IN SILICO PREDICTION OF *CAESALPINIA SAPPAN* L. SECONDARY METABOLITES TOWARDS PPARγ

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ABSTRACT

Chemotherapy can cause mitochondrial dysfunction and oxidative stress that induced Chemotherapy-induced Peripheral Neurotherapy (CIPN) condition. Inhibition of pro-inflammatory transcription factors by PPARγ agonist can be used for the basic development of CIPN treatment. To find potential compounds from plants, this study conducted an *in silico* study of the secondary metabolites of *Caesalpinia sappan* L. In this study, we conducted a molecular docking study of 27 secondary metabolites of *Caesalpinia sappan* L. using an *in silico* approach targeting PPARγ (PDB ID: 2PRG) using AutoDockVina software. ADMET characteristics were predicted using the SwissADME and pkCSM Online Tool. The results showed that metabolites from *Caesalpinia sappan* L. with the strongest affinity for PPARγ were Phanginin D, Phanginin E, Phanginin H, Phanginin A, Phanginin G, Phanginin B, Neosappanone A, and 8-Methoxybonducellin, compared to the native ligand. Therefore, that metabolites potentially to be developed as a treatment for CIPN.

Key words: *Caesalpinia sappan* L., CIPN, *in silico*, PPARγ.

INTRODUCTION

Neuropathic pain can be associated with a wide variety of conditions and syndromes, either the central or peripheral nervous system (Colloca *et al.*, 2017). One of the commonly known neuropathy conditions due to the use of antineoplastic agents called Chemotherapy-induced Peripheral Neurotherapy (CIPN). The prevalence of CIPN based on time is 68.1% in the first month after chemotherapy, 60% after 3 months, and 30% after 6 months (Seretny *et al.*, 2014). Mitochondrial dysfunction and oxidative stress are known to play important roles in the pathophysiology of platinum-induced neuropathy. Decreased mitochondrial physiological function causes decreased cellular metabolism, increased production of ROS (reactive oxygen species), and oxidative stress (Zajaczkowską *et al.*, 2019). In addition, previous studies have proven that cisplatin and oxaliplatin can induce mitochondrial dysfunction in Schwann cell cultures (Imai *et al.*, 2017).

A potential approach to reducing oxidative stress in CIPN is by increasing the endogenous antioxidant response in healthy cells, including neurons. PPARγ agonists reduce inflammation by promoting the inhibition of pro-inflammatory transcription factors such as NF-κB, STAT1, STAT3, AP-1, and NFAT, thereby reducing mRNA synthesis of enzymes and mediators that promote the formation of ROS (Villapol, 2018). The inhibition of NF-κB activity underlies the function of PPARγ in neuroprotection. Neuroprotective effects of PPARγ agonists have been reported in animal models of peripheral neuropathy, including nerve injury-induced neuropathic pain, trigeminal neuropathic pain, and diabetic neuropathy (Elkholy *et al.*, 2020)

Caesalpinia sappan L. is empirically used as an antipyretic and anti-inflammatory. Previous studies showed that *Caesalpinia sappan* L. possesses antioxidant and anti-inflammatory properties (Tewtrakul *et al.*, 2015; Suwan *et al.*, 2018). Brazilein as an active compound of *Caesalpinia sappan* L. had an antioxidant effect by scavenge DPPH (Liang *et al.*, 2013). There is still no research on *Caesalpinia sappan* L. as an antioxidant through PPAR gamma activation. Therefore, drug discovery as an alternative treatment of CIPN condition is urgently needed. In this study, in silico screening through molecular docking was performed to evaluate activity of *Caesalpinia sappan* L. towards PPARγ.

MATERIALS AND METHODS

1. Material
   The software used the windows 11 home single language 64-bit operating system Intel (R) Core (TM) i3-1005G1 CPU @ 1.20GHz (8 CPUs). The protein PPAR γ was downloaded on https://www.rcsb.org/ in PDB format, Protein Data Bank (PDB) code 2PRG (chain A) which had a Root Mean Square Deviation (RMSD) value of 1.789 Å. The test ligands were the metabolite secondary of *Caesalpinia sappan* L. obtained from http://www.knapsackfamily.com. This research had conducted using the application ChemDraw Ultra 12.0, Chem3D Pro 12.0, Biovia Discovery Studio 2016 Client®, and Autodock Tools 4.2. To find out the physicochemical properties and toxicity of the test ligand as a candidate for drug ingredients using SwissADME (http://www.swissadme.ch/index.php) and pkCSM (https://biosig.lab.uq.edu.au/pkcsm/prediction). Protein preparation was performed using AutoDockTools version 1.5.6. Docking protocol validation was analyzed using PyMOL software version 4.6.0 (Schrödinger LLC).
2. Method
   The grid box for the 2PRG receptor was 18 Å×18 Å×18 Å, centered at 50.345 -38.214 19.575. The docking protocol validity is accepted if the value of root mean square deviation (RMSD) ≤ 2.0 Å (Ramírez dan Caballero, 2018). Molecular docking was performed using Cygwin command for running AutoDock Vina and visualized using Biovia Discovery Studio Visualizer v21.1.0.20298 software (Dassault Systèmes, San Diego, California, USA).

RESULT AND DISCUSSION

Neuropathic pain therapy generally aims to improve the patient's quality of life. Several different therapies for neuropathic pain have been investigated. Based on clinical research studies, drugs recommended as first-line therapy for neuropathic pain include tricyclic antidepressants (TCAs), serotonin-norepinephrine reuptake inhibitors (SSRIs), calcium channel α2-δ ligands, and topical lidocaine (O'Connor dan Dworkin, 2009). In recent years, studies of adjuvant antioxidant therapy and ROS scavenger have been extensively studied, but clinical studies have shown controversial results. β-carotene, as a precursor of vitamin A, increases the incidence and mortality of lung cancer (Cockfield dan Schafer, 2019), and vitamin E supplementation increases the risk of prostate cancer in healthy men (Klein *et al.*, 2011). Moreover, the use of antioxidants also reduces the efficacy of chemotherapy and radiation therapy (Mut-Salud *et al.*, 2016). Therefore, the development of other drugs with targeted therapy toward PPARγ activation is important to be used as an alternative.

The docking method is used to determine the binding ability between the structure of metabolites with PPARγ as the pain receptor target. This study evaluates the potential of *Caesalpinia sappan* L. secondary metabolite in regulating PPARγ (2PRG). The structure of the 2PRG macromolecule is the ligand-binding domain of the human peroxisome proliferator-activated receptor gamma complex with 2,4-thiazolidinedione, 5-[[4-[2-(methyl-2-pyridinylamino)ethoxy]phenyl]methyl]-(9cl) or rosiglitazone. Rosiglitazone is the most potent and selective agent in the thiazolidinediones class, which binds the receptor with a higher affinity than other thiazolidinediones drugs, such as pioglitazone or ciglitazone (Liu *et al.*, 2005).

Table 1. Secondary metabolites docking scores against PPARγ

Compound	ΔG (kcal/mol)	Compound	ΔG (kcal/mol)
Rosiglitazone	-7,9	Phanginin G	-8,4
Phanginin D	-8,6	Phanginin B	-8,2
Phanginin E	-8,6	Neosappanone A	-8,1
Phanginin H	-8,6	8-Methoxybonducellin	-8,0
Phanginin A	-8,4		

The binding energy is related to the affinity value of ligand bonds with receptors (Fajrin *et al.*, 2018). The binding energy value is related to the amount of energy that is required by the ligand to bind to a receptor. The less the binding energy (more negative value), the more stable the bond between ligand and receptor. According to the analysis of predicted binding energy (Table 1), there are 8 of 27 metabolites from *Caesalpinia sappan* L. that have lower binding energy compared to rosiglitazone as a native ligand. Among the 27 compounds tested, Phanginin D, Phanginin E, and Phanginin H have the lowest binding energy (-8.60 kcal/mol), followed by Phanginin A and Phanginin G with a binding energy of -8,40 kcal/mol. Phanginin B (-8,20 kcal/mol), neosappanone A (-8,10 kcal/mol), and 8-Methoxybonducellin (-8,0 kcal/mol) have lower binding affinity but still higher than native ligand rosiglitazone. The other compounds have weaker binding energy prediction than native ligands. The receptor-ligand intermolecular interactions were shown in Figure 1.

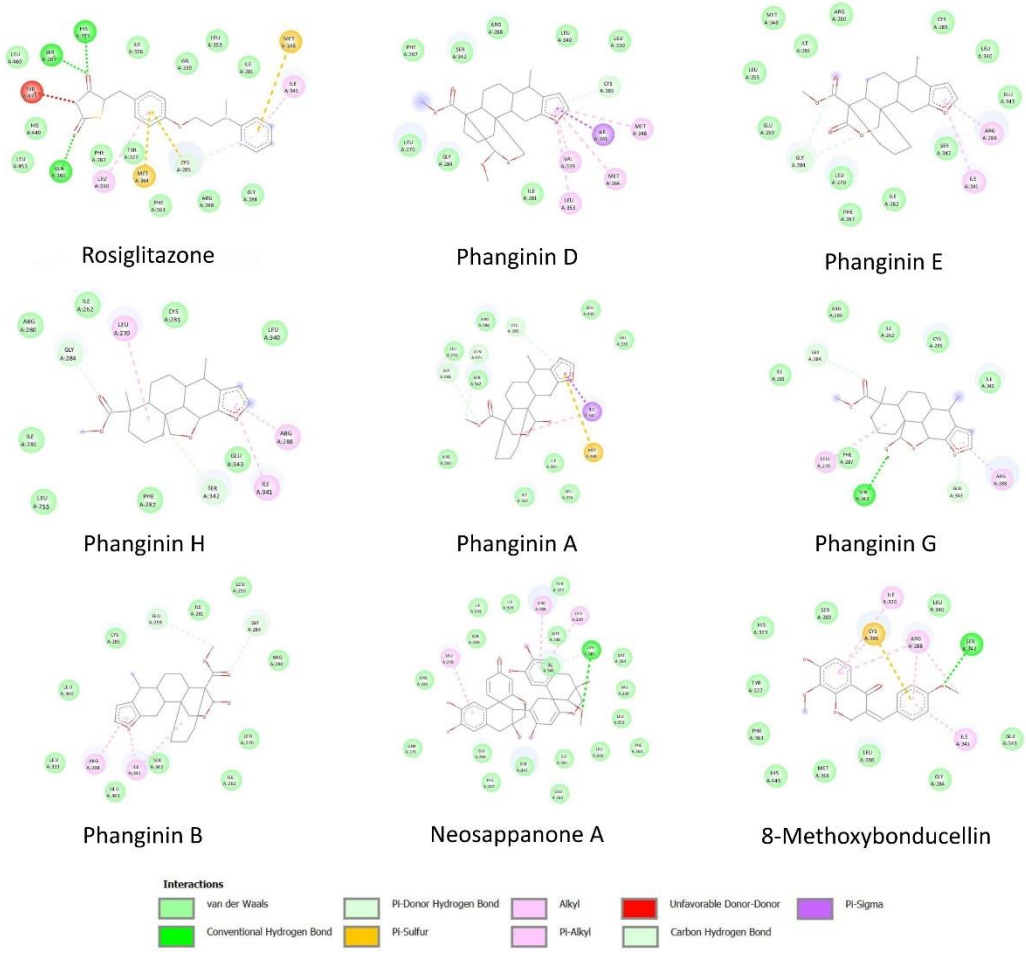

Figure 1. The ligand interactions toward 2PRG receptor.

Table 2. Physicochemical properties, drugability, and safety prediction of the compounds

Compounds	Physicochemical Properties					Drug-likeness Evaluation			Toxicity Prediction	
	MW (g/mol)	RBN	HBA	HBD	mLogP (Log $P_{o/w}$)	BA Score	Violation	RO5	LD_{50} (mol/kg)	Hepatotoxicity
Rosiglitazone	357.43	7	4	1	1.64	0.55	0	Yes	2.692	Yes
Phanginin D	374.47	3	5	0	2.78	0.55	0	Yes	2.313	No
Phanginin E	358.43	2	5	0	2.88	0.55	0	Yes	2.36	No
Phanginin H	344.44	2	4	0	2.98	0.55	0	Yes	2.375	No
Phanginin A	360.44	2	5	1	2.56	0.55	0	Yes	2.649	Yes
Phanginin G	360.44	2	5	1	2.56	0.55	0	Yes	2.55	No
Phanginin B	360.44	2	5	1	2.56	0.55	0	Yes	2.528	No
Neosappanone A	600.57	2	11	6	-0.55	0.11	3	No	2.502	No
8-Methoxybonducellin	312.32	3	5	1	1.36	0.55	0	Yes	2.243	No

The evaluation using SwissADME from 8 of 27 *Caesalphinia sappan* secondary metabolites showed that Neosappanone A unmeet Lipinski's rule (Table 2) by violating three of the 'Rule of Five' (RO5) criteria, which is molecular weight (MW) of less than 500 Da, no more than 10 hydrogen bond acceptors (HBA), and no more than five hydrogen bond donor (HBD) (Lipinski *et al.*, 2001). In drug discovery, violation of the RO5 setting leads to poor membrane permeation and absorbtion. However, this rule specifically states that RO5 applied only to compounds penetrate the cell membrane through passive diffusion (Benet *et al.*, 2016). The analysis using pkCSM showed that only rosiglitazon and phanginin A compounds predicted to have hepatotoxicity effect. It means that majority compounds could have a good safety.

CONCLUSION

This study concludes that phanginin D, phanginin E, phanginin H, phanginin A, phanginin G, phanginin B, neosappanone A, and 8-methoxybonducellin were predicted to have strong activity toward PPARγ and can be developed as a potent PPARγ agonists. However, further studies through in vitro and in vivo study are also needed to support the development of CIPN therapy.

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