

Effect of cosmetic whitening ingredients' lipophilicity on melanocyte cytotoxicity: A systematic review

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Abstract

Tyrosinase is an enzyme involved in the early stages of melanin synthesis. Melanin, which gives skin its pigment, is the most targeted molecule in the development of skin-whitening cosmetic products. Consequential risks of chemically induced skin diseases are associated with the usage of skin-whitening products that contain tyrosinase-inhibiting compounds. Currently, products containing tyrosinase inhibitors on the market lack collective analysis of their toxicity on melanocytes. Moreover, national regulatory agencies (e.g., FDA) place the burden of ensuring the safety of whitening products on manufacturers, risking bias and selective reporting. In fact, the ASEAN Cosmetic Directive does not include more than half of the 'novel' whitening agents currently on the market. This review describes the relationship of lipophilicity of cosmetic whitening ingredients (CWI) with melanocyte cytotoxicity. A systematic search of databases using the phrase (((Skin Whitening) OR (Skin Brightening) OR (Skin Lightening) OR (Skin Bleaching)) AND ((Melanocyte Cytotoxicity) OR (Cytotoxicity) OR (Cytotoxic Effect)) AND Mechanism)) was carried out. Data such as the assay and medium used, mechanism of action, cLogP, and cell viability were extracted from the 11 articles in the study. The cell viability data and respective concentration used for the MTT assays were used to calculate the IC₅₀ values of each CWI using the drc-R package in R. The identified CWIs were categorized by their respective lipophilicity; subsequently, linear regression analysis was conducted. It was revealed that the cLogP of the CWIs was able to exhibit a moderate positive correlation between IC₅₀ values ($R^2 = 0.3263$; $m = 57.85$). Lipophilic CWIs also exhibit moderate direct relationships between the variables ($R^2 = 0.3535$; $m = 83.36$). However, for hydrophilic CWIs ($R^2 = 0.00021$; $m = -3.263$), only a weak negative correlation exists, but the effect is negligible and not significant ($p\text{-value} > 0.05$; 0.8537). Out of the 18 identified CWIs, 7 were found to be potentially toxic (IC₅₀ value < 50 ug/mL); however, only hydroquinone is officially classified under the ASEAN Cosmetics Ingredient List (ACIL) to be banned in skin cosmetic products. The results of this study suggest that cytotoxicity is potentially influenced by cLogP and may also be attributed to other characteristics such as molecular structure, solubility, and cell line interaction. In addition, this study emphasizes the necessity for current safety regulations and guidelines and further investigation of in vitro safety data pertinent to human applications.

Keywords: Cosmetics, Cytotoxicity, Melanocyte, Skin lighteners, Skin whitening agents

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1. Introduction

White, fair skin is associated with beauty and youth, especially in women, and often attracts the attention of people. Light or white skin has emerged as a principal element of consumer culture in Asia [1]. An estimated statistic of 15% of the world's population is investing in skin whitening agents, with Asian countries dominating the market. However, the significance of skin-whitening products is also increasing in Western populations, who develop hyperpigmentation disorders, thereby making even white skin an aesthetic criterion that exists in both Western and Eastern cultures [2]. Franco and Roach (2023) highlights that in recent years there has been a dramatic increase in whitening products that are highly sought after, with the predicted market value to reach \$11.8 billion by 2026 [3]. The surge in popularity of whitening products has thrust the development and incorporation of various cosmetic whitening ingredients (CWIs) in these products with minimal regard to their safety profiles. These agents include hydroquinone and its derivatives, gentisic acid, licorice, azelaic acid, arbutin, retinoids, kojic acid, niacinamide, mequinol, vitamin C, corticosteroids, glutathione, alosein, glucosamine, and melatonin.

Melanin is an irregular light-absorbing polymer that contains indoles and other intermediate products of tyrosine oxidation [4]. Melanin is principally responsible for the pigmentation of human skin, producing a brownish pigment as a response to ultraviolet B irradiation as a photoprotection mechanism. Tyrosinase, an enzyme exclusively found in melanocytes, controls the rate-limiting reaction of melanogenesis, thereby making its inhibition a significant step in preventing melanin accumulation and the subsequent skin-lightening effects of CWIs. Melanocytes, which form the melanin units of the skin, are located in the basal layer of the epidermis, exclusively producing tyrosinase [5]. This property has led to tyrosinase being the most studied target for inhibiting melanin synthesis in melanocyte cells [5]. Other known mechanisms of skin whitening include melanosome transfer inhibition and dopaquinone synthesis inhibition, which occur predominantly in melanocytes. The chemical mechanism of melanogenesis is well-documented. Whereas the mechanism of dopaquinone synthesis inhibition principally operates via the oxidation of L-3,4-dihydroxyphenylalanine (L-DOPA) to o-dopaquinone by diphenolase action. However, the mechanism of melanosome transfer and pigment regulation via

keratinocyte–melanocyte interactions lacks scientific data and is simply not understood [6].

The consequence of the growing popularity and demand for skin-whitening products is the relative health and skin risks associated with their usage. The cytotoxicity of a CWI is typically assessed through cell viability assays, whereby the quantitative assessment is based on the survivability of cells. This approach has made cell viability the principal parameter in assessing the toxic effects of a compound on humans. On the other hand, lipophilicity (cLogP) is a key parameter to assess the expression of a compound's ability to permeate across biological membranes. For skin products, a cLogP of 1–3 is ideal to permeate the skin effectively [7]. However, whitening compounds currently marketed to the general public possess deviating cLogP values and equivocal data on cytotoxicity based on reports from available studies. The evaluation of these parameters is therefore essential in the safety assessment of whitening compounds for the skin. For instance, the whitening agent rhododendrol 4-(4-hydroxyphenyl)-2-butanol works by inhibiting melanin synthesis. A racemic form of rhododendrol has been marketed as a whitening agent; however, it was recalled due to users developing leukoderma, the total loss of epidermal melanin, caused by melanocyte cytotoxicity [8].

Whitening agents currently marketed in the industry suffer from a lack of toxicity and efficacy evaluation and the lack of guiding documents on their use and incorporation in cosmetic products. The ASEAN Cosmetic Directive, which consists of the ASEAN Cosmetic Ingredient Listing and the ASEAN Handbook of Cosmetic Ingredients, is not up-to-date and does not prescribe necessary specifications on the safety testing and profiles of both novel and marketed CWIs [9]. The conduct of the systematic review of the different whitening agents, with a highlight on their respective melanocyte cytotoxic activity assessed *in vitro*, provides insight into their safety properties. Furthermore, this systematic review examines the relationship between the cytotoxicity and lipophilicity of various skin whiteners to identify agents that possess safe and effective properties.

2. Methodology

2.1. Research design

A qualitative systematic review was conducted following the Preferred Reporting Items for

Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines (Fig. 1), in which ScienceDirect®, PubMed®, BioMed Central™, and Google Scholar™ databases were utilized to review and scrutinize relevant research articles regarding their relevance to the established research topic. Data from the chosen papers were examined to see how CWI's cLogP relates to cytotoxicity data, using IC50 values in B16F10 melanoma cells and normal human epidermal melanocytes (NHEM).

2.2. Data gathering procedure

The research question, “What skin whitening agents are, in addition to being effective, proven to be safe compounds that exhibit less melanocyte cytotoxicity or lack thereof?” was utilized in searching relevant studies from the aforementioned databases. The search phrase employed was (((Skin Whitening) OR (Skin Brightening) OR (Skin Lightening) OR (Skin Bleaching)) AND ((Melanocyte Cytotoxicity) OR (Cytotoxicity) OR (Cytotoxic Effect)) AND (Mechanism)). The inclusion and

exclusion criteria were applied in the selection of articles from the literature search. The researchers utilized the Mendeley Reference Manager and Mendeley Desktop to systematically collect, organize, and use bibliographic references. Duplicates were checked and removed using the Mendeley Desktop Duplicate Checker tool. Data such as the cLogP from PubChem (<https://pubchem.ncbi.nlm.nih.gov>) and cell viability were extracted from the qualified articles. Similarly, mechanisms of action of CWIs were extracted from the articles included in this study.

2.3. Selection and inclusion criteria

Fig. 2 lists the inclusion and exclusion criteria employed in this review.

2.4. Quality assessment

The Critical Appraisal Skills Programme (CASP) checklist was employed to evaluate the quality of studies included in this review. Initially, identified

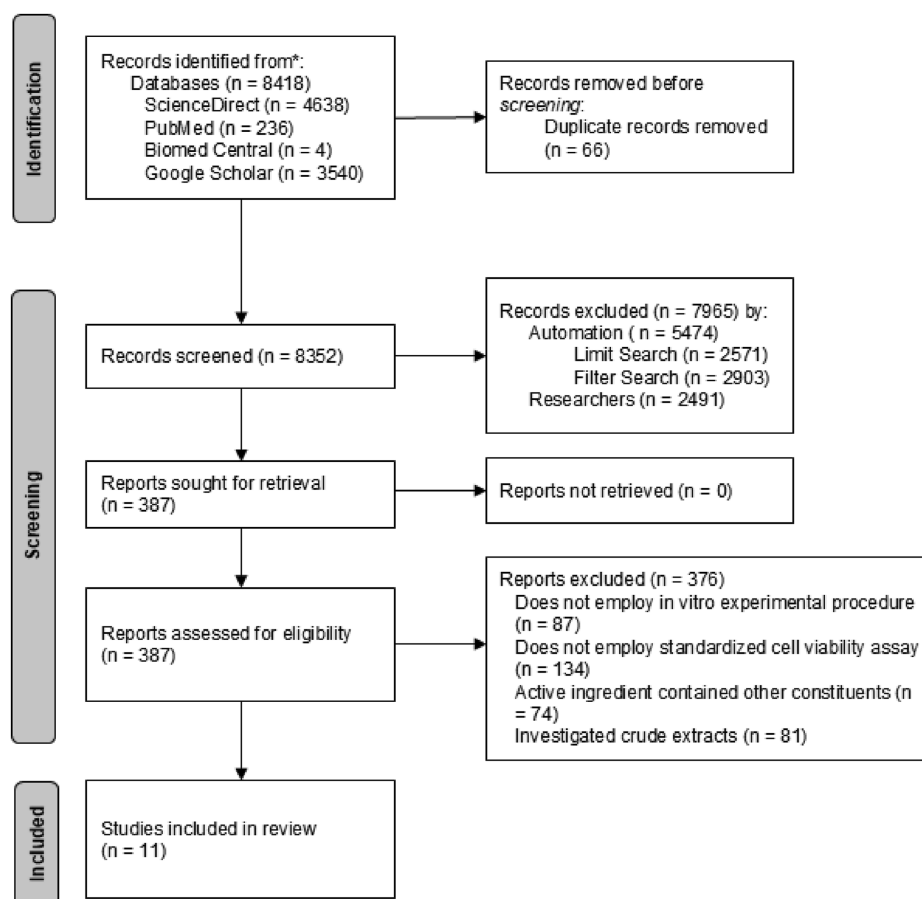


Fig. 1. PRISMA flow diagram.

Inclusion Criteria	Exclusion Criteria
1. Full research articles containing in vitro experimental studies of whitening agents 2. The whitening ingredients investigated are isolated compounds and not crude extracts. Natural product-derived ingredients are isolated compounds free from other constituents. 3. Studies that investigated cytotoxic activity through employing standardized MTT cell viability assay kits. 4. The article was published between 2008 to 2025. 5. The article was written in the English language or has an appropriate English translation.	1. Studies that investigated crude extracts. 2. Failed in the evaluation of a modified CASP quality assessment tool. 3. The article is a systematic review or meta-analysis. 4. <i>In vivo</i> studies. 5. The article employed a qualitative methodology approach. 6. Gray literature or incomplete research articles. 7. Publish exclusively in a non-English language

Fig. 2. Inclusion and exclusion criteria.

articles were manually and independently screened by the researchers using the inclusion and exclusion criteria. The articles that did not qualify based on the criteria were excluded, and the remaining articles were subject to quality assessment using the CASP checklist. The researchers conducted the assessment independently to determine the quality of the article and the publishing journal. A consensus-based discussion was conducted to resolve disagreements that arose when choosing or excluding the research publications. The full text of relevant studies was evaluated to exclude any that contained inadequate information. The studies deemed qualified for this review were then gathered for data extraction and analysis.

2.5. Data analysis

cLogP, cell viability data, and the corresponding concentrations of the CWIs were gathered and analyzed to determine the relationship between lipophilicity and cytotoxicity. The IC₅₀ values for each CWI were determined using the drc package in R (v4.4.2) with the retrieved concentrations and

respective cell viability data as seen in Tables 2 and 3 [10]. The relationship between IC₅₀ and cLogP was examined using a linear regression model, with ggplot2 (v3.3.3) employed for data visualization [11]. The analysis was performed on cell viability data, where IC₅₀ was calculated for each ingredient, and the results were plotted against cLogP to assess any potential correlation. The R-squared (R^2) value of the correlation analyses was presented as well as graphs for visualization.

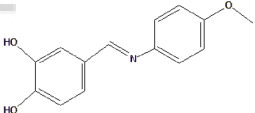
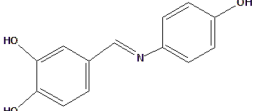
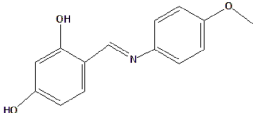
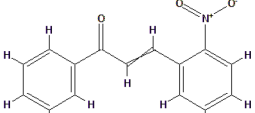
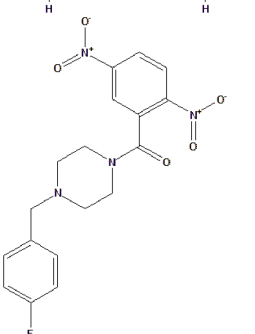
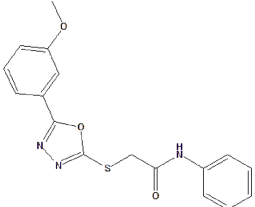
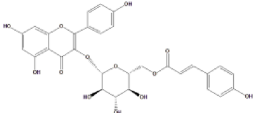
3. Results

The preliminary literature search resulted in a total of 8418 research articles from the different databases. A total of 387 research articles were manually screened for eligibility, after passing preliminary review of the search results, excluding 8352 articles by automated filter/limit search and apparent irrelevance to the research question based on initial screening of titles/abstracts. Ultimately, 11 articles passed the CASP quality assessment and were finally included in the systematic review following the PRISMA flow

Table 1. Mechanisms of action of identified CWIs.

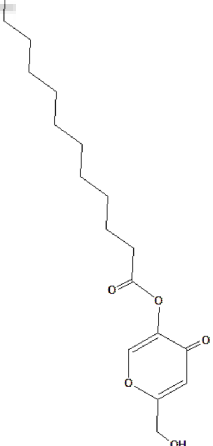
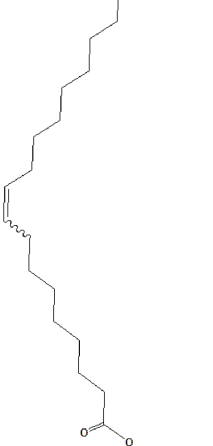
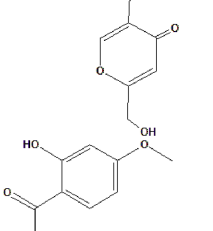
Mechanism of Action	Cosmetic Whitening Ingredient	References
Competitive tyrosinase inhibition (n = 15)	Kojic acid monooleate	[17]
	2-(5-(2-methoxyphenyl)-1,3,4-oxadiazol-2-ylthio)-N-phenylacetamide (1,3,4-oxadiazol derivative)	[15]
	Arbutin	[23]
	Paeonol	[18]
	Kojic acid monolaurate	[17]
	Tiliroside	[16]
	Nitrochalcone (2E)-3-(3-amino-4-methoxyphenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one	[13]
	N-Formylanonaine	[21]
	Salidroside	[18]
	Gallocatechin-3-gallate	[19]
	Epigallocatechin-3-gallate	[19]
	Epicatechin-3-gallate	[19]
	Hydroquinone	[23]
	4-(4-fluorobenzyl)piperazin-1-yl(2,4-dinitrophenyl)methanone	[14]
	Kojic acid	[22]
Noncompetitive tyrosinase inhibition (n = 3)	4-((E)-[(4-methoxyphenyl)imino]methyl)benzene-1,2-diol	[12]
	4-((E)-[(4-hydroxyphenyl)imino]methyl)benzene-1,2-diol	[12]
	4-((E)-[(4-methoxyphenyl)imino]methyl)benzene-1,3-diol	[12]

Table 2. CWIs with their respective dose range, cell viability data, IC50, and cLogP on B16F10 cells.

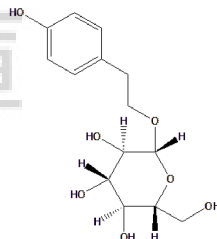
Cosmetic Whitening Ingredient	Chemical Structure	Dose (ug/mL)	Cell Viability (%)	IC50	cLogP	Reference
4-((E)-[(4-hydroxyphenyl)imino]methyl)benzene-1,2-diol		0.23 2.29 11.46 22.92	1 0.94 0.81 0.8	23.36	1.82	[12]
4-((E)-[(4-methoxyphenyl)imino]methyl)benzene-1,2-diol		0.24 2.43 12.16 24.32	0.88 0.95 0.8 0.79	13.16	2.22	[12]
4-((E)-[(4-methoxyphenyl)imino]methyl)benzene-1,3-diol		0.24 2.43 12.16 24.32	1 0.96 0.86 0.84	70.78	2.68	[12]
Nitrochalcone (2E)-3-(3-amino-4-methoxyphenyl)-1-(2-hydroxyphenyl) prop-2-en-1-one		0.25 2.5 12.7 25	1 0.93 0.82 0.76	219.88	1.28	[13]
4-(4-fluorobenzyl)piperazin-1-yl[(2,4-dinitrophenyl)methanone		0.39 3.88 9.7 19.42 38.83	0.94 0.83 0.72 0.51 0.13	20.25	1.2	[14]
2-(5-(2-methoxyphenyl)-1,3,4-oxadiazol-2-ylthio)-N-phenylacetamide (1,3,4-oxadiazol derivative)		1 5 10 20 30 40 50	0.96 0.92 0.92 0.92 0.91 0.89 0.87	33.41	4.8	[15]
Tiliroside		6.5 12.48 25 50 100	0.97 0.96 0.93 0.89 0.78	293.63	2.5	[16]

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Table 2. (continued)

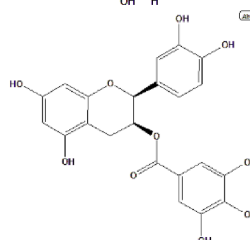
Cosmetic Whitening Ingredient	Chemical Structure	Dose (ug/mL)	Cell Viability (%)	IC50	cLogP	Reference
Kojic acid monolaurate		7.8 31.25 125 500	0.93 0.86 0.85 0.63	999.73	6.7	[17]
Kojic acid monooleate		7.8 31.25 125 500	0.95 0.99 0.95 0.88	298.73	6.7	[17]
Paeonol		8.3 16.62 83 166	0.95 0.93 0.85 0.79	299.53	2.16	[18]

Salidroside



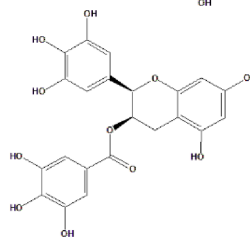
15	0.94	16.75	-1.23	[18]
30	0.9			
150	0.72			
300	0.69			

Epicatechin-3-gallate



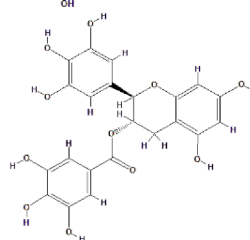
10	0.84	69.97	2.67	[19]
20	0.75			
40	0.64			
60	0.49			
80	0.36			
100	0.29			

Epigallocatechin-3-gallate



10	0.92	75.60	2.08	[19]
20	0.77			
40	0.68			
60	0.58			
80	0.46			
100	0.37			

Gallocatechin-3-gallate



10	0.95	68.27	2.08	[19,20]
20	0.85			
40	0.74			
60	0.5			
80	0.44			
100	0.42			

Table 3. CWIs with their respective dose range, cell viability data, IC₅₀, and cLogP on NHEM.

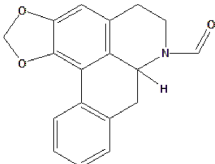
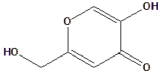
Cosmetic Whitening Ingredient	Chemical Structure	Dose (ug/mL)	Cell Viability (%)	IC ₅₀	cLogP	
N-Formylanonaine		3	1	185.79	3.33	[21]
		11.81	0.95			
		23.62	0.87			
		59	0.75			
Kojic acid		1	1	154.91	−0.64	[22]
		10	1			
		100	1			
		1000	0.76			
		10,000	0.02			

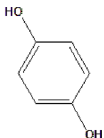
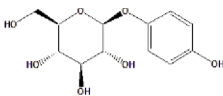
diagram illustrated in Fig. 1. This review has identified 18 CWIs retrieved from the eligible articles and classified them by their respective mechanism of action (Table 1).

Articles qualified for this review evaluated the cytotoxicity of CWIs through MTT assay using different cell lines, namely, B16F10 cells ($n = 14$), NHEM ($n = 21$) and murine melan-a melanocytes ($n = 2$), as listed in Tables 2–4, respectively. Different concentration ranges (0.03–10000 ug/mL) were also employed for the assay to determine cell viability, as several articles featured preliminary studies to investigate the cytotoxicity of the compounds. The percentage of surviving cells was calculated using the following equation: $\text{cell viability (\%)} = (A_{\text{sample}} - A_{\text{blank}}) / (A_{\text{control}} - A_{\text{blank}}) \times 100$. The cell viability data reported in this review were based on the experiments employing different concentrations of the CWI on the cell lines declared in each study, resulting in data being incomparable with other CWIs in this review. However, cell viability data alone may not suffice as metrics for the cytotoxicity of the compounds due to possible variability in cell growth and experimental and assay conditions leading to skewed analysis. Hence, IC₅₀ values were determined for each identified CWI to serve as a quantitative measure for cytotoxicity.

Based on the computed IC₅₀ values, CWIs considered to be safe are indicated by IC₅₀ values greater than 50 ug/mL [24]. In this review, the majority of the identified CWIs are non-toxic ($n = 11$), while only 7 are considered toxic. The CWIs that exhibited the least cytotoxicity, indicated by possessing the highest IC₅₀ value, is kojic acid monolaurate (999.73 ug/mL). In contrast, arbutin (4.65 ug/mL) was identified as the most toxic CWIs. Majority of the CWIs exerted competitive tyrosinase inhibition on cell lines to achieve pigment reduction compared to other identified mechanisms of action.

The collated cLogP values were then plotted against the IC₅₀ values of each CWI. Fig. 3A displays the association between cLogP and IC₅₀ values of the CWIs. Linear regression was employed to determine the strength of the correlation between the variables analyzed. The model indicates a statistically significant (p -value < 0.001 ; 9.27×10^{-9}) relationship between IC₅₀ and cLogP of the identified CWIs in this review, with a positive slope ($m = 57.85$), suggesting that an increase in cLogP is associated with higher IC₅₀ values. However, the strength of the relationship, as indicated by the R-squared value ($R^2 = 0.3263$), is moderate, indicating other factors may influence the IC₅₀ values. Furthermore, separate analyses were also

Table 4. CWIs with their respective dose range, cell viability data, IC₅₀, and cLogP on murine melan-a melanocytes.

Cosmetic Whitening Ingredient	Chemical Structure	Dose (ug/mL)	Cell Viability (%)	IC ₅₀	cLogP	
Hydroquinone		0.03	0.95	9.97	0.59	[23]
		0.11	0.86			
		0.33	0.77			
		1.1	0.73			
		3.3	0.67			
		11	0.46			
Arbutin		0.08	1	4.65	−1.35	[23]
		0.3	1			
		0.82	0.91			
		2.7	0.85			
		8.2	0.96			

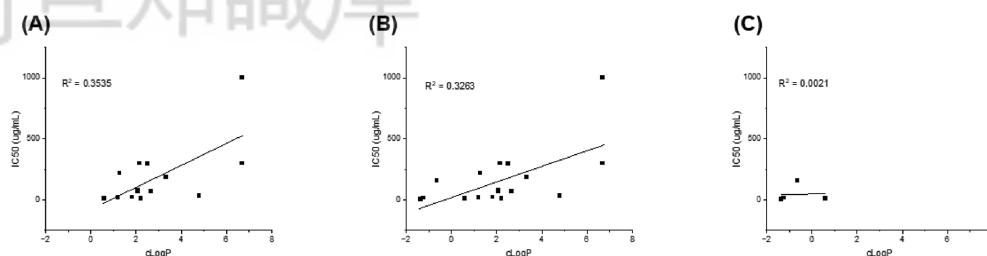


Fig. 3. The correlation between the cLogP and IC₅₀ of the CWIs (A), lipophilic CWIs (B), and hydrophilic CWIs (C).

conducted for lipophilic ($\text{cLogP} > 1$) and hydrophilic ($\text{cLogP} < 1$) CWIs to determine if the same relationship can be observed as seen in Fig. 3B and C, respectively. The analysis conducted for lipophilic drugs exhibited a significant (p -value < 0.001 ; 1.13×10^{-7}) linear relationship between the variables within the group, indicating that cLogP has a significant influence on the IC₅₀ value of the CWI in line with the previous analysis. In addition, the estimated slope ($m = 83.36$) indicates that as cLogP increases, IC₅₀ tends to increase significantly, and the multiple R-squared value ($R^2 = 0.3535$) suggests that approximately 35% of the variation in IC₅₀ can be explained by cLogP. On the other hand, hydrophilic CWIs show that there is no significant (p -value > 0.05 ; 0.8537) linear relationship between IC₅₀ and cLogP in this range. In addition, the analysis also shows a weak correlation between IC₅₀ and cLogP ($R^2 = 0.00021$) of hydrophilic CWIs, with only 0.21% of the variation in IC₅₀ explained by cLogP in this subset. The negative slope ($m = -3.263$) suggests a weak inverse relationship, but the effect is negligible and not significant.

The review identified 7 potentially toxic (IC₅₀ value < 50 $\mu\text{g/mL}$) CWIs, namely 4-[(E)-[(4-hydroxyphenyl)imino]methyl]benzene-1,2-diol, 4-[(E)-[(4-methoxyphenyl)imino]methyl]benzene-1,2-diol, 4-(4-fluorobenzyl)piperazin-1-yl(2,4-dinitrophenyl)methanone, 2-(5-(2-methoxyphenyl)-1,3,4-oxadiazol-2-ylthio)-N-phenylacetamide(1,3,4-oxadiazol derivative), salidroside, hydroquinone, and arbutin. These CWIs were then cross-referenced with the ASEAN Cosmetics Ingredient List (ACIL). Out of the aforementioned potentially toxic CWIs, only hydroquinone is currently included under the ACIL Annex II, which enumerates the substances that are prohibited in the manufacture of cosmetic formulations [9]. Despite this, ingredients such as salidroside and arbutin are currently in marketed cosmetic products. In the absence of regulatory guidelines, this study highlights the need for up-to-date safety assessments and review of in vitro safety data relevant to human use.

4. Discussion

Cosmetic whitening ingredients are abundant in the market, exhibiting various mechanisms of action in imparting skin lightening effects. The incorporation of these ingredients in formulations is based on their efficacy, particularly on the extent of melanogenesis. Various methods are employed in the measurement of their efficacy, such as cell-free enzymatic assays, melanocyte cultures, and reconstructed epidermis bioassays. However, safety properties of these ingredients, particularly data on their melanocyte cytotoxicity, are uncommon. Analyzing the existing data on the lipophilicity (i.e., cLogP) and melanocyte cytotoxicity (i.e., IC₅₀) of CWIs is a method for identifying their safe and effective use in formulations.

4.1. Determinants of cytotoxicity of CWIs

In the selection of CWIs, the whitening effect mechanism, structure, dose, and lipophilicity are factors that exert influence on the compounds' cytotoxicity. The mechanism of the whitening effect can be accomplished through various methods, with tyrosinase inhibition as the principal mechanism [5]. Tyrosinase, which is exclusively produced by melanocyte cells, makes these cells the primary target for tyrosinase inhibition and other mechanisms for skin lightening. As an important structural and functional component of the skin, the cytotoxicity of CWI, particularly to melanocyte cells, should be examined. Tyrosinase inhibition disrupts the melanogenesis pathway by targeting tyrosinase (also called phenolase or polyphenol oxidase), the key enzyme that controls the rate-limiting steps of the pathway. The majority of the CWIs' structure that utilizes competitive tyrosinase inhibition ($n = 15$), as observed in the study, contains a phenol group or diphenyl group, which contributes to improving whitening action by allowing greater affinity towards tyrosinase binding sites.

Phenolic compounds can act as competitive inhibitors by binding to the active site of tyrosinase,

thereby preventing tyrosine from accessing the enzyme and undergoing conversion to melanin precursors [25]. Structurally, these phenol groups also promote lipophilicity of CWIs, which allows better penetration and absorption into the skin, wherein the compound can effectively exert its inhibitory effects towards tyrosinase activity [26]. Thus, disrupting the melanogenesis pathway. However, improved permeation through the skin may also increase the bioavailability of the CWIs, which both increases efficacy and risk for toxicity, especially in excess amounts. This warrants an analysis of the aforementioned factors in order to determine their respective effect on the safety properties of the CWIs.

4.1.1. Lipophilicity (cLogP)

cLogP is directly proportional to hydrophobicity; this means that the higher the cLogP of a compound, the more lipophilic it is in nature. The existing literature lacks a consensus regarding the appropriate lipophilicity range, typically represented by the calculated n-octanol/water partition coefficient value (cLogP), that facilitates effective permeation through the skin [27]. However, it is generally acknowledged that molecules exhibiting moderate lipophilicity (cLogP 1–3) possess the ability to diffuse through the skin. Excessive lipophilicity can lead to inadequate solubility and permeation challenges, whereas limited lipophilicity may hinder the ability to traverse the hydrophobic barriers of the epidermis and cellular membranes [28]. Hence, it is utilized as a parameter of a compound's ability to permeate the skin and reach the target site in the context of whitening agents. Cell viability data are then indirectly influenced through absorption mechanisms, which are determined by the CWI's cLogP value. A high cLogP value, but within 1–3 makes CWIs highly lipophilic, which increases absorption and efficacy but also the likelihood of excessive accumulation within the cell membrane, which can contribute to increased cytotoxicity in melanocyte cells [7].

In this study, IC₅₀ and cLogP values were analyzed through linear regression analysis to determine the strength of the relationship of cytotoxicity and lipophilicity as presented in Tables 2–4. The analysis has shown that high lipophilicity of compounds elicited low cytotoxicity as indicated in the direct relationship of IC₅₀ and cLogP values. Fig. 3A and B have shown, through the slope value, that a significant moderate positive correlation exists between the variables, suggesting that the ingredients with higher lipophilicity (higher cLogP) may require higher concentrations to reach 50%

inhibition, resulting in increased IC₅₀ values, possibly indicating that more hydrophobic compounds are less bioavailable or less active in cellular environments, resulting in low cytotoxicity. This finding presents challenges to the predominant understanding of lipophilic CWIs being highly cytotoxic. In addition, the excessive lipophilicity of the compound is also attributed to decreased permeability, as it is unable to pass through lipid-rich cell membranes. The consistent positive correlation between IC₅₀ and cLogP values of the overall and lipophilic CWIs indicates that in CWI selection, lipophilicity can be employed as one measure among other physicochemical properties of a compound. The results of analyzing cLogP against IC₅₀ values using linear regression analysis on hydrophilic drugs, however, revealed that there is a weak inverse correlation between the two values, but the effect is negligible and not significant (Fig. 3C).

These findings merit investigation into various parameters that influence the lipophilicity of CWIs, which in turn influence cellular permeability and transport, resulting in cytotoxic effects. Cytotoxicity is regulated by a variety of parameters, including compound-specific characteristics such as chemical structure and mechanism of action. Other aspects that influence cell viability include drug solubility, chemical reactivity, and interactions with intracellular targets [29].

4.1.2. Structure

The molecular structure of CWIs determines the permeability of the compound into melanocyte cells to inhibit tyrosinase or impede melanin formation pathways. The structural groups of the compound contribute to its depigmentation activity and influence its lipophilicity. The importance of tyrosinase in melanogenesis has led to numerous reports of structure-activity relationships as a means of identifying tyrosinase inhibitors from both natural (e.g., fungi, bacteria, plants) and synthetic sources. Tyrosinase inhibitors are often evaluated using a monophenolic substrate like tyrosine or a diphenolic substrate such as L-DOPA, with action measured through dopachrome production [30]. Thus, the structures of major tyrosinase inhibitors have been extensively researched to determine their efficacy and safety as skin whitening treatments, notably their physicochemical properties. Kojic acid, arbutin, and hydroquinone, recognized as efficient tyrosinase inhibitors, have various limitations that restrict their use, including instability, toxicity, and inadequate solubility or skin permeability [5,7,26]. Phenolic compounds, including p-

coumaric, caffeic, ferulic, and hydroxybenzoic acids, have been investigated for their antimelanogenic properties; however, the presence of the carboxylic acid group results in inadequate skin permeability and hence diminished *in vivo* efficacy [31]. To circumvent this constraint, literature indicates the use of ester or amide derivatives, as the incorporation of these groups yields a neutral molecule with enhanced lipophilicity, hence facilitating improved skin permeability [32].

The CWIs included in this review consist not only of the well-known active ingredients but also novel CWIs, both natural and synthetic. These active compounds possess similar structural components that account for their respective activity. These components, aside from hypopigmentation activity, also contribute to the physicochemical properties of the drug, such as lipophilicity, stability, and solubility. Hence, categorizing the CWIs on the basis of their molecular structure provides insights into the effect of molecular structures on the relationship between lipophilicity and cytotoxicity. The identified CWIs can be classified, based on their core structures, as hydroquinone derivatives, kojic acid derivatives, phenolic compounds, flavonoids, fatty acid esters, and heterocyclic compounds.

4.1.2.1. Hydroquinone derivatives. Hydroquinone (HQ) is a simple diphenol (benzene-1,4-diol) with high lipophilicity and reactivity. Whereas arbutin (4-hydroxyphenyl- β -D-glucopyranoside) is a glucosylated derivative of hydroquinone, where a β -D-glucose moiety is attached to the hydroxyl group of hydroquinone [26]. Both of the compounds share the hydroquinone core chemical structures, which competitively inhibit tyrosinase. However, the glucose modification significantly reduces the lipophilicity of arbutin compared to hydroquinone, affecting its penetration, metabolism, and toxicity. Glycosidic linkages have also been modified to produce different derivatives of arbutin in the hopes of enhancing the inhibitory effect on tyrosinase and regulating the lipophilicity of the compound to reduce cytotoxicity [33]. Hydroquinone and arbutin possess cLogP values of 0.59 and -1.35 , respectively (Table 4). Despite the disparity of their lipophilicity, they both exhibit similar IC₅₀ values of 9.97 and 4.65 $\mu\text{g/mL}$, which suggests that the cytotoxicity of these CWIs is not dependent on lipophilicity.

4.1.2.2. Kojic acid derivatives. Kojic acid is a well-known antityrosinase agent, efficiently used for skin-lightening cosmetic products and widely used to treat hyperpigmentation, melasma, and wrinkles

[17]. However, the lipophilicity of the compound presents a significant barrier, hindering optimal skin permeation to reach melanocytes located in the basal layer of the epidermis. Therefore, attempts had been made to improve the physical properties and biological activities of kojic acid (KA) via esterification with fatty acids aimed to increase the lipophilicity of the compound. The physical properties of kojic acid esters are an important factor for inhibition of melanin synthesis, where it must penetrate into the cell membrane to inhibit cellular tyrosinase and melanin synthesis [34]. Thus, an appropriate hydrophobic and hydrophilic balance of the derivatives is important for the inhibition of melanin synthesis. The lipophilicity of the compounds relies heavily on the length of the ester formed through the combination of KA with a fatty acid. Lauric acid (C12) and oleic acid (C18) attached to kojic acid resulted in varying lipophilic properties wherein cLogP was increased proportional to the length of alkyl chains. In this review, KA and its derivatives (monopalmitate, monooleate, and monolaurate) have been assessed on the basis of their safe dose and cLogP to determine the effect of increasing lipophilicity on cytotoxicity and, to an extent, the efficacy of its skin-lightening activity. The results showed that cytotoxicity, based on IC₅₀ values, of kojic acid monolaurate (IC₅₀ = 999.73 $\mu\text{g/mL}$) and kojic acid monooleate (IC₅₀ = 298.73 $\mu\text{g/mL}$) were significantly lower than kojic acid (IC₅₀ = 154.91 $\mu\text{g/mL}$). Furthermore, the cellular tyrosinase inhibitory effect of kojic acid monolaurate and kojic acid monooleate was found to be similar to kojic acid at nontoxic doses [34]. These findings reveal that the increase of lipophilicity led to the decreased cytotoxicity of the KA esters as they possessed higher IC₅₀ values than KA, consistent with findings of the linear regression analysis. It can also be attributed to the substitution of the hydroxyl group with an ester functional group, making the compound less prone to oxidation [35]. Hence, the introduction of long alkyl chains (C12 and C18) significantly increases lipophilicity, improves membrane penetration and increases the depigmenting effect, making kojic acid esters a viable alternative to kojic acid as skin-whitening agents and potential depigmenting agents to treat hyperpigmentation. The lipophilicity and cytotoxicity of kojic acid derivatives highlight the impact of lipophilicity on the cytotoxicity of CWIs in addition to the structural components that impart stability to the compound.

In a similar study, Ayuhastuti et al. (2024) examined the chemical structure of kojic acid dipalmitate, which comprises two molecules of palmitic

acid, a saturated fatty acid, linked to the two hydroxyl groups of kojic acid [36]. Kojic acid dipalmitate possesses lipophilic properties, resulting in greater solubility in oils and fats compared to kojic acid. Derivatives have been shown to enhance the stability and solubility of kojic acid in oil-based cosmetic formulations.

4.1.2.3. Polyphenolic compounds. Polyphenolic compounds are used widely as whitening agents owing to their excellent tyrosinase inhibitory activity, adaptive chemical structures, and antioxidant properties [37]. These compounds share hydroxyl (-OH) functional groups, which influence hydrophilicity and interaction with tyrosinase. However, the lipophilic nature of compounds varies based on functional group attachments, such as glycosylation (sugar moieties), flavonoid frameworks, catechol units, and alkyl substitutions. Tiliroside (cLogP = 2.5) is a polyphenolic compound with moderate lipophilicity, attributed to its glycosylated flavonoid structures, wherein the hydrophobic aglycone regions contrast with the hydrophilic sugar moieties [37]. Catechin derivatives like gallicocatechin-3-gallate (cLogP = 0.07), epigallocatechin-3-gallate (cLogP = 1.2), and epicatechin-3-gallate (cLogP = 1.5) also exhibit moderate hydrophobicity owing to their galloyl groups. These compounds exhibit remarkable antioxidant activity; however, their lower cLogP values hinder cutaneous absorption. Salidroside (cLogP = -1.23) stands out as one of the most hydrophilic compounds in the group, due to its glucoside structure. Meanwhile, paeonol exhibits significant lipophilicity, with cLogP values of 2.16, attributed to the methoxy (-OCH₃) functional group.

Taking into account both the cLogP values and IC₅₀, from most lipophilic to least, the polyphenolic compounds are arranged as follows: tiliroside (IC₅₀ = 293.63 µg/mL), paeonol (IC₅₀ = 299.53 µg/mL), epicatechin-3-gallate (IC₅₀ = 69.97 µg/mL), epigallocatechin-3-gallate (IC₅₀ = 75.60 µg/mL), gallicocatechin-3-gallate (10 µg/mL), and salidroside (IC₅₀ = 68.27 µg/mL). The trend shows that despite increased lipophilicity, which is thought to increase cytotoxicity risks if bioaccumulated in tissues, IC₅₀ values were greater with the lipophilic compounds, such as tiliroside, indicating better safety profiles. Paeonol, a phenolic compound extracted from *Paeonia suffruticosa*, is considered less cytotoxic as a whitening agent due to its structural properties, selective tyrosinase inhibition, and strong antioxidant activity [18]. Unlike hydroquinone and other aggressive depigmenting agents, paeonol functions through mild and reversible inhibition of

melanogenesis, reducing long-term damage to melanocytes. This shows that despite the importance of lipophilicity, the mechanism of action of CWIs also plays a vital role in determining cytotoxicity, suggesting a non-injurious function in melanocyte physiology in the skin.

4.1.2.4. Aromatic enones/amides and chalcone derivatives. The CWIs classified under this group share a conjugated, unsaturated aromatic framework. The physicochemical properties of these compounds are influenced by their lipophilic aromatic cores and substituents imparting hydrophilicity in particular regions of the molecule [38]. This impacts the overall solubility and ability of the compound to permeate across membranes. Radhakrishnan et al. (2015) synthesized a chalcone derivative, namely, (2E-3-(3-amino-4-methoxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one) (cLogP = 1.28), which has an added amino substituent and hydroxyl group capable of forming hydrogen bonds, imparting further polarity to the already polar chalcone backbone structure [13]. This increased polarity results in lower lipophilicity than compounds attached with nonpolar substituents. N-formylanonaine (cLogP = 3.33), isolated by Wang et al. (2010) from the leaves of *Michelia alba* D.C. (Magnoliaceae), features an aromatic system with an N-formyl substituent [21]. It has a relatively higher lipophilic character attributed to the dominant aromatic core, where the electron-withdrawing formyl group only imparts partial hydrophilicity. Extrapolated IC₅₀ values of N-formylanonaine (185.79 µg/mL) are relatively lower than those of (2E-3-(3-amino-4-methoxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one) (219.88 µg/mL). In contrast to the findings of the regression analysis, the lipophilic N-formylanonaine exhibits higher cytotoxicity compared to the more hydrophobic (2E-3-(3-amino-4-methoxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one). This can be attributed to the N-formyl moiety capable of producing reactive intermediates that bind to cells, thereby, increasing its cytotoxic properties. On the other hand, (2E-3-(3-amino-4-methoxyphenyl)-1-(2-hydroxyphenyl)prop-2-en-1-one) is a stable compound due to the hydrogen bonding of the amino and hydroxyl groups and the antioxidant properties imparted by the phenolic hydroxyl group.

4.1.2.5. Heterocyclic and imine-containing compounds. The presence of multiple heterocyclic rings and imine functional groups in the identified CWIs (e.g., oxadiazole and benzyldiene-aniline derivatives) reveals the factors influencing both the lipophilicity and cytotoxicity. The molecular structure of CWIs reflects

the distribution of polar and nonpolar moieties, which determine their physicochemical properties.

In the case of, 2-(5-(2-methoxyphenyl)-1,3,4-oxadiazol-2-ylthio)-N-phenylacetamide, an oxadiazole derivative with an improved cytotoxic profile; its oxadiazole ring imparted the metabolic stability [15]. Further, its phenylacetamide moiety and methoxy-substituted phenyl group enhanced the lipophilicity of the compound. A study by Bae et al. (2012) designed, synthesized, and evaluated a series of (E)-N-substituted benzylidene-aniline derivatives for inhibitory activity against tyrosinase [12]. It includes 4-((E)-[(4-methoxyphenyl)imino]methyl)benzene-1,2-diol, 4-((E)-[(4-hydroxyphenyl)imino]methyl)benzene-1,2-diol, and 4-((E)-[(4-methoxyphenyl)imino]methyl)benzene-1,3-diol. The structure-activity relationship of these compounds shows how substituents and substitution patterns can affect lipophilicity and cytotoxicity [12]. These Schiff bases, characterized by a double bond linking nitrogen and carbon atoms, form a conjugated system resulting in electron delocalization, and in combination with a methoxy substituent, it increases lipophilicity. The hydroxyl group balances the lipophilicity by increasing polarity while also reducing cytotoxicity by decreasing reactivity against cellular nucleophiles. In addition, positional isomerism (1,2- or 1,3-arrangement) of the diol affects the hydrogen-bonding potential and overall molecular planarity, imparting a hydrophilic region to the molecule.

The heterocyclic or imine groups introduced a distinct balance between hydrophilic and lipophilic domains, often yielding compounds with moderate to high lipophilicity that are designed to interact with the active site of tyrosinase via multiple binding modes. Lipophilicity of the compounds, as represented by their cLogP values, shows that 2-(5-(2-methoxyphenyl)-1,3,4-oxadiazol-2-ylthio)-N-phenylacetamide (cLogP = 4.8) is the most hydrophobic, followed by 4-((E)-[(4-methoxyphenyl)imino]methyl)benzene-1,3-diol (cLogP = 2.68), 4-((E)-[(4-methoxyphenyl)imino]methyl)benzene-1,2-diol, and (cLogP = 2.22), and 4-((E)-[(4-hydroxyphenyl)imino]methyl)benzene-1,2-diol (cLogP = 1.82). The IC₅₀ values of the compounds are 33.41, 70.78, 13.16, and 23.37 µg/mL, respectively. In contrast to earlier findings, however, no evidence of a relationship between the lipophilicity and cytotoxicity as indicated by their cLogP and IC₅₀ values was detected. IC₅₀ values did not correspond with an increase or decrease of the cLogP values.

4.1.2.6. Miscellaneous small molecule. The review also includes structurally unique compounds that do not possess similarities with other identified CWIs,

which consist of small molecules and vitamin derivatives found to possess depigmenting activity.

4-(4-fluorobenzyl)piperazin-1-yl[(2,4-dinitrophenyl)methanone] is a lipophilic compound owing to the piperazine moiety, multiple aromatic rings, and electron-withdrawing substituents (e.g., fluorine and dinitrophenyl groups). However, the presence of strong electron-withdrawing groups may also render it more reactive, potentially leading to higher cytotoxicity if the compound interacts non-specifically with cellular components [14].

4.2. Dose

Cytotoxicity in cells and tissues, in almost all cases, increases alongside the dose of any compound that is administered [39]. Therefore, the decrease of cell viability occurs as a dose-dependent phenomenon. The same principle applies to cytotoxicity studies of novel active ingredients and compounds employed by the articles for the identified CWIs in this review. It is a generally accepted principle that the manifestation of any cytotoxic effects in cells or tissues requires a certain number of primary damaging reactions in excess of a threshold [39]. This rationalizes the methods employed by the studies wherein MTT assays were conducted on a wide range of concentrations to determine the safe and effective threshold of the compound and, most importantly, compare cell viability data with depigmenting activity in order to rule out cytotoxicity as a contributing factor to the skin whitening effect of CWIs.

Cytotoxic doses of the CWIs included in this review vary greatly, ranging from 0.03 to 10,000 µg/mL, with varying extents of cell viability (Tables 2–4). The cell viability measured through the MTT assays was utilized in determining the IC₅₀ value of the identified CWIs in this review through a dose-response curve. IC₅₀ values indicate the concentration of a substance being investigated that results in the death of 50% of the cell population. This value provides a quantitative measure of the CWIs' toxicity at various concentrations, whereas a lower IC₅₀ value indicates significant biological effects that result in cell death at lower concentrations, which potentially pose safety risks. It also enables comparative analysis across different cell types involved in this review, which allows us to determine the toxic effects of the CWIs on various cells. The examination of IC₅₀ values in relation to cLogP underscores the distinct characteristics of each CWI, beyond lipophilicity, that influence the compound's toxicity. This variability in IC₅₀ values necessitates further investigation to ascertain the

concentrations of CWIs used in the manufacture of cosmetics are deemed safe for application as depigmentation agents for the skin, thereby minimizing the risk of adverse effects. The correlation between dosage and toxicity is, therefore, crucial in establishing the safe and effective therapeutic dose of CWIs to be incorporated into formulations.

4.3. Cell lines in cytotoxicity studies

Various cell lines are employed in cytotoxicity assays in order to determine the effect of test compounds on either the integrity of the cells, leading to cell death, or the proliferation of cells. In the context of this review, multiple cell lines were employed in the articles in order to quantify the anti-melanogenic effect of the CWIs on target sites in the skin - melanocyte cells. NHEM cells and B16F10 cells simulate the homeostatic and abnormal production of melanin in melanocyte cells on the skin, respectively, allowing the evaluation of the anti-melanogenic properties. These cell lines differ in origin, structure, and properties, which result in distinct interactions when the tested CWIs in terms of cell permeation and cytotoxic properties.

4.3.1. B16F10 cell line

B16F10 cells are cultured from murine melanoma models known for high metastasis potential. Heterogeneous morphology of B16F10 cells consists of melanocytes with spindle-shaped and epithelial-like configurations, indicating the dynamic state of the malignant cells engaged in epithelial-mesenchymal transitions [40]. These cells also tend to demonstrate a less organized actin cytoskeleton compared to other B16 sublines. The lack of organization of the actin filaments results in the cell being more pliable and deformable. This can facilitate the diffusion and endocytic uptake of compounds such as nanoparticles and lipophilic drugs via cell membrane distortion and endocytosis [41]. Furthermore, the altered lipid composition of cholesterol and sphingolipid in B16F10 cells is linked to improved fluidity, causing improved partitioning of lipophilic compounds into the membrane. However, potential integration of these compounds in the plasma membrane can occur due to their solubility in lipids, which deters their entry into the cell. In addition, melanin, which is abundant in these cells, possesses a strong affinity with lipophilic compounds. As these compounds interact with melanin, the free concentration of the compound in the intracellular space is reduced through sequestration [40]. The binding of lipophilic CWIs,

for instance, results in decreased bioavailability to enable interaction with critical cellular targets (e.g., DNA, enzymes, or organelles), thereby decreasing cytotoxic potential. Melanosomes, where the synthesis of melanin occurs and subsequent storage also provides a physical compartment where lipophilic compounds accumulate after melanin binding, further isolate them and prevent intracellular interactions, reducing their ability to disrupt cellular processes [42]. In contrast, hydrophilic compounds are less bound to melanin and lipid and remain free in the aqueous cytoplasm, making them more bioavailable for intracellular targets, resulting in increased risk for cell damage and death.

As seen in Fig. 3, an inverse relationship was observed between lipophilicity and cytotoxicity as the increasing cLogP values of CWIs necessitated higher IC₅₀ values. This indicates that the characteristics of B16F10 cells significantly facilitated the effect of CWIs, as lipophilic compounds exhibited lower in vitro cytotoxicity in the MTT assays employed using this cell line compared to hydrophilic counterparts. For instance, a highly hydrophilic CWI, namely, salidroside (cLogP = -1.23), was identified in this review to be a cytotoxic compound with an IC₅₀ value of 16.75 ug/mL. Furthermore, CWIs identified with the least cytotoxic potential possess high cLogP values such as kojic acid monolaurate (cLogP = 6.7) and paeonol (cLogP = 2.16), possessing IC₅₀ values of 999.73 and 299.53 ug/mL, respectively. The results show that CWIs, depending on their lipophilicity, present different cytotoxic profiles on B16F10 cells related to their lipid-rich cellular membrane. Hence, the results of the analysis corroborate that high lipophilicity of a compound may result in decreased cell bioavailability, pointing to possible entrapment within the cell membrane, reducing its toxic activity in the cell.

4.3.2. Normal human epidermal melanocytes

Normal human epidermal melanocytes are human skin tissue isolated most commonly from excised neonatal foreskin and adult skin biopsies of consenting donors. This remains as the gold standard of many studies involving melanin synthesis and melanosome transfer due to being able to closely replicate morphological and signaling properties of melanocytes [43]. NHEMs express relevant genes and regulatory proteins in signaling pathways to accurately observe and measure tyrosinase expression of CWIs. The structural and biochemical properties of NHEM cells are similar with B16F10 cells, particularly in melanin synthesis,

the presence of melanosomes, and the lipid composition of the plasma membrane. The unique characteristic of NHEM cells lies in their ability to produce cytochrome P450 (CYP) enzymes responsible for metabolizing a variety of compounds [44].

As melanosomes are also present in NHEM cells, they possess the same sequestering potential with B16F10 cells between lipophilic compounds and melanin due to the aforementioned binding ability of melanin. The binding capacity also plays a vital role in reducing the cytotoxicity of lipophilic molecules in NHEM cells [40]. On the other hand, hydrophilic compounds, as previously discussed, are less prone to binding with melanin and becoming entrapped in melanosomes, resulting in higher free concentration in the cytoplasm, thereby increasing the risk for altering cellular processes, resulting in damage or death of cells. In addition, they share a lipid-rich plasma membrane that has a high propensity for lipophilic compounds to accumulate into the membrane, preventing passage into the intracellular space, which affects its cytotoxic impact, whereas hydrophilic compounds may enter via diffusion through aqueous channels and specific transporters, potentially resulting in higher cytoplasmic concentration. Local metabolism of compounds entering NHEM these cells, including CWIs through oxidation reactions produce more polar metabolites, facilitating elimination. CYP450 enzymes also exhibit higher affinity with lipophilic compounds, thereby making fat-soluble CWIs become primary targets for metabolism and elimination from NHEM cells, making them less cytotoxic compared to hydrophilic CWIs. However, the subsequent metabolism of lipophilic substances can occur either through bioactivation or detoxification, which determines the cytotoxicity of the resulting metabolite [44]. In bioactivation, CYP450 enzymes may convert a lipophilic CWI into a reactive metabolite (e.g., electrophile or free radical). This results in reactive metabolites binding to organelles and other cellular components, affecting their vital functions within the cells. This underscores the structure-dependent cytotoxic profile of CWIs as metabolism pathways differ depending on the compound. Another pathway is through detoxification, wherein lipophilic compounds are modified through the addition or removal of functional groups such as hydroxyl groups. Increasing the polarity of compounds, which facilitates uninhibited excretion from the cell or phase II metabolism reaction, reduces cytotoxicity. A study conducted by Wang et al. (2010) investigated the cytotoxicity of (-)-N-formyl-anonaine in human epidermal melanocytes

employing the MTT assay and showed, through the cell viability data, an IC₅₀ value of 185.79 µg/mL, indicating the low cytotoxicity potential of the compound in vitro [21]. Being lipophilic (cLogP = 3.33), the propensity of fat-soluble CWIs to undergo the aforementioned sequestration into melanin and melanosomes or either of the metabolism pathways renders the substance non-cytotoxic in NHEM cells. In contrast, hydrophilic compounds are less likely to be metabolized by CYP450 enzymes as they do not conform with the enzyme's binding preferences with their active site favoring hydrophobic interactions [44]. Hence, the cytotoxicity of hydrophilic CWIs likely remains unchanged despite the presence of these enzymes.

4.3.3. Murine melan-a melanocyte

Murine melan-a cells are a type of cell line characterized by nontumorigenic mouse melanocyte cells isolated from embryonic skin, first established as an immortal, pigmented line for investigating melanocyte biology and pigmentation disorders [45]. This cell line is syngeneic with B16F10 melanoma cells, facilitating comparative melanoma research. The distinguishing feature of murine melan-a cells lies in their inability to form tumors compared to B16F10 cells. In terms of structural and biochemical properties, murine melan-a melanocyte cells are identical to B16F10 cells, both having a phospholipid bilayer and being capable of melanin synthesis and storage through melanosomes.

These shared characteristics reflect similar interactions with lipophilic and hydrophilic CWIs in terms of cellular permeation and consequent cytotoxic effects as discussed from the previous two cell lines. Melanin binding and sequestering of lipophilic compounds still remain possible with this cell line, reducing the cytotoxic potential of the substance attributed to decreased free concentration in the cytoplasm, mitigating cell disruption. In addition, the plasma membrane of murine melan-a cells is comparable with NHEM and B16F10 cells, indicating a similar propensity for accumulation of lipophilic compounds among these cells. In contrast, hydrophilic compounds when introduced into murine melan-a, melanocytes lack these mechanisms to cause changes in the cytotoxic potential of the compound. In the study conducted by Hu et al. (2009), hydroquinone and arbutin were studied for their effects on melanogenesis and cytotoxicity, which revealed that these hydrophilic compounds possessed a higher cytotoxic potential compared to their lipophilic glucoside derivative – deoxyarbutin [23]. The analysis shows that

hydroquinone ($\text{cLogP} = 0.59$) and arbutin ($\text{cLogP} = -1.35$) possess IC_{50} values of 9.97 and 4.65 $\mu\text{g/mL}$, respectively, indicating high cytotoxicity. These compounds both inhibit tyrosinase to reduce melanin synthesis and skin pigmentation. This study shows that in CWI selection for formulation, lipophilicity can be considered to ensure safety and efficacy; however, the conduct of a cytotoxicity assay remains essential to determine the appropriate concentration of CWIs in the formulation.

4.4. Regulation of cosmetic whitening ingredients

The ASEAN Cosmetic Association, in cooperation with the ASEAN member states, developed the ASEAN Cosmetic Directive (ACD). This directive serves as a framework for harmonization of cosmetic regulation and is not intended to enforce implementation or override ASEAN member states' national regulatory authorities. This directive encompasses the ACIL, which enumerates compounds prohibited in cosmetics, those permitted with restrictions, and acceptable coloring and preservation agents. The lists facilitate the establishment of a uniform and secure standard for cosmetic items throughout ASEAN member states [9].

The most striking finding of this review is the proportion (85%) of potentially toxic CWIs not classified in ACIL [9]. In the absence of regulatory guidance or published restrictions, the lack of classification could encourage the development of cosmetic products containing cytotoxic CWIs. Of the seven CWIs identified in this review, six possess IC_{50} values less than 50 $\mu\text{g/mL}$ —considered the cytotoxic threshold—but remain unlisted in ACIL. Among these, salidroside and arbutin present the highest risk to consumers, as these CWIs are already incorporated in various formulations in the market. This study identified CWIs with low IC_{50} that parallels the list of banned agents (e.g., deoxyarbutin, anthraquinone, etc.). It asserts that regulatory screening of CWIs is necessary in the development of cosmetic formulations especially in the incorporation of novel compounds. In addition, dose and formulation concentrations should also be evaluated, as they also impact the cytotoxic properties of the resulting product.

The burden of testing and evaluating the melanocyte cytotoxicity profile of whitening compounds used in the production of cosmetics is a responsibility of the manufacturer as part of the product information file (PIF) to provide cytotoxicity and safety data. Regulatory bodies do not issue a standard test for cytotoxicity profile testing but

rather rely on internationally recognized protocols such as Organization for Economic Cooperation and Development (OECD) guidelines or in vitro cytotoxicity tests such as, but not limited to, MTT assay, melanin content assay, and LDH leakage assay [24]. The role of the regulatory authorities is not to conduct and generate safety data; instead, they verify safety assessments provided by the manufacturer. Manufacturers are expected to follow good manufacturing practices (cGMP) and established rules and regulations to assure the safety and quality of their products. This policy presents an inherent conflict of interest, which may have an impact on the objectivity and impartiality of their safety assessments. To support product claims and marketability, there is a risk of downplaying potential adverse effects. Nevertheless, the national regulatory authorities are responsible for verifying the documentation of conducted cytotoxicity testing. Depending on the jurisdiction and the type of product, the specific testing standards and processes may differ.

Variations in the research design across researchers can have a negative impact on the reliability and comparability of study results. Accuracy and comparability can be derived from research procedures that are consistent and standardized. In this view, the lack of FDA-imposed standardized cytotoxicity methods could lead to inconsistent analysis or comparability of IC_{50} values. In addition, this review considered the data obtained from the in vitro model, which limits its extrapolation in cosmetic in-use conditions may not reflect the actual physiological condition. Further studies are needed to investigate cosmetic whitening agents, using harmonized testing methods, with attention on experimental conditions such as concentration (e.g., nominal and in-use) and the selection of cell lines. Standardization will help reduce variability and bias, thereby enabling more reliable conclusions about the safety of CWIs.

Molecular initiating events of CWIs presented in this paper have been previously reported through macromolecular interactions with tyrosinase up until cellular responses (i.e., cell viability) in tested cell lines [46–49]. A key limitation of this review is the unavailability of data on the full extent of the effect of metabolic by-products of a CWI and their role in key events leading to adverse outcome pathways (AOP). Similarly, the full observation of the CWIs' adverse health effects on the living systems has not been elucidated in this study. This recognizes the significance of investigating the specific relationship of CWIs and their metabolites on key events (e.g., tyrosinase reduction, melanin production,

melanosome transfer) along with molecular initiating events and their consequential adverse outcomes (e.g., leukoderma). Finally, our findings underscore the need to systematically map key events and outcomes to build comprehensive AOPs specific to a CWI, which could support mechanistic risk assessments and inform regulatory guidance.

5. Conclusions

The selection of safe and effective CWIs proves to be difficult due to the lack of up-to-date guidance and regulation. With the discovery of novel whitening agents, there is an increasing risk of incorporating potentially harmful CWIs into cosmetic products as their cytotoxic properties remain unclassified in published regulatory guidance. In-vitro cytotoxicity assays (e.g., MTT assays) provide quantitative measures to the toxic potential through their IC₅₀ values. Lipophilicity presents as a major factor that influences the in vitro cytotoxic properties of CWIs, as it dictates the mechanism and rate of penetration through cell membranes (p -value <0.001). The findings of this research also provide insights on CWI's molecular structure, dose, and cell lines as factors affecting in vitro cytotoxicity.

In conclusion, this review shows that a consistent moderate direct relationship exists between cLogP and IC₅₀ values ($R^2 = 0.3236, 0.3535$; $m = 57.85, 83.36$) of the 18 identified CWIs, indicating an inverse relationship between lipophilicity and cytotoxicity. The linear regression analysis shows that in the identified CWIs in this review, the variation in their IC₅₀ values is significantly influenced by cLogP values (p -value <0.001). However, molecular structure, particularly in the presence of various functional groups (e.g., $-OH$, $-OCH_3$, and $-NH$) and moieties (e.g., sugar, N -formyl, piperazine), impacts both the cytotoxicity and lipophilicity of CWIs. The differences in the physicochemical properties of the employed cell lines also affect the penetration pathway of the compounds and their ability to disrupt cell physiology. Hence, these findings underscore the need for intensive analysis of CWIs prior to incorporation into topical formulation to ensure safety and efficacy. Future research should aim to assess other characteristics that influence cell viability, such as chemical structure, chemical reactivity, and adverse outcome pathways, to conclude the relationship between cLogP and the IC₅₀ value of whitening compounds.

Conflicts of interest statement

The authors declare no known conflict of interest. The research did not receive any external funding.

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