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Characterization of Purple Corn Anthocyanin Components and Their Pharmacokinetic Profiles through In Silico Study

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Abstract: Purple corn contains high levels of bioactive anthocyanins with potential health-promoting effects. This study aimed to quantitatively characterize the anthocyanin profile of an Indonesian purple corn variety and evaluate the pharmacokinetic properties of its major constituents using in silico modelling. The anthocyanin extract was analyzed by HPLC (high-performance liquid chromatography), and concentrations were determined using authentic standards. The pharmacokinetic parameters, including absorption, distribution, metabolism, excretion, and toxicity (ADMET), were predicted using the pkCSM platform, and drug-likeness properties were evaluated based on Lipinski's Rule of Five. Cyanidin-3-glucoside (0.82 mg/100 g DW), peonidin-3-glucoside (0.38 mg/100 g DW), and pelargonidin-3-glucoside (0.35 mg/100 g DW) were identified as the predominant anthocyanins. In silico ADMET predictions using pkCSM revealed low intestinal absorption, moderate peripheral tissue distribution, and violations of Lipinski's Rule of Five, indicating limited suitability as conventional oral pharmaceuticals. Nevertheless, the compounds exhibited low predicted toxicity, minimal CYP450 inhibition, and favorable excretion profiles, supporting potential safe use as nutraceuticals or functional food ingredients. This integrated approach demonstrates that experimental phytochemical profiling combined with computational pharmacokinetic analysis provides valuable insight into the properties of anthocyanins beyond standard bioavailability studies. The findings provide a foundation for future formulation strategies, such as encapsulation or delivery enhancement, to improve bioavailability and functional activity in foods or dietary supplements. Overall, anthocyanins from Indonesian purple corn are promising safe natural bioactives, warranting further experimental and translational research.

Keywords: anthocyanins, pharmacokinetics prediction, pkCSM, purple corn

1. INTRODUCTION

Anthocyanins are a subclass of plant phenolics and serve as natural, water-soluble pigments, frequently employed as alternatives to synthetic colorants. They are commonly found in the cell sap of plants and are key contributors to the red, blue, and purple hues observed in various vegetables, fruits, and seeds [1], including purple corn. Anthocyanins belong to the phenolic compound subclass

of phytochemicals. These compounds are generally present in the form of glycosides, while their sugar-free forms are referred to as anthocyanidins. Anthocyanins possess a range of biological activities, including roles in reducing the risk of diabetes, cancer, inflammation, microbial infections, and obesity, along with providing protective benefits for cardiovascular health [2].

Various anthocyanin compounds identified in purple corn, examining different parts of the plant from multiple regions worldwide. These compounds have been documented in regions such as Peru [3], Mexico [4], China [5], United States [6], and Thailand [7]. Despite the well-documented biological potential of purple corn anthocyanins, the qualitative and quantitative composition of these compounds can vary considerably depending on several factors. Previous studies have demonstrated that anthocyanin accumulation in purple maize is influenced not only by genetic factors but also by environmental conditions such as temperature, ultraviolet radiation, water availability, and soil characteristics. These environmental parameters regulate the biosynthesis and stability of anthocyanins and can significantly alter the phytochemical profile of the crop [8]. Fertilization regimes, soil nutrient composition, and cultivation systems have been reported to affect anthocyanin biosynthesis and accumulation in purple maize. Furthermore, different genotypes or locally adapted cultivars may exhibit distinct anthocyanin compositions and concentrations due to variations in metabolic pathways and genetic regulation of flavonoid biosynthesis [9]. Furthermore, anthocyanin composition may vary substantially depending on genetic background, geographical origin, and cultivation conditions, which can influence both biological activity and pharmacokinetic behavior [10].

Most pharmacokinetic and bioavailability studies on anthocyanins have focused on compounds derived from berries, grapes, or other widely consumed fruits, while purple corn has received comparatively less attention in this context, particularly those cultivated in Indonesia. Pharmacokinetic testing of drugs and drug candidates is optimally performed using in vivo methods with animal subjects or through in vitro techniques. However, this process often involves substantial costs and extensive time commitments, particularly when evaluating multiple drug candidates. To enhance efficiency, computational methods (in silico) are routinely employed, utilizing advanced software designed specifically to simulate pharmacokinetic properties. Among the most effective tools in this regard are pkCSM, which have demonstrated significant value in numerous studies for predicting the pharmacokinetic profiles and toxicity of various compounds [11].

By integrating chromatographic identification of the predominant anthocyanins with structure-based ADMET prediction, the present study provides a preliminary assessment of how the specific anthocyanin profile of Indonesian purple corn may influence its pharmacokinetic and safety properties. This integrative approach contributes to a more contextualized understanding of the potential health applications of anthocyanins derived from regionally cultivated purple corn.

2. MATERIALS AND METHODS

2.1. Plant Materials

Purple corn seeds (*Zea mays* L. var. ceratina) were obtained from the Faculty of Agriculture, Universitas Brawijaya, Malang, East Java, Indonesia. The plant determination process was conducted at the Laboratory of Plant Systematics, Faculty of Biology, Universitas Gadjah Mada. Cultivation of purple corn, intended as raw material for extract preparation, was carried out at the Agro-Technology Innovation Center (PIAT), Universitas Gadjah Mada, Yogyakarta, Indonesia. The corn was harvested

at full maturity, approximately three months after planting. Post-harvest processing included husk removal, followed by drying of the corn ears for one day until the kernels could be easily separated from the cobs. The kernels were then shelled and further dried for three to four days, with drying conducted before 12:00 noon each day. The dried kernels were ground with a grinder, passed through a 60-mesh sieve, and kept in a freezer (-10°C) in maximal six months for future use [12] .

2.2. Preparation of Anthocyanin Extract of Purple Corn

Seventy-five grams of purple corn flour were combined with 750 ml of a 3% ethanol-citrate solution in a 2500 ml Erlenmeyer flask, stirred with a magnetic stirrer set to 1000 rpm at room temperature for a duration of three hours. The mixture was then subjected to centrifugation at 3,000 × g for 20 minutes at ambient temperature to separate the supernatant. This supernatant was then evaporated to dryness at a temperature of 40°C and a pressure of 175 mbar utilizing a Buchi-3000 rotary evaporator, resulting in a concentrated thick extract. The single-step evaporation process yielded 31.4 g of extract with a density of 1.16 g/ml. The thick extract was subsequently packaged in a dark bottle with a secure seal and stored in a dark environment in the freezer to ensure optimal preservation. The same procedure was carried out using 1 N ethanol-HCl in the extraction process [13].

2.3. Anthocyanin Characterization using High-Performance Liquid Chromatography

The analysis of anthocyanin components in purple corn anthocyanin extract was performed using High-Performance Liquid Chromatography (HPLC), which was equipped with a photodiode array detector and an autosampler. The procedure utilized a C18 Symmetry 5 μm reversed-phase column (4.6 × 150 mm) in conjunction with a Symmetry 2 micro guard column (4.6 × 22 mm). For the analysis, the solvents employed included: A) a mixture comprising 1% phosphoric acid, 10% acetic acid, and 5% acetonitrile in water, and B) 100% acetonitrile. Both solvents were filtered through a 0.45 μm poly(tetrafluoroethylene) membrane filter, while the samples were filtered with a 0.45 μm polypropylene filter provided by Whatman Inc. Separation of anthocyanins was carried out through a 35-minute linear gradient, with solvent A concentration rising from 0% to 30%. The injection volume was maintained at 50 μL, with a flow rate set at 1 ml/min. Spectral data were collected over a wavelength range of 260-600 nm [14].

2.4. Pharmacokinetic and Toxicity Prediction of Purple Corn Anthocyanin

A total of three anthocyanin compounds were analyzed for their pharmacokinetic properties and toxicity predictions. The structural information for these anthocyanin compounds was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). To evaluate the physicochemical properties that influence pharmacokinetics and toxicity, we employed the pkCSM (http://biosig.unimelb.edu.au/pkcsm/prediction) online tool. The physicochemical properties, as well as the pharmacokinetic and toxicity predictions of nine anthocyanin compounds in purple corn, were conducted using pkCSM. The structures of the nine compounds, obtained from PubChem, were drawn as two-dimensional molecular structures using MarvinSketch (https://docs.chemaxon.com/display/lts-europium/marvinsketch-downloads.md) and saved in *.pdb format. Subsequently, the files were converted to SMILES format and uploaded into the pkCSM online tool for analysis of physicochemical properties, pharmacokinetics, and toxicity [11].

3. RESULTS AND DISCUSSION

3.1. Characterization of Anthocyanin Compounds

Purple corn anthocyanin extract is characterized as a thick, dark brown solution, which is subsequently analyzed for its anthocyanin components using HPLC. The characterization results using ethanol-HCL 1 N and ethanol-citrate 3% are presented in Figure 1 and Figure 2.

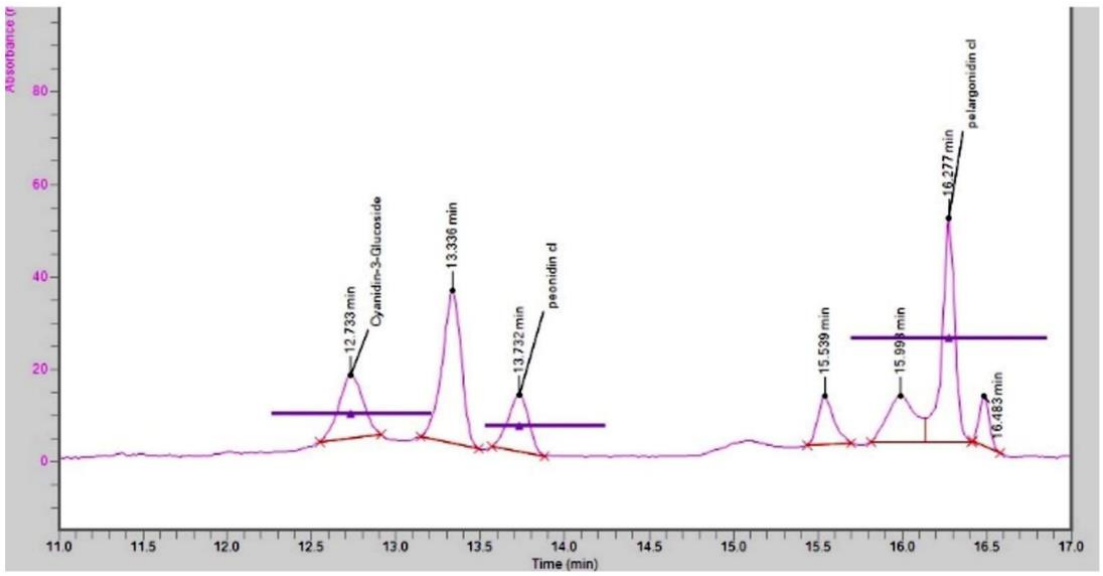

Figure 1. Profile and intensity of anthocyanin compounds in purple corn extract using ethanol–1 N HCl as the solvent

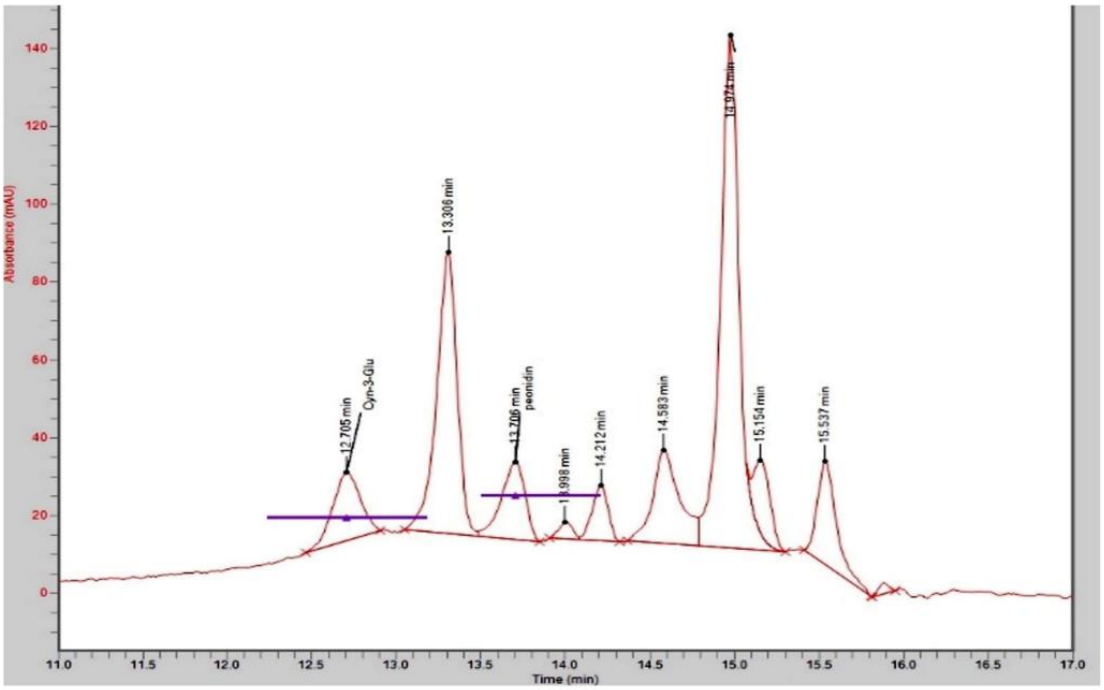

Figure 2. Profile and intensity of anthocyanin compounds in purple corn extract using 3% ethanol–citrate as the solvent

The HPLC profiling of the purple corn anthocyanin extract displayed multiple prominent peaks, each representing distinct anthocyanin compounds, including cyanidin-3-glucoside, peonidin-3-

glucoside, and pelargonidin-3-glucoside. The chromatographic profiles and peak intensities varied depending on the extraction solvent used, specifically 1 N ethanol-HCl and 3% ethanol-citrate. The 1 N ethanol-HCl solvent produced a higher intensity peak for pelargonidin-3-glucoside (retention time ≈ 16.27 minutes) compared to the peaks for cyanidin-3-glucoside and peonidin-3-glucoside (see Figure 1). In contrast, the 3% ethanol-citrate solvent showed the highest intensity peak for cyanidin-3-glucoside (retention time ≈ 13.386 minutes), suggesting that this solvent is more selective or efficient in extracting this particular compound (see Figure 2). Moreover, the consistent retention times across both chromatograms support the accurate identification of the extracted anthocyanin compounds. Based on the HPLC results, three major anthocyanins were identified in the purple corn extract, as summarized in Table 1.

Table 1. Profile of Anthocyanin Compounds in Purple Corn Extract

No	Compound	Rt (ethanol-HCl)	Rt (3% ethanol-citrate)	Intensity	Note
1	Cyanidin-3-glucoside	12.738 min	12.705 min	High (Citrate)	More efficient in ethanol–citrate
2	Peonidin-3-glucoside	13.727 min	13.286 min	Moderate	Consistent across both solvents
3	Pelargonidin-3-glucoside	16.272 min	17.274 min	High (HCl)	More efficient in ethanol–HCl

The selection of two ethanol-based solvents—1 N HCl in ethanol and 3% citric acid in ethanol—for the extraction process and HPLC analysis of anthocyanidin compounds from purple corn was based on the chemical properties of anthocyanins, which are highly influenced by pH. Anthocyanidins, as the aglycone form of anthocyanins, tend to be more stable and readily detectable under acidic conditions, as they exist predominantly in the flavylium ion form at low pH, which is both colored and spectrophotometrically stable [2]. 3% ethanol-citrate creates a highly acidic environment ideal for hydrolyzing the glycosidic bonds between the aglycone and sugar moieties in the anthocyanin structure, then facilitating the detection of anthocyanins such as cyanidin, peonidin, or pelargonidin. This condition enhances chromatogram clarity and supports the identification of target molecules in their free forms. Meanwhile, 3% ethanol-citrate was selected as a safer, more environmentally friendly, and food-compatible solvent alternative, as citric acid is a food-grade organic acid [15].

The presence of three major anthocyanin compounds, namely cyanidin-3-glucoside, peonidin-3-glucoside, and pelargonidin-3-glucoside, has been widely reported as key bioactive components in various types of purple-pigmented plants [3,16]. This study also found that a 3% ethanol-citrate solvent is more effective in extracting cyanidin-3-glucoside, which is known for having one of the highest antioxidant activities among anthocyanins. In contrast, the 1 N ethanol-HCl solvent was able to extract a larger quantity of pelargonidin-3-glucoside. The differences in intensity for anthocyanins between the ethanol-HCl and 3% ethanol-citrate solvents highlight the significant impact that solvent composition has on the extraction efficiency and stability of anthocyanin compounds. This finding aligns with previous research demonstrating that the pH of a solvent influences the structural form of anthocyanins, which in turn affects their solubility [17].

The concentrations of cyanidin-3-glucoside, peonidin-3-glucoside, and pelargonidin-3-glucoside in purple corn were 0.82, 0.38, and 0.35 mg per 100 g of dry weight, respectively. In contrast,

the concentrations of cyanidin-3-glucoside, peonidin-3-glucoside, and pelargonidin-3-glucoside in the 3% ethanol–citrate extract were 27.8, 10.4, and 9.53 ppm, respectively (Table 2).

Table 2. Anthocyanin Content in Purple Corn and Purple Corn Anthocyanin Extract

No	Compound	Content (mg/100 g dry weight)	Content in 3% alcohol–citrate solvent (ppm)
1	Cyanidin-3-glucoside	0.82	27.8
2	Peonidin-3-glucoside	0.38	10.4
3	Pelargonidin-3-glucoside	0.35	9.53

These findings are consistent with previous studies reporting the presence of these three major anthocyanins in purple corn, although their concentrations vary depending on cultivar and geographical origin. Purple corn from China has been reported to contain cyanidin-3-glucoside, peonidin-3-glucoside, and pelargonidin-3-glucoside at levels of 41.45, 6.46, and 17.64 mg/100 g, respectively [18], while another study reported proportions of 75.7%, 16.0%, and 8.3% of total anthocyanins for these compounds [19]. Purple corn from Peru has been reported to contain 15.43, 4.44, and 2.33 mg/g in aqueous extract [20]. Meanwhile, purple corn from Lebanon contains 2.8 and 1.6 mg/g dry extract for cyanidin-3-glucoside and peonidin-3-glucoside, respectively, while pelargonidin-3-glucoside was not quantified [21]. In the United States, concentrations of 268.8, 19.6, and 16.4 mg/g dry weight for cyanidin-3-glucoside, peonidin-3-glucoside, and pelargonidin-3-glucoside, respectively, have been reported [10].

The variation in anthocyanin composition and concentration among purple corn samples from different regions may be influenced by several factors, including genetic differences among cultivars, environmental conditions such as climate and soil composition, agricultural practices, and post-harvest handling. These factors are known to significantly affect the biosynthesis and accumulation of anthocyanins in plant tissues [9].

3.2. Prediction of Physicochemical Properties, Pharmacokinetic, and Toxicity Profiles of Purple Corn Anthocyanins

In this study, the prediction of physicochemical properties, pharmacokinetic, and toxicity profiles for anthocyanin compounds conducted utilizing one recognized tools, pkCSM, which has been extensively applied in various in silico analyses. The predicted pharmacokinetic profile (absorption, distribution, metabolism, excretion, toxicity/ADMET) of anthocyanin compounds are presented in Table 3.

Table 3. Pharmacokinetic and Toxicity Profiles of Anthocyanin Compounds from Purple Corn

Parameter	Req	Compound		
		Cyanidin-3-glucoside	Peonidin-3-glucoside	Pelargonidin-3-glucoside
Absorption				
Water solubility	-	–2.929	–2.734	–2.816
Caco2 permeability	> 0.90	0.058	0.366	0.14
Intestinal absorption	> 30%	45.392	34.632	48.354
Distribution				
VDss	> –0.15	1.464	0.881	0.984

BBB permeability	> –1	–1.713	–1.406	–1.525
CNS permeability	> –3	–3.813	–4.345	–3.628
Metabolism				
CYP2D6 substrate		-	-	-
CYP3A4 substrate		-	-	-
CYP1A2 inhibitor		-	-	-
CYP2C19 inhibitor		-	-	-
CYP2C9 inhibitor		-	-	-
CYP2D6 inhibitor		-	-	-
CYP3A4 inhibitor		-	-	-
Excretion				
Total clearance		0.522	0.646	0.564
Renal OCT2 substrate		-	-	-
Toxicity		-	-	-
AMES toxicity		-	-	-
Max. tolerated dose		0.562	0.573	0.526
hERG I inhibitor		-	-	-
hERG II inhibitor		+	+	+
Oral rat acute toxicity (LD50)		2.549	2.577	2.572
Oral rat chronic toxicity (LOAEL)		4.201	4.584	4.298
Hepatotoxicity		-	-	-
Skin sensitization		-	-	-
T. pyriformis toxicity		0.285	0.285	0.285
Minnow toxicity		6.398	5.797	5.17

3.2.1. Absorption

The results demonstrated that all compounds exhibited low water solubility (log S ranging from –2.929 to –2.734), with poor predicted Caco-2 cell permeability values (<0.9), indicating limited passive absorption across the intestinal membrane. Despite this, cyanidin-3-glucoside and pelargonidin-3-glucoside showed moderate predicted human intestinal absorption rates (34.6–48.4%). A drug candidate is considered to have favorable absorption when the intestinal absorption exceeds 80% and the Caco-2 permeability is greater than 0.90, whereas absorption is deemed poor if the intestinal absorption falls below 30%. Based on the predictions from pkCSM, all anthocyanin compounds derived from purple corn extract exhibited Caco-2 permeability values below the optimal threshold (≤ 0.9 log Papp), indicating poor transcellular absorption across the intestinal epithelium. Caco-2 cell monolayers are widely recognized as a gold standard model to estimate passive drug diffusion across the intestinal epithelium. This low permeability can be attributed to the hydrophilic nature of anthocyanins, their glycosidic structures, and relatively high molecular weights, which collectively limit their passive transcellular transport [22].

Furthermore, the predicted human intestinal absorption (HIA) values indicated that all compounds exceeded the 30% threshold typically associated with acceptable oral absorption. These compounds may have simpler structures, lower molecular weights, or fewer glycosylation moieties, which could facilitate limited diffusion or improve recognition by intestinal transporters.

3.2.2. Distributions

All compounds demonstrated a high volume of distribution (VDss > 0.85 log L/kg), suggesting extensive distribution across peripheral tissues. The unbound plasma fraction ranged from 0.161 to 0.293, indicating that a moderate portion of each compound remains free and potentially pharmacologically active. Predicted blood-brain barrier (BBB) and central nervous system (CNS) permeabilities were low for all compounds, with log BB values below –1 and log PS values below –3, consistent with limited CNS penetration. These results indicate that while the anthocyanin compounds are unlikely to cross the BBB efficiently, they are still predicted to achieve broad distribution in systemic tissues. Taken together, the pkCSM predictions suggest that the anthocyanins derived from purple corn extract possess favorable peripheral tissue distribution, which may support their potential biological activity outside the CNS.

A steady-state volume of distribution (VDss) greater than -0.15 log L/kg indicated a moderate to high potential for extravascular tissue penetration. This implies that the compounds are not confined to the plasma compartment and may exert biological effects in various peripheral. The predicted blood-brain barrier (BBB) permeability values for anthocyanins, particularly those derived from purple corn, suggest a moderate ability for these compounds to traverse the BBB, with values typically exceeding -1 logBB for various assessed anthocyanins. While this permeability does not imply robust penetration into the central nervous system (CNS), it indicates the potential for achieving pharmacologically relevant concentrations within this critical area [23]. Collectively, these distribution characteristics suggest that anthocyanins from purple corn may not only possess systemic bioavailability but may also have therapeutic relevance in targeting CNS-related pathologies, such as neuroinflammation and oxidative stress-induced neuronal damage.

3.2.3. Metabolism

No compound was predicted to interfere with primary cytochrome P450 enzymes, suggesting that the risk of pharmacokinetic interactions is low. Predicted total clearance values were moderate (ranging from 0.522 to 0.759 log mL/min/kg), and all compounds were not identified as renal OCT2 substrates. The finding that anthocyanin compounds derived from purple corn extract do not interact with CYP enzymes suggests that they are neither substrates nor inhibitors of these metabolic pathways.

This observation aligns with their chemical structure, as anthocyanins are characterized as polar and hydrophilic due to the presence of glycosyl and hydroxyl groups in their flavylium structure [24, 25]. This in silico predictions are consistent with established biological mechanisms and support the hypothesis that anthocyanins extracted from purple corn exhibit a relatively safe metabolic profile, thereby suggesting a low likelihood of involvement in CYP-mediated drug interactions.

3.2.4. Excretion

The predicted total clearance (CLTOT) values for anthocyanin compounds, which range from 0.522 to 0.759 log mL/min/kg and none of the anthocyanin compounds had any effects on OCT2 substrate. Measurements of renal Organic Cation Transporter 2 (OCT2) substrate activity and total clearance (CLTOT) are useful for predicting how a substance is eliminated from the body. CLTOT represents the combined contribution of hepatic clearance (metabolism in the liver and biliary excretion) and renal clearance (elimination via the kidneys) [11]. OCT2, present in renal tissue, contributes significantly to the movement and excretion of both therapeutic agents and internal compounds. Because of their impact on bioavailability, these parameters are essential for determining

the dosage necessary to attain and sustain steady-state drug levels. CLTOT values of anthocyanins and no effect on OCT2 substrate in this study indicate moderate systemic elimination characteristics [26].

Such clearance rates suggest that these compounds are not cleared rapidly and may afford a therapeutic window, which could be advantageous if sufficient plasma concentrations can be achieved over time. The moderate clearance rates point towards relatively short-to-intermediate half-lives, thereby facilitating their potential use for chronic administration with more regular dosing intervals. The pharmacokinetics of these anthocyanins suggest that while therapeutic effects can be sustained, the timing of dosing will be crucial for maintaining effective plasma levels [27].

3.2.5. Toxicity

The predicted toxicological profiles of the anthocyanin compounds from purple corn extract were generally favorable. All compounds tested negative for AMES mutagenicity, hepatotoxicity, and skin sensitization, indicating low genotoxic and general toxicity risks. Three compounds were predicted to inhibit hERG II channels, which play a critical role in cardiac repolarization and are associated with the risk of QT prolongation and arrhythmias [28]. However, it is important to contextualize this prediction within realistic dietary or nutraceutical exposure scenarios. Typical daily anthocyanin intake from purple corn-derived products is on the order of tens to hundreds of milligrams, far below the concentrations predicted to produce significant hERG II inhibition in silico. Therefore, while hERG II inhibition is noted, the likelihood of clinically relevant cardiac effects under normal consumption conditions is minimal.

Acute and chronic oral toxicity predictions further support the safety of these compounds. The predicted LD50 values (~2.5 log mg/kg, equivalent to ~316 mg/kg body weight) indicate low acute toxicity, and the LOAEL values for chronic exposure (3.5–4.6 log mg/kg, equivalent to ~3,200–40,000 mg/kg) suggest a wide therapeutic index. When compared with typical dietary anthocyanin intake, the safety margins exceed several orders of magnitude, implying that normal consumption of purple corn extracts or nutraceutical formulations is unlikely to pose significant toxicity risks. These findings highlight that, despite in silico predictions of hERG II inhibition, the anthocyanin compounds are generally safe for oral consumption within realistic dietary or nutraceutical exposure levels, supporting their potential application as functional food ingredients or adjunctive nutraceuticals [29].

3.3. Drug-likeness of Anthocyanin Compounds

The drug-likeness potential of anthocyanin compounds is presented in Table 4.

Table 4. Lipinski's Rule of Five Profile of Anthocyanidin Compounds in Purple Corn

Compound	MW	Log P	Rotable bonds	HBA	HBD	Violations
Cyanidin-3- glucoside	449.388	0.382	4	10	8	1 (HBD)
Peonidin-3-glucoside	498.868	-2.311	5	10	7	1 (HBD)
Pelargonidin-3-glucoside	433.389	0.6764	4	9	7	1 (HBD)

Note: MW=molecular weight; HBA=hydrogen bond acceptors; HBD=hydrogen bond donors

This result suggests that these compounds remain within the acceptable limits as potential drug candidates as per Lipinski's criteria. A single violation related to the number of hydrogen bond

donors (HBD > 5), is a common feature in polyphenolic natural products and may still be acceptable under certain pharmacokinetic optimization strategies [30]. Notably, compound peonidin-3-glucoside has a significantly low log P value of -2.311, indicating a highly hydrophilic nature.

While the Lipinski's Rule of Five analysis highlights certain pharmaceutical limitations of the anthocyanin compounds, alternative evaluation frameworks provide a more appropriate perspective for natural products and nutraceuticals. Polyphenolic compounds such as cyanidin-, peonidin-, and pelargonidin-based anthocyanins frequently violate one or more Lipinski criteria due to high molecular weight, multiple hydrogen bond donors and acceptors, and low lipophilicity, yet they remain biologically active. Concepts such as the "beyond the Rule of Five" (bRo5) and the Veber rule—considering topological polar surface area and molecular flexibility—are better suited to assess the drug-likeness and absorption potential of natural polyphenols [31] .

From a nutraceutical perspective, anthocyanins can exert biological effects through antioxidant activity, modulation of oxidative stress pathways, metabolite-mediated mechanisms, and interactions with gut microbiota, despite low systemic bioavailability [32]. Several formulation strategies have been developed to enhance their stability and absorption, including nanoencapsulation, liposomal delivery, polymeric nanoparticles, and microencapsulation, which protect anthocyanins from degradation and improve intestinal uptake. Clinical and intervention studies further support the potential health benefits of anthocyanin-rich foods, showing improvements in endothelial function, blood pressure, and lipid profiles [33].

By integrating these alternative evaluation frameworks, mechanistic insights, and formulation strategies, anthocyanin compounds can be more appropriately considered as functional food ingredients or nutraceuticals rather than conventional oral pharmaceuticals, aligning their pharmacokinetic properties and safety profiles with realistic dietary and preventive health applications.

3.4. Implications for Health and Medical Applications: Repositioning of Anthocyanins

The pharmacokinetic and toxicity profiles predicted in this study, along with the analysis based on Lipinski's Rule of Five, provide valuable insights into the application of anthocyanins from purple corn in health and medicine. This study have shown that several anthocyanin compounds violate two or more of Lipinski's criteria, which is often due to their high molecular weight, excessive numbers of hydrogen bond donors and acceptors, and low lipophilicity. These characteristics indicate poor oral drug-likeness from a traditional pharmaceutical perspective.

However, it is crucial to note that natural compounds, such as flavonoids and polyphenols like anthocyanins, often do fall outside Lipinski's parameters yet still exhibit biological activity and clinical utility. This is especially true when these compounds function as nutraceuticals, adjuvant therapies, or locally acting agents. The violations of Lipinski's criteria highlight the challenges associated with systemic delivery, suggesting that formulation improvements—such as encapsulation, prodrugs, or nanoemulsions—may be necessary to enhance absorption and targeted delivery. Here is the summary table comparing the prediction results of pkCSM and Lipinski's Rule of Five for anthocyanin compounds derived from purple corn, along with their implications for formulation development and clinical application (Table 4).

Table 4. Summary of Pharmacokinetic Predictions, Lipinski's Rule Assessment, and Implications for Health Applications

Aspects	pkCSM Results	Lipinski's Rule Assessment	Interpretation	Health Application Implications
Absorption	Low Caco-2 permeability; Moderate intestinal absorption (>30% for some compounds)	Violations: H-bond donors >5, H-bond acceptors >10, MW >500 Da	Poor passive absorption; some GI uptake possible	Suitable for functional food or nutraceutical formulations; requires delivery enhancement for systemic drug use
Distribution	High VDss (>0.8 L/kg), Low BBB and CNS permeability	Not CNS-permeable; lipophilicity (logP) < 5	Favors peripheral distribution; unlikely to cross blood-brain barrier	Effective for peripheral conditions (e.g., cardiovascular, anti-inflammatory)
Metabolism	Not substrates or inhibitors of CYP450 enzymes	Not applicable	Low risk of hepatic metabolism interaction	Safe for use with other medications; suitable for chronic use
Excretion	Moderate clearance (0.5–0.7 mL/min/kg); not renal OCT2 substrates	Not applicable	Clearance through hepatic/biliary routes likely	May require controlled-release systems to prolong action
Toxicity	No AMES toxicity, no hepatotoxicity, no skin sensitization; minor hERG II inhibition	Acceptable safety profile	Generally non-toxic and environmentally safe	Safe for long-term supplementation; minimal side effects expected
Lipinski's Rule of Five	Multiple violations (MW >500, HBD/HBA excess, low LogP)	Not drug-like per Lipinski	Limited oral bioavailability as classic drug	More appropriate as dietary bioactives or with nanocarrier systems
Overall Implication	Limited as conventional drugs, strong as bioactive compounds	—	Challenges in absorption and delivery	Good candidate for functional foods, adjuvants, and preventive health strategies

While the anthocyanin compounds identified in this study do not fully conform to conventional drug-likeness criteria, including Lipinski's Rule of Five, this limitation does not necessarily preclude their biological relevance. Anthocyanins are widely recognized to exhibit relatively low oral bioavailability and rapid metabolism, resulting in limited systemic concentrations after dietary intake [34]. Consequently, these compounds are generally considered less suitable as traditional high-potency pharmaceutical agents. However, extensive research has demonstrated that anthocyanins and other dietary polyphenols may exert beneficial biological effects through multiple complementary mechanisms, including antioxidant activity, modulation of inflammatory pathways, and regulation of cellular signaling processes [35]. Rather than acting as single-target pharmacological agents, polyphenols often function as pleiotropic bioactive compounds that contribute to health maintenance through cumulative and synergistic effects when consumed as part of the diet. In this context, the translational potential of anthocyanins may be more appropriately positioned within the framework of nutraceuticals and functional foods rather than conventional systemic pharmaceuticals. Indeed, anthocyanins derived from purple corn have attracted increasing

interest as natural bioactives with potential applications in dietary supplements and health-promoting food products [10]. Nevertheless, the pharmacokinetic limitations identified in the present study highlight the importance of formulation strategies—such as nanoencapsulation, carrier-based delivery systems, or structural modification—to enhance stability, absorption, and overall bioavailability [36]. Future experimental and clinical studies are therefore necessary to validate these computational predictions and to further elucidate the translational potential of purple corn anthocyanins in human health applications.

To address the relevance of the computational analysis to the Indonesian purple corn variety studied, all in silico predictions in this work were performed using the anthocyanin compounds experimentally identified in the extract through HPLC analysis. Cyanidin-3-glucoside, peonidin-3-glucoside, and pelargonidin-3-glucoside, which were confirmed as the predominant constituents of the purple corn extract, were used as the input structures for pkCSM and Lipinski Rule of Five evaluations. This approach ensures that the predicted pharmacokinetic, drug-likeness, and toxicity profiles directly reflect the phytochemical composition of the Indonesian variety rather than generic literature-derived structures. Consequently, the computational predictions provide insights that are specifically applicable to this regional crop, supporting the potential development of Indonesian purple corn as a source of bioactive nutraceuticals and functional food ingredients.

4. CONCLUSION

This study characterized the major anthocyanin compounds in an Indonesian purple corn extract and predicted their pharmacokinetic profiles, drug-likeness, and toxicity using the pkCSM platform. HPLC analysis identified cyanidin-3-glucoside, peonidin-3-glucoside, and pelargonidin-3-glucoside as the predominant compounds. Computational predictions suggest moderate intestinal absorption, favorable peripheral tissue distribution, and low toxicity, but limited membrane permeability, poor blood-brain barrier penetration, and multiple violations of Lipinski's Rule of Five, indicating that these compounds are unlikely to function as conventional systemic pharmaceuticals.

Despite these pharmacokinetic limitations, the predicted safety profiles and absence of major CYP450 interactions indicate that anthocyanins may be suitable for further investigation as bioactive constituents in nutraceuticals, functional foods, or dietary supplements, where effects may occur locally or systemically through indirect mechanisms. These findings highlight the potential of purple corn anthocyanins as safe natural bioactives, while also underscoring the need for experimental validation, in vivo studies, and formulation strategies to improve bioavailability. Overall, the study provides a preliminary pharmacokinetic and safety framework to guide future research on the health-promoting potential of anthocyanin-rich purple corn products.

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