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RESEARCH ARTICLE

Theobroma cacao L. (Cocoa) pod husk as a new therapy for transient receptor protein vanilloid-1 (TRPV1)-targeted diabetic neuropathy: An *in silico* study

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Abstract

Backgrounds: *Theobroma cacao* L. (cocoa) is one of the leading commodities found in Indonesia. Cocoa pod husk has many bioactive compounds with antinociceptive properties. One of the targets in treating pain, especially painful diabetic neuropathy, is the transient receptor potential vanilloid-1 (TRPV1). **Aim:** This study aimed to investigate the activity of active compounds from cocoa pod husk extracts against TRPV1 and their toxicity. **Methods:** Molecular docking was used to predict the activity of the test ligands, and the results were analysed with Molegro Virtual Docker 6.0. The TRPV 1 structure was taken from the Protein Data Bank (ID: 5IS0), with capsazepine as a native ligand. The toxicity prediction was evaluated using pkCSM. **Results:** The results showed that the active chemical compounds from cocoa pod husks with the strongest affinity for TRPV1 were phlorofucofuroeckol-A (-95.7785 ± 1.868), catechins (-92.6868 ± 2.681), 7-phloroeckol (-91.9788 ± 0.356), and resveratrol (-91.1921 ± 0.579), and the safest compounds were catechins, resveratrol, and 7-phloroeckol. **Conclusion:** Catechins, resveratrol, and 7-phloroeckol from cacao pod husks are safe and potential therapy for diabetic neuropathy.

Introduction

Painful Diabetic Neuropathy (PDN) is a common comorbidity among diabetic neuropathy patients, with a reported prevalence in Europe ranging from 0.7-34% in Type 1 or Type 2 diabetes mellitus patients overall (Alleman *et al.,* 2015). PDN can even be classified as an incurable disease due to its poor response to conventional analgesics and lack of working drugs based on the pathogenesis of the underlying illness. Current clinical strategies for PDN management include tricyclic compounds, serotonin noradrenaline reuptake inhibitors (SNRIs), anticonvulsants, opiates, and topical capsaicin, often inefficient because of their significant side effects (Carrasco *et al.,* 2018).

Transient receptor potential vanilloid-1 (TRPV1) is an ion channel in sensory neurons activated by protons, capsaicin, heat, and various endogenous lipids called endovanilloids (Palazzo *et al.,* 2010). TRPV1 activation is associated with chronic inflammatory and peripheral neuropathic pain, thereby controlling inflammation and reducing pain in diabetic patients (Brito *et al.,* 2014).

Of the 25,000-30,000 plant species in Indonesia, around 39,000 are estimated to include therapeutic substances. Only 6,000 plants have been recorded as ingredients for herbal medicine, among which roughly 1,000 varieties have been utilised in herbal medicine (Mustofa *et al.,* 2021). Biodiversity holds a lot of promise for discovering novel chemicals, such as cocoa pod husks, previously considered a waste product.

The most often studied part of cocoa was the seeds which are reported to contain active antioxidant compounds, such as phenolic compounds, procyanidins, and flavonoids (Urbanska & Kowalska, 2019), with a demonstrated antidiabetic activity (Olasope et al., 2016). Previous research showed that

cocoa pod husks contain 45.6-46.4 mg of GAE phenolic compounds, 32.3% carbohydrates, 21.44% lignin, 19.2% sugar, 8.6% protein, and 27.7% minerals and pectin in ranges of 6% to 15% from the dry weight (Karim *et al.,* 2014). A recent study could detect 49 bioactive compounds in cocoa extracts, including polyphenols (resveratrol, esculetin, catechins, (-)-epigallocatechin 3-O-Gallate), carbohydrates (pectin), and phlorotannin (phlorofucofuroeckol-A, 7-phloroeckol, DDBT, eckol, and 6,6'-dieckol) (Cadiz-Gurrea *et al.,* 2020). The specific mechanism of these compounds is uncertain, and the compounds involved have not been studied. This study aims to predict the activity of the test ligand against TRPV-1 and its toxicity.

Materials and methods

Hardware and software

The study was conducted *in silico* using the molecular docking method. The hardware used was an ASUS notebook with Intel(R) Core(TM), CPU at 1.80 GHz processor and Windows 8 operating system (64 bit, 4GB RAM). The software used included Molegro Virtual Docker 6.0 (free trial) and ChemOffice 2010, consisting of ChemBio Draw Ultra 12.0 and ChemBio3D Ultra 12.0. The IBM SPSS Statistics version 22 was used to analyse the data.

Molecular Structure and Optimisation

The molecular structure of test ligands was drawn using ChemBio Draw Ultra 12.0 2010 (Figure 1). Furthermore, optimisation was carried out on the geometry of the molecular structure using MM2 tools on ChemBio3D ultra 12.0 2010. The structure of the TRPV1 complex with capsazepine (PDB ID 5IS0) was obtained through the Protein Data Bank and saved in PDB format (Panggalih *et al.,* 2019).

Docking method validation

The re-docking method using Molegro Virtual Docker 6.0 was performed to validate the docking process. Validation was carried out on the active site of the result of 5IS0 crystallography. The 6ET 801 (B) ligand in the conformation found in the complex structure of crystal-ligand (Figure 2B) was extracted and put into the active side (cavity 5 vol. 228.352).

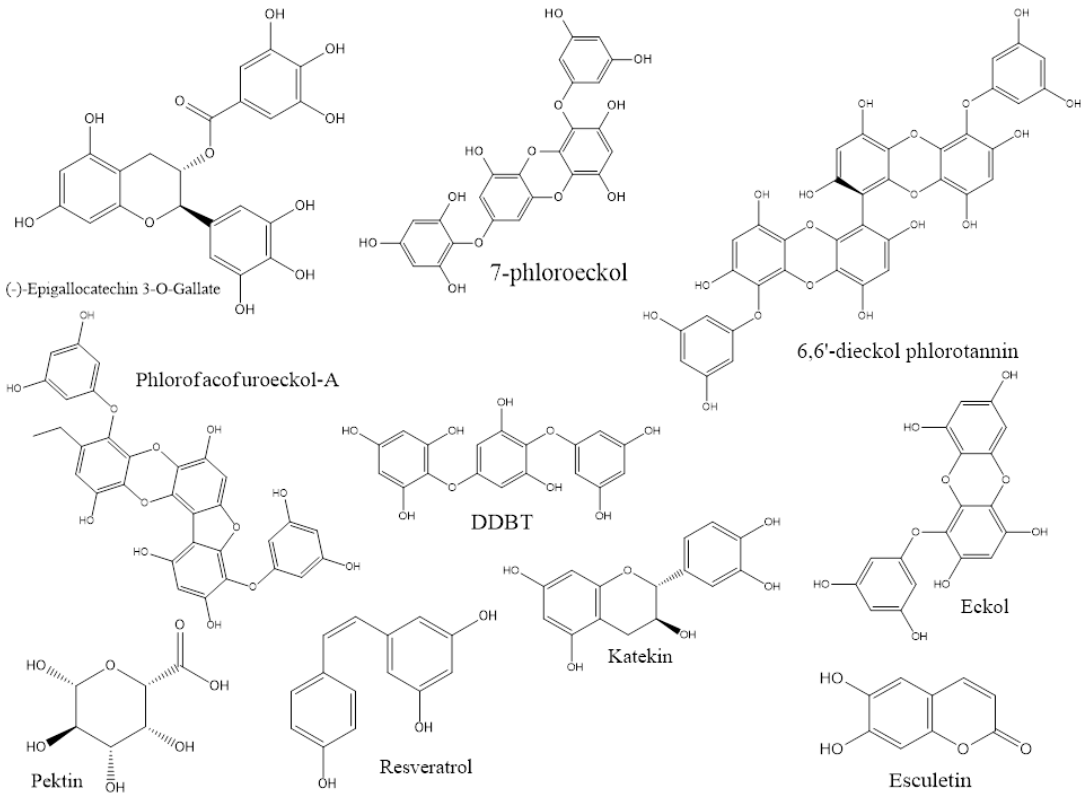
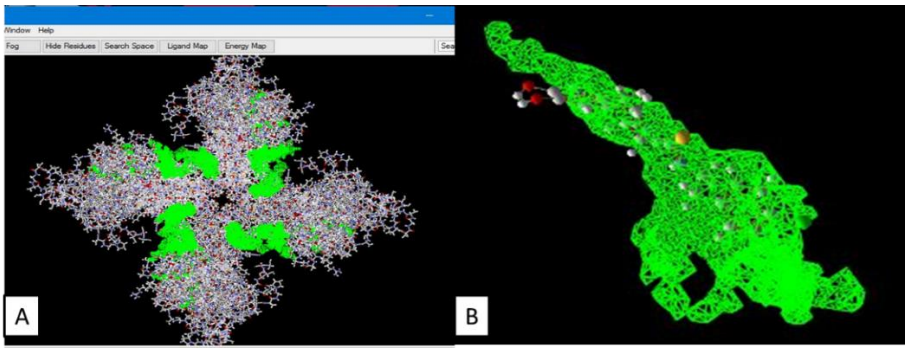


Figure 1: Chemical structure of bioactive compounds from cocoa pod husk



A) TRPV1 after detecting cavities and B) Capsazepin as a native ligand

Figure 2: Interaction between Capsazepine and TRPV1 in Cavity 5

Molecular docking

The molecular docking process was carried out to determine the binding location of the ligand to the TRPV1 as a receptor target in PDN. The test ligands were resveratrol, catechins, esculetin, pectin, 7-phloroeckol, phlorofucofuroeckol-A, (-)-epigallocatechin 3-O-Gallate, DDBT, eckol, and 6,6'-dieckol. Capsazepine, a TRPV1 antagonist, was chosen as a native ligand. The affinity of the test ligands against TRPV1 was indicated by the rerank score using Molegro Virtual Docker 6.0. The determination of the hydrogen bond was also evaluated.

Prediction of toxicity

All the test ligands were predicted for toxicity online using pkCSM. LD_{50} and ADMET values were the parameters used in pkCSM.

Statistical analysis

The rerank score was shown in mean±SD. It was analysed using one-way ANOVA, followed by Tukey's post hoc analysis with a 95% confidence level to evaluate the compound with the best affinity to TRPV1. The *p* - value of 0.05 indicated that the results were significantly different. Toxicity prediction analysis used pkCSM by comparing the lethal dose 50 (LD_{50}) of the tested compounds with ADMET values, such as the lowest-observed-adverse-effect level (LOAEL).

Results

Docking methods validation

The results of the docking method validation showed an average of Root Mean Square Deviation (RMSD) of 1.63271 ± 0.175. This value indicates that the docking method is valid.

Interaction prediction of bioactive compound from cocoa pod husk with TRPV1

The results of the docking analysis of the test ligands with their receptors (Table I) were calculated as a rerank score value described as the affinity of the ligand binds with the receptor. The rerank score is a value that reflects the energy required to form a bond between a ligand and its receptors and thus the compound's predictable activity. The stronger the ligand-receptor bond, the lower the rerank score value.

The test ligands with the strongest bond with TRPV1 are phlorofucofuroeckol-A (-95.7785 ± 1.868), catechins (-92.6868 ± 2.681), 7-phloroeckol (-91.9788 ± 0.356), and resveratrol (-91.1921 ± 0.579). The rerank score of the test compounds is higher than that of capsazepine (-96.3851 ± 1.047), as shown in Table I.

Table I: Rerank score of the test ligands and capsazepine on TRPV1 receptor

Compounds	Rerank score ± SD	No	Compounds	Rerank score ± SD
Capsazepine	-96.3851 ± 1.047[a]	7	Phlorofucofuroeckol-A	-95.7785 ± 1.868[a]
Resveratrol	-91.1921 ± 0.579[b]			
Catechin	-92.6868 ± 2.681[b]	8	(-)-Epigallocatechin 3-O-Gallate	-81.7869 ± 0.979[b]
Esculetin	-67.8644 ± 0.012[c]	9	DDBT	-85.3081 ± 6.531[c]
Pectin	-76.1119 ± 0.052[d]	10	Eckol	-82.3129 ± 0.116[c]
7-phloroeckol	-91.9788 ± 0.356[b]	11	6.6'-dieckol	-71.9141 ± 1.720[c]

Superscript letters indicate that there is a significant difference between groups using One Way ANOVA at the 95% confidence level.

The toxicity prediction of bioactive compounds from cocoa pod husks with TRPV1

The pkCSM study used the LD_{50} and LOAEL to predict toxicity (Table II). Oral LD_{50} (median lethal dose) is an acute toxicity parameter for oral rats. Based on the literature of the criteria for classifying test preparations, the range of LD_{50} values with mild toxicity is >2000-5000 mg and >5000 mg for non-toxic compounds (WHO, 2019). The compound with the highest LD_{50} value was eckol (2.704), indicating it has relatively low toxicity.

Table II: Toxicity prediction of compounds from cocoa pod husks using pkCSM

Compounds	Ames Toxicity	Max tolerated dose (log mg/kg/day)	LD_{50} (mol/kg)	LOAEL (log mg/kg bw/day)	Hepatotoxicity	Skin Sensitisation
Capsazepine	No	0.275	2.314	0.982	No	No
(-)-Epigallocatechin 3-O-Gallate	No	0.439	2.601	4.13	No	No
6.6-dieckol	No	0.438	2.482	6.96	No	No
7-phloroeckol	No	0.417	2.497	4.279	No	No
Catechin	Yes	0.197	2.261	1.587	No	No
DDBT	No	0.425	2.541	3.489	No	No
Eckol	No	0.497	2.704	3.571	No	No
Esculetin	No	0.6	2.054	2.886	No	No
Pectin	No	0.43	2.482	6.49	No	No
Phlorofucofuroeckol-A	No	0.438	2.482	5.458	No	No
Resveratrol	Yes	0.561	2.216	1.761	No	No

LOAEL (Lowest Observed Adverse Effect Level) was defined as the lowest dose at which chronic toxic effects of ingested compounds were observed in rats (WHO, 2017). The highest LOAEL value was 6.96 for 6.6-dieckol, indicating this compound is safer than others. However, capsazepine showed high toxicity.

The physicochemical properties of a ligand when it crosses the cell membrane in the body can be determined by performing the Lipinski test (Table III). The conditions that a ligand must have based on the Lipinski rule are molecular weight <500 Da (this value must be met so that the ligand penetrates the cell membrane more easily), LogP value <5 (related to the polarity of the ligand in fat, oil, or non-polar solvents; a negative LogP value cannot pass through the lipid bilayer membrane), donor hydrogen bonds <5, hydrogen bond acceptor <10, and a molar refractivity range from 40-130 (Benet *et al.,* 2016). Based on the prediction of toxicity according to the Lipinski rule, catechins, resveratrol, and 7-phloroeckol are the safest among all the compounds from cocoa pod husks.

Table III: The ligand properties based on the Lipinski rule through pkCSM

Ligand	Weight Molecule	LogP	Acceptor	Donor
(-)-Epigallocatechin 3-O-Gallate	458.375	2.2332	11	8
6,6-dieckol	742.554	7.2014	18	12
7-phloroeckol	496.38	4.814	12	8
Capsazepin	338.451	3.45792	4	3
Catechin	290.271	1.5461	6	5
DDBT	374.301	3.2104	9	7
Eckol	372.285	3.6105	9	6
Esculetin	178.143	1.2042	4	2
Pectin	588.468	-5.8995	17	8
Phlorofucofuroeckol-A	614.515	7.2758	13	8
Phlorotanin	498.396	3.1548	12	12
Resveratrol	228.247	2.9738	3	3

Discussion

PDN occurs as a result of damage or dysfunction of the nervous system that mediates pain. PDN is associated with metabolic changes due to long-term hyperglycemia. Glucose accumulation causes higher production of ROS (Farmer et al., 2012).

ROS formation activates TRPV1, distributed in the skin, dorsal root ganglia (DRG), and spinal cord dorsal horn (Pabbidi *et al.,* 2008). TRPV1 activation causes depolarisation and stimulation of the NMDA receptor subunit NR2B (NMDAR2B) in the spinal cord's dorsal horn, resulting in chronic pain (Luongo *et al.,* 2012; Zhuo, 2013).

This study explained that some compounds from cocoa pod husks, such as phlorofcofuroeckol-A, catechins 7-phloroeckol, and resveratrol, have a high affinity with TRPV-1. Antagonists or agonists at these receptors reduce pain in diabetic neuropathy (Carrasco *et al.,* 2018). Previous studies demonstrated that substances from

plants, such as capsaicin (Morera *et al.,* 2012) and 6-shogaol (Fajrin *et al.,* 2018), had a strong affinity with TRPV1 *in silico*. Deactivation of TRPV1 causes decreased NMDAR2B activation.

In drug development, activity and safety are the most important to find new candidate substances. This research showed that among ten compounds in cocoa pod husks, phlorofucofuroeckol-A had the best affinity with TRPV1. However, it did not follow the Lipinski rule, making it harder to explore further compared to catechins, resveratrol, and 7-phloroeckol.

Conclusion

This research found that phlorofucofuroeckol-A, catechins, 7-phloroeckol, and resveratrol have the strongest affinity for TRPV1. Moreover, catechins, resveratrol, and 7-phloroeckol are the safest among all the compounds from cocoa pod husks.

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