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IN SILICO TOXICITY OF ANTHOCYANIN COMPOUNDS (CYANIDIN AND PEONIDIN)

Ni Made Pitri Susanti[1*], I Nyoman Triadhi Wisesa[1], Chenme Juwianti[1]

[1]Study Program of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Udayana, Badung-Indonesia

Corresponding author email: dekpitsusanti@unud.ac.id

ABSTRACT

Backgrounds: Cyanidin and peonidin, anthocyanin compounds, have many in silico, in vitro, and in vivo activities, including antioxidant, anticancer, antihyperlipidemic, and antidiabetic. Toxicity testing is carried out to determine the potential hazard that may be produced by the test compound. **Objective:** This study was aimed to determine the in silico toxicity of anthocyanins (cyanidin and peonidin) using Toxtree v3.1.0 software. **Methods:** In silico toxicity testing was carried out using 2D structures of cyanidin and peonidin with Cramer rules, Verhaar scheme, Benigni/Bossa rulebase, Kroes TTC decision tree, Eye Irritation/Corrosion and Skin Irritation/Corrosion parameters. Data analysis on the results of the tested toxicity parameters was carried out descriptively. **Results: T**he results showed that the two compounds have the same category for the toxicity parameters of Cramer Rules (class III), Kroes TTC Decision Tree (substance would not be expected to be safety concern), Benigni/Bossa Rulebase (Negatif for genotoxic and nongenotoxic carcinogenicity), Eye Irritation/Corrosion and Skin Irritation/Corrosion (not irritating or corrosive). Different results are shown in the parameters of the Verhaar Scheme, where cyanidin is included in class 5 (cannot be classified based on this parameter), while peonidin is included in class 1 (narcosis or basic toxicity). **Conclusion:** Based on in silico toxicity, cyanidin and peonidin have a chemical structure that has the potential for toxicity, but these compounds are neither potentially genotoxic nor non-genotoxic carcinogenicity, and are not potentially toxic to the skin and eyes. The toxicity mechanism of cyanidin cannot be classified based on the test parameters while peonidin is narcosis or basic toxicity.

Keywords: Anthocyanin; Cyanidin; Peonidin; Toxicity; In Silico

INTRODUCTION

Anthocyanins are a class of flavonoids found in plants with the characteristic of giving bright colors such as orange, red and blue. There are six types of anthocyanins commonly found in higher plants, namely: cyanidin, pelargonidin, petunidin, malvidin, peonidin, and delvinidin[1]. Many plants contain anthocyanins, one of which is the purple sweet potato (Ipomoea batatas L.). Anthocyanin in purple sweet potato is known to have activity as an antioxidant, anti-inflammatory, anticarcinogenic, antiulcer, hepatoprotective, and hypouricemia[2-7]. In purple sweet potato root, the highest anthocyanin content is cyanidin and peonidin [2]. In silico, cyanidin and peonidin also have the potential to inhibit porcine pancreatic α-amylase as anti-diabetic type 2, inhibit superoxide dismutase as an antioxidant, and HMG-CoA reductase inhibitors as antihyperlipidemia[8-10].

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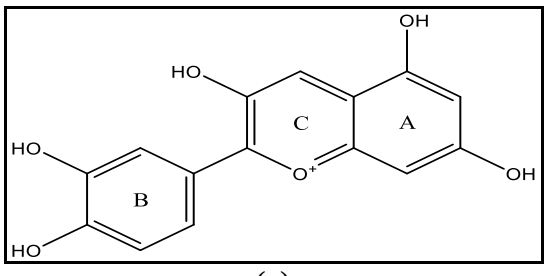
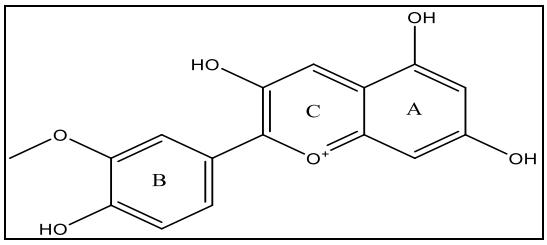
In drug development, besides activity, toxicity also needs to be considered. Toxicity tests determine the potential hazard of compounds that may be produced[11]. Initial toxicity testing can be carried out in silico without using experimental animals, so that toxicity tests can be carried out more quickly and efficiently in predicting the toxicity of a compound[12]. In silico method was developed to predict the toxicity of a compound based on the relationship between its physicochemical properties and biological activity including its toxicity effects, using certain algorithms and database models[13]. In this study, toxicity test of the anthocyanin compounds, cyanidin and peonidin, was carried out using Toxtree v3.1.0. to predict toxicity levels, mutagenesis and carcinogenesis, as well as irritation or corrosion of skin and eyes in silico[14-15].

METHODS

The 2D structures of cyanidin and peonidin were downloaded from https://pubchem.ncbi.nlm.nih.gov. with SDF file format (*.sdf) (Figure 1). Toxicity prediction was performed using Toxtree v3.1.0 software. Tests were carried out using six parameters, namely Cramer Rules, Verhaar Scheme, Benigni/Bossa Rulebase, Kroes TTC Decision Tree, Eye Irritation/Corrosion, and Skin Irritation/Corrosion. The data obtained were analyzed descriptively.



(a)



(b)

Figure 1. 2D Structure of Cyanidin (a) and Peonidin (b)

RESULTS

The results of the cyanidin and peonidin toxicity tests for the 6 test parameters are shown in Table 1. Cyanidin and peonidin showed the same category for the toxicity parameters of Cramer Rules, Benigni/Bossa Rulebase, Kroes TTC Decision Tree, Eye Irritation/Corrosion, and Skin Irritation/Corrosion. Different results are shown in the parameters of the Verhaar Scheme, where cyanidin is included in class 5 (cannot be classified based on this parameter), while peonidin is included in class 1 (narcosis or basic toxicity).

DISCUSSION

Toxtree has many test parameters with different toxicity test results[16]. The Cramer rules, Verhaar scheme, and Benigni/Bossa rulebase toxicity parameters only require the 2D structure of the test compound, while the Kroes TTC decision tree, Eye Irritation/Corrosion and Skin Irritation/Corrosion parameters require additional data, estimated daily intake (μg/day) and melting point.

Cramer rule parameters are used to classify and rank compounds for oral toxicity. This parameter classifies compounds into three classes, namely: Class I, Class II, and Class III. Questions about this parameter are based on knowledge of toxicity and metabolism in mammals[17]. Cyanidin and peonidin are classified into class III, a group of compounds with a chemical structure that

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allows for significant toxicity or toxicity[15]. This is based on the structure of cyanidin and peonidin which have heterocyclic, heteroaromatic, substituted rings, and more than one aromatic ring. Based on Figure 1, the heterocyclic and heteroaromatic structures of cyanidin and peonidin are ring C which is an oxidation form of the pyran ring of flavonoids, while the aromatic rings are rings A and B[18]. Compounds with heterocyclic or heteroaromatic rings have the intermediate metabolism product of arene oxide, which is reactive compound, react with nucleophiles, and react spontaneously to DNA causing carcinogenesis and mutagenesis. The benzene ring with -OH substituent is metabolized through oxidative reaction to become reactive electrophile compound that can cause oxidative stress. Increasing the number of aromatic rings will increase the irreversible inhibitory effect of cytochrome P450 thereby increasing the risk of toxicity [19-21].

The Kroes TTC decision tree is based on Cramer's rules to estimate the exposure threshold based on dose-response for compounds with a carcinogenic risk[14,22]. This parameter requires daily intake data. The daily intake of cyanidin and peonidin compounds is unknown, therefore the daily intake data is used from the results of the Cramer rule parameter test, which is 90 µg/day[23]. The test results show that cyanidin and peonidin are included in compounds with substances that would not be expected to be a safety concern. Cyanidin and peonidin do not have structures indicating potential genotoxic carcinogenicity based on the 39 structural alerts used in this parameter [23,24].

The Verhaar scheme is used to predict the mechanism of toxicity in acute toxicity[25]. This parameter can be divided into five classes, namely: Class 1, 2, 3, 4, and 5. The test results show that cyanidin belongs to class 5, which is a group of compounds that cannot be classified based on the parameters of the Verhaar scheme so that further testing is required[14].

Table 1. Toxicity Test Results of Cyanidin and Peonidin

Test Parameter	Compound	
	Cyanidin	**Peonidin**
Cramer rules	Class III (High)	Class III (High)
Kroes TTC decsion tree	Substance would not be expected to be safety concern	Substance would not be expected to be safety concern
Verhaar scheme	Class 5 (Not Possible to classify according to these rules)	Class 1 (narcosis or baseline toxicity)
Benigni/Bossa rulebase	Negatif for genotoxic and nongenotoxic carcinogenicity	Negatif for genotoxic and nongenotoxic carcinogenicity
Skin Irritation/ Corrosion	Non irritating or corrosive to skin (does not cause burns and severe burns)	Non irritating or corrosive to skin (does not cause burns and severe burns)
Eye Irritation/ Corrosion	Non irritating or corrosive to eye (does not cause burns and severe burns)	Non irritating or corrosive to eye (does not cause burns and severe burns)

Peonidin belongs to class 1 which is a compound with basic toxicity. Compounds with basic toxicity have a toxicity mechanism called narcosis, which is a reversible suppression of physiological functions due to the hydrophobic bond of the compound to cell membranes and proteins. Compounds with this toxicity mechanism have toxic effects through disruption of membrane function[26]. Class 1 compounds are not

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reactive and do not interact specifically with receptors on organisms[14].

Benigni/Bossa rulebase parameters are used to predict the carcinogenic and mutagenic potential of a compound based on the structural alerts model[14.24.27]. The mechanism of carcinogenicity consists of genotoxic and non-genotoxic. Based on the test results, cyanidin and peonidin are predicted not to cause genotoxic or non-genotoxic carcinogenicity. This result was obtained because the cyanidin and peonidin compounds did not contain any of the structural alerts present in this parameter.

Parameters Eye Irritation/Corrosion and Skin Irritation/Corrosion are used to assess the potential toxicity of the compounds to the skin and eyes. This parameter uses the physicochemical properties of the melting point. The test results show that cyanidin and peonidin are not irritating or corrosive (do not cause burns and severe burns). This is because both compounds have high melting points, above 200ºC[28]. The melting point is related to the permeability of compounds on the skin as well as their solubility and ability to penetrate the skin[29]. Compounds with melting points below 100ºC are more easily absorbed through the skin, so the possibility of causing corrosion or irritation is higher[30,31].

CONCLUSION

Based on in silico toxicity, cyanidin and peonidin compounds have a chemical structure that allows for toxicity with a cyanidin toxicity mechanism that cannot be classified based on test parameters, while peonidin is marcosis or basic toxicity. These two compounds do not have the potential to cause carcinogenicity either genotoxic or nongenotoxic, and are not potentially toxic to the skin or eyes.

CONFLICT OF INTEREST

There is no conflict of interest in the preparation of this article.

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