin, alogliptin, linagliptin, anagliptin, teneligliptin, saxagliptin, trelagliptin and omarigliptin [6]. These inhibitors have diverse structures suggesting the enzyme accepts various shapes of inhibitors. The ability of DPP-4 to accept different structures of inhibitors may be due to its large cavity and its multiple binding subsides (S1, S2 and S3) [6]. This opens the opportunity for the discovery of new inhibitors with different structures.

The phytocompounds from plants have been long used as the lead compounds for drug development. About a quarter of all Food and Drug Administration (FDA) and/or the European Medical Agency (EMA) approved drugs are derived from plants [7]. Owing to this, we opted for phytocompounds from *E. longifilia and C. sativus* to screen the potential candidates for DPP-4 inhibitors. *E. longifolia* is a popular traditional herbal medicine that native to Indonesia, Malaysia, Vietnam and also Cambodia, Myanmar, Laos and Thailand. Traditionally, the root of *E. longifilia* is widely used in folk medicine to treat various illness such as sexual dysfunction, aging, malaria, cancer, diabetes, anxiety, aches, constipation, exercise recovery, fever, increased energy, increased strength, leukemia, osteoporosis, stress, syphilis and glandular swelling. The primary compound *of E. longifolia* is quassinoids [8]. *Crocus sativus* is cultivated in many countries such as Iran, India, Spain, Greece and Turkey [9]. It is extensively used in cosmetics and culinary preparation because to its unique colour and fragrance. *Crocus sativus* has been used in folk medicine as antispasmodic, eupeptic, to treat menstrual cramps, lumbar pain, cough, broncial spasms, asthma, heart disease, small pox, scarlet fever and colds The important bioactive component of *C. sativus* includes apocarotenoids such as crocetin, crocin, safranal and picrocrocin [9]. In this study we used *in silico* pharmacokinetic and molecular docking analyses to screen the potential lead compound for DPP-4 inhibitor since they enable us to screen the compounds in an affordable time, thus reducing the initial costs and improving the chances to find the desired drug candidates.

MATERIALS AND METHODS

Selection of Ligands

Phytocompounds from *E. longifilia and C. sativus* were curated from Biopurify Phytochemicals database (https://www.phytopurify.com).

Preparation of the Ligands

The 2D structures of the compounds were extracted from PubChem (https://pubchem.ncbi.nlm.nih.gov/) or drawn using ChemDraw software. The 2D structures of the compounds were then converted into 3D structures using Discovery Studio Client and subjected to MM2 energy minimization using Chem3D software.

Preparation of the Target Protein

The three-dimensional X-ray crystallographic structures of DPP-4(PBD ID:2P8S) was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/) and saved in .pdb format. The structure of the protein was prepared using Discovery Studio Client software by removing the water molecules and complexes. The hydrogen atoms were then added to the protein structures using MolProbity tool. Following the protein preparation, the protein was subjected to energy minimization using Swiss PDB viewer software.

Prediction of Drug Likeness

The drug likeness of the compounds was predicted using Lipinski Rule of Five using web tool developed by Supercomputing Facility for Bioinformatics and Computational Biology, IIT Delhi (SCFBio) (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp). The compounds were distinguished between drug like or nondrug like based on molecular weight, total number of hydrogen bond acceptor, total number of hydrogen bond donor, water-octanol coefficient ratio logP value and molar refractivity. The compounds that did not comply with the rules were discarded.

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

The Absorption, Distribution, Metabolism, Excretion and Toxicity of the compounds were screened using SwissADME web tool (http://www.swissadme.ch/). The parameters used for screening were human intestinal absorption (HIA), water solubility, brain blood barrier (BBB) penetration, CYP2D6 inhibition and both Egan and Veber filters. Compounds that had poor pharmacokinetic properties and potentially toxic were excluded.

Table 1. Molecular properties of phytocompounds against DPP-4

Source	Compound	PubChem CID	Molecular Formula
Eurycoma longifolia	Eurycomanone	49798945	$C_{20}H_{24}O_9$
	13alpha(21)-epoxyeurycomanone	13936702	$C_{20}H_{24}O_{10}$
	13alpha(21)-dihydroeurycomanone	433873	$C_{20}H_{24}O_8$
	Eurycomalactone	441793	$C_{19}H_{24}O_6$
	14,15beta-dihydroxyklaineanone	10883790	$C_{20}H_{28}O_8$
Crocus Sativus	Crocin	5281233	$C_{44}H_{64}O_{24}$
	Crocin 1	24721245	$C_{44}H_{64}O_{24}$
	Crocin 5	100956919	$C_{22}H_{26}O_9$
	Crocin 6	101924085	$C_{50}H_{74}O_{29}$
	Crocetin	5281232	$C_{20}H_{24}O_4$
	Crocusatin D	11063077	$C_{10}H_{16}O_3$
	Beta-D-glucosyl crocetin	10368299	$C_{26}H_{34}O_9$
	Kaempferol	5280863	$C_{15}H_{10}O_6$
	Picrocrocin	130796	$C_{16}H_{26}O_7$
	Safranal	61041	$C_{10}H_{14}O$

Molecular Docking Analysis

Molecular docking was carried out using Easydock vina 2.0 [1]). The active site residue was selected within a grid box of dimensions X: 18, Y: 24 and Z: 26 spaced at 1Å. The bond energies such as van der walls interaction, hydrogen bond and electrostatic energy between the receptor and ligands were investigated and their interactions were ranked according to their binding free energy. The resulting dock poses were visualized using Discovery Studio Client.

RESULTS AND DISCUSSION

Type 2 diabetes has become a major worldwide concern due to the rise in its prevalence and number of cases over the last decades. In order to combat this metabolic disease, several

sulfonylureas, biguanides, meglitinides, thiazolidinediones, bile acid sequestrants, sodium-glucose transport protein, dopamine agonists, sodium-glucose transport protein 2 (SGLT2) inhibitors, oral glucagon like peptide 1 (GLP-1) receptor agonists and DPP-4 inhibitors [11]. DPP-4 inhibitors were introduced for the treatment of type 2 diabetes in 2006 and currently there are several DPP-4 inhibitors have been approved by the Food and Drug Administration (FDA) such as sitagliptin, saxagliptin, linagliptin, and alogliptin [2, 12]. However, the number is still limited and more DPP-4 inhibitors need to be explored. Thus, in this study we were aiming to screen the phytocompounds from *E. longifilia* and *C. sativus* which potentially could be used as a lead to develop a new DPP-4 inhibitor. The list of compounds from both plants was taken from Biopurify Phytochemicals database since they are available to be purchased in the pure form.

Drug-likeness Screening

Prior to molecular docking analysis, the phytocompounds were screened based on Lipinski Rule of Five. The rule sets the relevant properties of the phytocompounds in order to be considered as drug such as molecular weight should be less than or equal to 500 Da, number of hydrogen bond donor should be less than five, the number of hydrogen bond acceptor should be less than or equal to ten and the water-octanol coefficient ratio logP should be less than 5. A compound is considered to comply the drug-like if violates not more than one criterion. Apart from that, the additional feature, molar refractivity was also accessed in this study. The molar refractivity is measurement of overall polarity of the molecule and a drug-like compound usually has molar refractivity between 40 to 130. The results obtained are shown in Table 2. There are three phytocompounds that found to violate more than one criterion, which are crocin, crocin 1 and crocin 6. All of these compounds violate three criteria, which are having molecular weight more than 500 Da, hydrogen donor more than five, and hydrogen acceptor more than ten. Molecular weight of the compound that found to be less than 500 is anticipated to have poor transportation, absorption, and diffusion in human body, while the number of hydrogen bond donors and acceptors that more than five and 10 correlates to the poor permeation of the compounds across the membranes of cells [13]. Moreover, the molar refractilities of these compounds also do not comply with the drug likeness property (Table 2). Thus, crocin, crocin 1 and crocin 6 were discarded from further analyses.

ADMET Screening

The ADMET profiles of the 13 compounds were next established using SwissADME server to evaluate further their pharmacokinetics properties. The study was aimed to obtain the compounds that have high HIA, impermeable to BBB, inhibitive to CYPD26 enzyme, have high solubility in water, and pass the Egan and Veber filters. Table 3 shows the results. Six candidate compounds were found to have poor HIA which are 14,15-betadihydroxyklaineanone, 13alpha(21)-epoxyeurycomanone, 13alpha(21)-dihydroeurycomano, eurycomanone, crocin 5 and beta-D-glucosyl crocetin. Human intestinal absorption is one of the most influential ADME properties in the early stages of lead discovery and optimization [14]. As for BBB permeability, all compounds are found to be impermeable to BBB except safranal. The blood-brain barrier is a microvascular endothelial cell layer that plays a critical role in separating the brain from the blood. Drugs may penetrate BBB through different mechanisms such as passive diffusion, carrier-mediated transport, endocytosis, and active transport [15]. The drugs that are not targeting the central nervous system (CNS) should not permeable to BBB to avoid unwanted side effects. In the CYPD26 enzyme inhibition study, it was found that just Kaempferol is inhibitive. Cytochrome P450 2D6 is an enzyme that primarily expressed in the liver which responsible in the metabolism of xenobiotics. The enzyme involves in the elimination of approximately 25% of clinically used drugs. Thus, candidate compounds needs to be inhibitive towards the enzyme. As for water solubility, nine compounds are found to be very soluble (eurycomanone, 13alpha(21)-epoxyeurycomanone, 13alpha(21)-dihydroeurycomanone, eurycomalactone, 14,15-betadihydroxyklaineanone, crocusatin D, kaempferol, picrocrocin and safranal), two compounds are soluble (crocin 5 and crocetin) and one has poor solubility which is beta-D-glucosyl crocetin. One of the most critical elements influencing drug bioavailability is aqueous solubility. In order to be absorbed, a drug must be soluble in water to pass the biological membranes [16]. Next, the compounds were also filtered using Egan and Veber filters. The filters are used to predict the passive gut absorption and oral bioavailability of the compounds, respectively [17, 18]. The results show only eurycomalactone, crocetin, crocusatin D, kaempferol, picrocrocin and safranal passed both filters. Together, only eurycomalactone, crocetin, picrocrocin and crocusatin D that fulfil all criteria and were taken for further analysis.

Molecular Docking

Four compounds, which three from *E. longifilia* (Crocetin, Picrocrocin and Crocusatin D) and one *from C. sativus* (eurycomalactone) proceeded to molecular docking to analyze their interactions with DPP-4. The binding free energy was calculated and the compound with lowest energy is considered to have highest binding affinity towards DPP-4. The results in Table 4 show the highest binding affinity was found in the interaction between DPP-4 and eurycomalactone, followed by crocetin, picrocrocin and crocusatin D with binding free energy -8.1, -7.3, -6.6 and -6.0 kcal/mol, respectively. In order to further analyze the interaction between eurycomalactone and DPP-4, their dock posed was visualized in Discovery Studio Client.

Eurycomalactone was found to interact with DPP-4 by forming hydrogen bonds with amino acids Arg356, Phe357 and Arg358. Apart from that, the compound also forms π-alkyl interaction with His363, Ile407 and Ile374 of the enzyme. Previously, it was discovered that one of DPP-4 active site (s2) consists of the Val207, Ser209, Phe357, and Arg358 [19]. The DPP-4 Arg358 was found to involve in hydrogen bond formation while Phe357 in hydrophobic interactions [20, 21]. Our results show that eurycomalactone binds to these two of amino acids. Interestingly, however, we found that eurycomalactone forms hydrogen bond with Phe357 instead of hydrophobic interaction. The study on commercial DPP-4 inhibitor, sitagliptin also shows the inhibitor interact with Arg358 and Phe357 [21]. The interaction of eurycomalactone with the amino acid residues within this site may hinder the enzyme from binding its natural ligands and thus inhibiting it. These results suggest eurycomalactone can potentially be further developed as inhibiting drug for DPP-4.

Table 2. Pharmacokinetic parameters for drug-likeness properties of phytocompounds

Compound	Molecular Weight (mol/g)	Hydrogen Bond Donor	Hydrogen Bond Acceptor	Molar Refractivity	water-octanol coefficient ratio (logP)	status
Eurycomanone	408.0	5	9	93.79	-1.83	Accepted
13alpha(21)-epoxyeurycomanone	424.0	5	10	93.34	-2.62	Accepted
13alpha(21)-dihydroeurycomanone	408.0	5	9	93.79	-1.82	Accepted
Eurycomalactone	348.0	2	6	66.13	0.65	Accepted
14,15-betadihydroxyklaineanone	396.0	5	8	94.60	-1.09	Accepted
Crocin	976.0	14	24	227.43	-5.23	Not Accepted
Crocin 1	976.0	14	24	227.43	-5.23	Not Accepted
Crocin 5	434.0	5	9	93.79	-1.83	Accepted
Crocin 6	1139.1	17	23	259.58	-4.60	Not Accepted
Crocetin	328.0	2	4	97.72	4.61	Accepted
Crocusatin D	184.0	2	3	49.20	0.66	Accepted
Beta-D-glucosyl crocetin	490.0	5	9	129.94	1.87	Accepted
Kaempferol	286.0	4	6	72.39	2.31	Accepted
Picrocrocin	330.0	4	7	80.42	-6.49	Accepted
Safranal	150.0	0	1	46.30	2.49	Accepted

Table 3. ADMET profiling of the selected phytocompounds

Compound	Adsorption Rate (HIA)	BBB Permeant	CYP2D6 Inhibitor	Water Solubility	EGAN	VEBER
Eurycomanone	Low	No	No	Very Soluble	No	No
13alpha(21)-epoxyeurycomanone	Low	No	No	Very Soluble	No	No
13alpha(21)-dihydroeurycomanone	Low	No	No	Very Soluble	No	No
Eurycomalactone	High	No	No	Very Soluble	Yes	Yes
14,15-betadihydroxyklaineanone	Low	No	No	Very Soluble	No	No
Crocin 5	Low	No	No	Soluble	No	No
Crocetin	High	No	No	Soluble	Yes	Yes
Crocusatin D	High	No	No	Very Soluble	Yes	Yes
Beta-D-glucosyl crocetin	Low	No	No	Poorly Soluble	No	No
Kaempferol	High	No	Yes	Very Soluble	Yes	Yes
Picrocrocin	High	No	No	Very Soluble	Yes	Yes
Safranal	High	Yes	No	Very Soluble	Yes	Yes

Table 4. Binding free energy of selected compounds against DPP-4

Compound	Binding Free Energy (kcal/mol)
Eurycomalactone	-8.1
Crocetin	-7.3
Picrocrocin	-6.6
Crocusatin D	-6.0

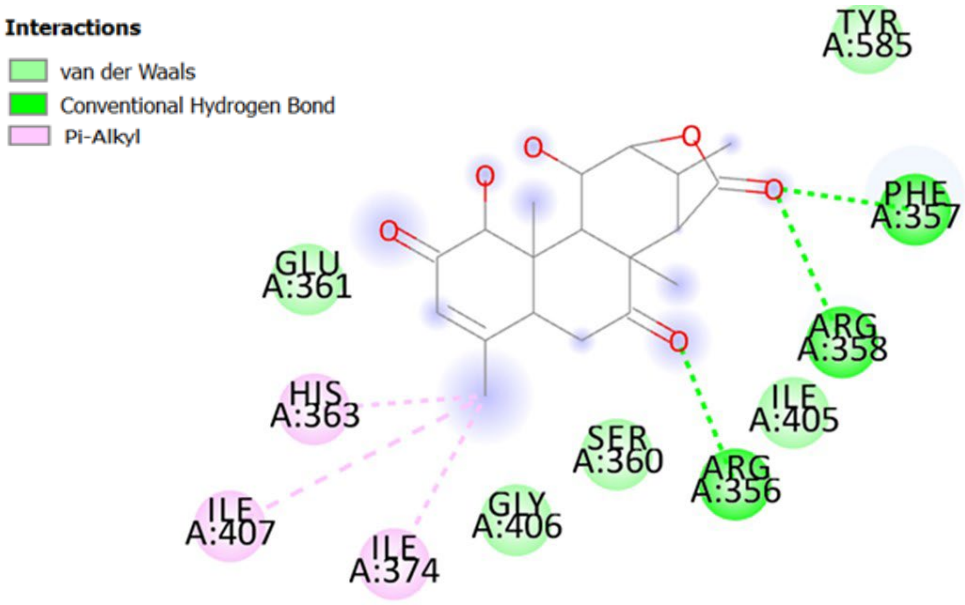

Figure 1. Molecular interactions of eurycomalactone against DPP-4

CONCLUSION

In this study, we explored the structurally diverse compounds from *E. longifilia and C. sativus* from *the* Biopurify Phytochemicals database and performed computational pharmacokinetics and molecular docking analyses to discover the candidates for DPP-4 inhibitors. Following the pharmacokinetics screening using Lipinski rule of five and ADMET, four compounds were found to meet the criteria of an actively oral drug. Among these compounds, eurycomalactone shows the highest binding affinity towards DPP-4 by forming hydrogen bonds within the active site of the enzyme, potentially inhibiting it. Hence, it is concluded that eurycomalactone from *E. longifilia* can potentially be developed into a drug for DPP4 inhibition.

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