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Assessment of Systemic Safety of *Althaea rosea* Flower Extract for Use in Cosmetics: Threshold of Toxicological Concern and History of Safe Consumption Approaches

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Abstract

Althaea rosea flower extract (ARFE) is widely used as a food and cosmetic ingredient. However, the systemic safety of ARFE for use in cosmetics has not been confirmed, yet. Here, we adopted the threshold of toxicological concern (TTC) and history of safe food consumption approaches to evaluate the systemic safety of ARFE as a cosmetic ingredient. A systematic literature review identified 48 chemical constituents in ARFE, 92.6% of which are common food components. Through a literature review, 48 chemical constituents of ARFE were identified. To exclude the potential genotoxicity issues, in silico predictions of an in vitro AMES test and additional literature reviews were performed, demonstrating that all the chemical constituents of ARFE have no genotoxicity issues. To evaluate the systemic toxicity of ARFE, a comparison with the dietary intake of ARFE was performed. The daily dietary intake of ARFE through tea products was estimated to be 66.67 mg/kg/day. Since exposure to ARFE through cosmetic use ranges from 0.0045 to 5.380 mg/kg/day, which is far lower than dietary intake, it is unlikely to pose any additional health risk. The TTC approach along with in silico predictions of dermal absorption also revealed that systemic exposure doses (SEDs) of all the chemical constituents are below TTC thresholds, further supporting its systemic safety for use in cosmetics.

Keywords: safety assessment; cosmetics; threshold of toxicological concern approach (TTC); next-generation risk assessment (NGRA); *Althaea rosea* flower extract; hollyhock

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Academic Editor: Vasil Georgiev

Received: 23 April 2025
Revised: 13 June 2025
Accepted: 18 June 2025
Published: 24 June 2025

Citation: Gil, S.; Lim, K.-M. Assessment of Systemic Safety of *Althaea rosea* Flower Extract for Use in Cosmetics: Threshold of Toxicological Concern and History of Safe Consumption Approaches. *Cosmetics* **2025**, *12*, 133. https://doi.org/10.3390/cosmetics12040133

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1. Introduction

Traditionally, cosmetic safety assessments have relied on conventional animal testing as the sole source of toxicological data. The European Union implemented a stepwise ban on animal testing for cosmetics: finished product testing was banned in 2004, ingredient testing in 2009, and a complete marketing ban (including repeated-dose toxicity) in 2013 (EU No. 1223/2009) [1]. Following this regulatory shift, over 40 countries have established full or partial bans on cosmetics animal testing [2]. Consequently, alternative strategies are required to evaluate the safety of cosmetic ingredients without reliance on animal testing [3].

The safety of cosmetic ingredients must be assessed with respect to local and systemic toxicity [4]. Efforts has been directed to the development of non-animal test methods to evaluate local toxicity. As a result, key local toxicity endpoints including skin irritation, eye

irritation, phototoxicity and skin sensitization can be evaluated with officially accepted non-animal based in vitro test methods [5,6]. However, due to the complexity of systemic toxicity endpoints, there is no regulatory accepted non-animal test method to replace conventional animal tests yet.

Next-generation risk assessment (NGRA) methods have emerged as promising approaches in this regard [7]. These include the threshold of toxicological concern (TTC), Read-Across, and Quantitative Structure-Activity Relationship (QSAR) models. Among these approaches, the TTC approach has gained significant attention as a viable option for assessing the safety of cosmetic ingredients, particularly fragrance and botanical extracts [8–15]. The TTC approach systematically evaluates the systemic safety of substances with limited toxicological data based on their chemical structure and systemic exposure dose (SED) [16]. In June 2019, the European Food Safety Authority (EFSA) introduced new guidelines for applying the TTC approach to food safety assessments [17]. In addition to EFSA, the TTC approach is also recommended for the safety assessment of cosmetic ingredients, as stated in the guidance issued by the major authorities like the EU Scientific Committee on Consumer Safety (SCCS) [18], the US Cosmetic Ingredient Review (CIR) [19] and China's National Institutes for Food and Drug Control (NIFDC) [20].

The TTC approach categorizes chemicals into five classifications [21]. For substances identified as potentially DNA-reactive mutagens or carcinogens, the TTC value is set at 0.0025 μg/kg·bw/day. For organophosphates and carbamates, the TTC value is 0.3 μg/kg·bw/day, and according to the Cramer classification system, which estimates the theoretical toxicity of a compound based on its chemical structure, TTC values are set to 30 μg/kg·bw/day, 9 μg/kg·bw/day, and 1.5 μg/kg·bw/day for Cramer Class I (low toxicity), Cramer Class II (moderate toxicity), and Cramer Class III (high toxicity), respectively. Substances with exposure levels below the TTC threshold are considered unlikely to pose a significant health risk. Yang et al. [9] refined these TTC values through considering over 1000 chemicals used in cosmetics and the EU SCCS adopted 2.3 μg/kg·bw/day for Cramer Class II and III, and 46 μg/kg·bw/day for Cramer Class I [18].

The TTC approach has been successfully employed for the systemic safety evaluation of botanical extracts used in cosmetics, owing to the generally low abundance of bioactive constituents in them [8,13,14,22]. Another approach to ensure the safety of a botanical extract used in cosmetics without animal experiments is to prove its history of safe use as food, medicine or other related product types [19,23]. Especially, by demonstrating the history of safe food consumption of a botanical extract and its equivalence with the food materials with respect to species, source, process, composition, exposure and usage, mandatory systemic toxicological test data for safety assessment can be exempted. Since many botanical extracts used in cosmetics are used as food materials, this approach may be effective in ensuring the systemic safety of a botanical extract used in cosmetics

Althaea rosea, commonly called hollyhock, is a perennial ornamental plant that is included in the *Malvaceae* family [24–26]. The root and flowers of *Althaea rosea* have been used as folk medicine and herbal tea in Asian countries for many years. Several studies reported the pharmacological and biological effects of *Althaea rosea* such as antioxidant, anti-inflammatory, immunomodulatory, anti-urolithiatic and anti-cancer activities [27–32]. Recently, the cosmetic function of *Althaea rosea* flower extract (ARFE) was discovered [33], paving the way for the use of ARFE in cosmetics. ARFE contains various bioactive phytochemicals including anthocyanins and flavonoids [28]. However, it is yet unclear whether ARFE is safe to use in cosmetics.

The aim of this study is to evaluate the systemic safety of ARFE for its use in cosmetics, using the TTC method and food consumption assessment. For the TTC approach, full chemical constituents' identification using instrumental analysis is necessary. Fortunately,

the chemical constituents of *Althaea rosea* flower powder were recently reported [25,28,34]. We used this information to apply the TTC approach and food consumption assessment in an effort to provide a useful example of the systemic safety assessment methods for botanical extracts used in cosmetics.

2. Materials and Methods

2.1. Literature Search Strategy and Selection Criteria for ARFE Constituent Analysis

We searched PubMed, the Web of Science and Google Scholar with search terms ("*Althaea rosea*" OR "hollyhock") AND (extract OR flower). We identified 84 papers with relevant information. Three papers had detailed information on the chemical constituents of *Althaea rosea* flower powder [25,28,34], which we used to identify the ARFE constituents.

2.2. Classification of Chemical Compounds in A. rosea Flower Extract into Cramer Class

SMILES codes for analysis were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/, accessed on 1 June 2025) and input into ChemTunes® (https://mn-am.com/products/chemtunestoxgps/, accessed on 1 June 2025) for TTC calculation. Toxtree package 3.1 was incorporated in ChemTunes® to assign TTC Cramer Classes based on the extended decision tree [35].

2.3. In Silico Prediction of Genotoxicity Assay

The genotoxicity of *A. rosea* flower was evaluated using in silico predictions by inputting the constituents identified through the literature review and their corresponding chemical structures in SMILES format into ChemTunes®.

2.4. Estimation of Systemic Exposure Dosages

The daily systemic exposure levels for cosmetics were estimated using the daily exposure value for each cosmetic product type provided by the Scientific Committee on Consumer Safety (SCCS) [18]. Daily exposure to a cosmetic product category per kg (body weight) was calculated based on a 60 kg adult (female).

Systemic exposure dosages (SEDs, μg/kg/day) of the chemical constituents of ARFE from cosmetic use were calculated using the following equation:

$$\mathbf{SED} = \mathbf{Eproduct} \times \frac{\boldsymbol{C}}{\mathbf{100}} \times \frac{\boldsymbol{E}}{\mathbf{100}} \times \frac{\boldsymbol{B}}{\mathbf{100}} \times \frac{\boldsymbol{DAp}}{\mathbf{100}}$$

Eproduct (mg/kg bw/day): Estimated daily exposure to a cosmetic product category per kg (body weight) based on the applied quantity and frequency of application.

C (%): Concentration of ARFE used in the finished cosmetic product.

E (%): Extraction ratio (ratio of extractants vs. *A. rosea* flower powder).

B (%): Content of the constituent in *A. rosea* flower powder.

DAp (%): Dermal absorption of the component as a percentage.

The usage concentration of *A. rosea* flower in cosmetics (***C*** (%)) was referenced from the Chinese Cosmetic Ingredient Regulatory Database (China CosIng [36]).

For ***E*** (%), since it is common to add solvent (water) to a plant at 1:9 or higher for extraction [10], it was assumed to be 10%. ***DAp*** (%) was initially set at 100% as a worst-case scenario of Tier 1 to be conservative, even though it was overly conservative for high-molecular-weight or highly polar constituents such as flavonoid glycosides and mucilages. For the constituents that needed a refined dermal absorption rate, we used the CDC Finite Dose Skin Permeation Calculator, which considers physicochemical properties.

2.5. Food Consumption Assessment

An equivalence of *A. rosea* flower powder used as the food material was examined through comparing the preparation of tea and the manufacture of cosmetic raw material. The safe exposure level was evaluated by comparing the dietary intake level of *A. rosea* L. flower powder through tea with the cosmetic exposure level.

3. Results

3.1. Identification of Chemical Constituents of A. rosea Flower Extract

The chemical composition of *A. rosea* flower powder was recently analyzed and published [28], as presented in Table 1. A total of 51 chemical constituents of *A. rosea* flower powder was identified from the literature review, accounting for 105.24% of *A. rosea* flower extract on mass basis. Among them, 92.6% were classified as common food or nutritional ingredients that are considered safe and assumed to pose no health concerns to consumers. *A. rosea* flower powder has the high contents of fat, proteins, ash, and fiber. *A. rosea* flower powder contains mucilages composed of glucuronic acid, galacturonic acid, rhamnose, and galactose, which are part of high-molecular-weight dietary fiber [37].

Table 1. Chemical constituents of *A. rosea* flower powder [28].

No.	Chemicals	CAS	Chemical Class	Contents (%)
1	Water	7732-18-5		8.67
2	Fat			2.67
3	Proteins			12.1
4	Ash			7.62
5	Carbohydrates (Total carbohydrates)			68.95
6	Hexanoic acid	142-62-1	Fatty Acids	0.00
7	Octanoic acid	124-07-2	Fatty Acids	0.00
8	Decanoic acid	334-48-5	Fatty Acids	0.00
9	Dodecanoic acid	143-07-7	Fatty Acids	0.00
10	Tridecanoic acid	638-53-9	Fatty Acids	0.00
11	Tetradecanoic acid	544-63-8	Fatty Acids	0.02
12	Pentadecanoic acid	1002-84-2	Fatty Acids	0.00
13	Hexadecanoic acid	57-10-3	Fatty Acids	0.39
14	Heptadecanoic acid	506-12-7	Fatty Acids	0.01
15	Octadecanoic acid	57-11-4	Fatty Acids	0.10
16	Arachidic acid	506-30-9	Fatty Acids	0.09
17	Docosanoic acid	112-85-6	Fatty Acids	0.04
18	Tricosanoic acid	2433-96-7	Fatty Acids	0.01
19	Tetracosanoic acid	557-59-5	Fatty Acids	0.02
20	(Z)-tetradec-9-enoic acid	544-64-9	Fatty Acids	0.01
21	(Z)-hexadec-9-enoic acid	373-49-9	Fatty Acids	0.00
22	(Z)-heptadec-10-enoic acid	29743-97-3	Fatty Acids	0.02
23	(Z)-octadec-9-enoic acid	112-80-1	Fatty Acids	0.18
24	(Z)-icos-9-enoic acid	29204-02-2	Fatty Acids	0.01
25	(9Z,12Z)-octadeca-9,12-dienoic acid	60-33-3	Fatty Acids	0.29
26	(9E,12E)-octadeca-9,12-dienoic acid (Linolaidicacid)	506-21-8	Fatty Acids	0.05
27	(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	463-40-1	Fatty Acids	0.50
28	(6Z,9Z,12Z)-octadeca-6,9,12-trienoic acid (Gamma-Linolenic acid)	506-26-3	Fatty Acids	0.04
29	11,14-eicosadienoic acid	5598-38-9	Fatty Acids	0.10
30	(5Z,8Z,11Z,14Z)-icosa-5,8,11,14-tetraenoic acid	506-32-1	Fatty Acids	0.06
31	4-Hydroxybenzoic acid	99-96-7	Phenolic Acid	0.00148
32	Gallic acid	149-91-7	Phenolic Acid	0.00081

Table 1. *Cont.*

No.	Chemicals	CAS	Chemical Class	Contents (%)
33	Protocatechuic acid	99-50-3	Phenolic Acid	0.00041
34	Syringic acid	530-57-4	Phenolic Acid	0.00204
35	Caffeic acid (+derivatives)	331-39-5	Phenolic Acid	0.0501
36	Chlorogenic acid	327-97-9	Phenolic Acid	0.02
37	Ferulic acid	1135-24-6	Phenolic Acid	0.00494
38	P-Coumaric acid	501-98-4	Phenolic Acid	0.0359
39	Hydrocinnamic acid	501-52-0	Phenolic Acid	0.0234
40	Cyanidin (Cy-3-O-β-glucopyranoside)	13306-05-3	Anthocyanins	0.0147
41	Malvidin (Mv-3-O-β-glucopyranoside+ Mv-3,5-O-β-glucopyranoside)	10463-84-0 (643-84-5)	Anthocyanins	0.1301
42	Pelargonidin (Pg-3-O-β-glucopyranoside)	134-04-3	Anthocyanins	0.057
43	Petunidin (Pt-3-O-β-glucopyranoside)	13270-60-5	Anthocyanins	0.100
44	Anthocyanin I-III	-	Anthocyanins	0.2395
45	Gallocatechin gallate	5127-64-0	Flavonoid	0.0535
46	Naringin (Naringin glycoside I+II)	10236-47-2	Flavonoid	1.6070
47	Apigenin (Apigenin glycoside II+III)	520-36-5	Flavonoid	0.0171
48	Luteolin (Luteolin glycoside II)	491-70-3	Flavonoid	0.7224
49	Kaempherol (+Kaempherol glycoside II+III)	520-18-3	Flavonoid	0.0258
50	Quercetin (Quercetin glycosides)	117-39-5	Flavonoid	0.1260
51	Rutin			0.0620
	Total (%)			105.24%

The fatty acid constituents of *A. rosea* flower powders include linoleic acid and palmitic acid. In addition, flower oil contained significant amounts of oleic acid and eicosadienoic acid.

Besides these food components, *A. rosea* flower powder contains a total of twenty-one potentially bioactive constituents; nine phenolic acids, five anthocyanins, and seven flavonoids.

3.2. Assessment of Genotoxicity of A. rosea Flower Extract

According to the literature review conducted so far, no publicly available genotoxicity test results have been reported for extracts of *A. rosea* flower. It is essential to exclude the genotoxic potential of botanical extracts, so we searched or predicted in silico the genotoxicity potential of the 20 non-food chemical constituents, except for anthocyanins, the structure of which was not available with ChemTunes®, based on the SMILES format. Even though we could not use an in silico tool to predict the genotoxicity of anthocyanins, it is widely known that anthocyanins protect against DNA damage [38], allowing us to conclude that they do not have genotoxicity potential. As a result, most chemical constituents were concluded as non-genotoxic, except for luteolin (Table 2).

Table 2. Constituents of *Althaea rosea* flower extract (µg/kg/day), in silico and literature-based assessment of genotoxicity and systemic exposure dose estimation.

No.	Cramer Class	Chemical	Classification	Genotoxicity		TTC Threshold (µg/kg bw/Day)	Leave-on Skin and Hair (SCCS) 20.786 mg/kg
				Results	Detail		Realistic (µg/kg bw/Day)
1	I	4-Hydroxybenzoic acid	Phenolic acid	-	AMES	46	0.00615
2	I	Gallic acid	Phenolic acid	-	AMES	46	0.00337
3	I	Protocatechuic acid	Phenolic acid	-	IN SILLICO	46	0.00170
4	I	Syringic acid	Phenolic acid	-	IN SILLICO	46	0.00848
5	I	Caffeic acid	Phenolic acid	-	AMES	46	0.20828
6	I	Ferulic acid	Phenolic acid	-	IN SILLICO	46	0.08314
7	I	P-Coumaric acid	Phenolic acid	-	IN SILLICO	46	0.02054
8	I	Hydrocinnamic acid	Phenolic acid	-	IN SILLICO	46	0.14924
9	II	Chlorogenic acid	Phenolic acid	-	AMES	2.3	0.09728
10	III	Cyanidin	Anthocyanins	-	IN SILLICO	2.3	0.06111
11	III	Malvidin	Anthocyanins	-	IN SILLICO	2.3	0.54085
12	III	Pelargonidin	Anthocyanins	-	IN SILLICO	2.3	0.23696
13	III	Petunidin	Anthocyanins	-	IN SILLICO	2.3	0.41572
14	III	Anthocyanins I-III	Anthocyanins		IN VIVO	2.3	0.99565
15	III	Gallocatechin gallate	Flavonoid	-	IN SILLICO	2.3	0.22241
16	III	Naringenin (Naringin glycoside)	Flavonoid	-	IN SILLICO	2.3	6.68062
17	III	Apigenin (Apigenin glycoside)	Flavonoid	-	AMES	2.3	0.07109
18	III	Luteolin (luteolinglycosideii)	Flavonoid	+	IN SILICO (genotox concern)	2.3	3.00316
19	III	Kaempherol	Flavonoid	-	IN VIVO	2.3	0.10726
20	III	Quercetin (Quercetin glycoside)	Flavonoid	-	IN VIVO	2.3	0.52381
21	III	Rutin	Flavonoid	-	IN VIVO (Carcino-genesis study)	2.3	0.25775

To assess the genotoxicity issue of luteolin, we reviewed relevant genotoxicity studies and in vitro experimental data. Numerous studies have reported conflicting results regarding its genotoxic potential. For example, the literature review revealed that several studies have demonstrated that these constituents may actually protect cells against genetic damage. Taj and Nagarajan reported that luteolin protected cells in micronucleus assays and reduced chromosomal aberrations in rats [39]. Moreover, luteolin derived from *Clerodendrum cyrtophyllum* Turcz leaves exhibited no genotoxic effect towards HepG2 cells but rather showed antioxidant activity by increasing cell viability [40]. Also, dietary flavonoids including quercetin, luteolin and genistein reduce oxidative DNA damage and lipid peroxidation in vivo [41,42]. Recently, Fernando et al. demonstrated that luteolin at 2.5 µg/mL does not induce DNA damage but rather protects against H_2O_2-induced DNA damage in lung fibroblasts with COMET and phosphor-H2A.X assays [43], further supporting the absence of the genotoxicity of luteolin.

3.3. Systemic Exposure Dosages of the Chemical Constituents of A. rosea Flower Extract from the Use in Cosmetics

According to the ChinaCosING database, ARFE (CAS. No. 90045-76-4) was used at concentrations of up to 2% in 2021 [36]. More recently, in 2024, registered products have been reported to contain ARFE at concentrations ranging from 0.0001% to 0.005%. We assumed the worst-case scenario considering that ARFE is used at 2%. Using these data and the content levels in Table 1, we estimated systemic exposure dosages (SEDs, μg/kg/day) of the chemical constituents of ARFE from cosmetic use (see Section 2.3).

For our calculations, we defined C (%) as 2% and set Eproduct at 207.86 mg/kg/day (207,860 μg/kg/day, reflecting the daily use of leave-on type cosmetics: body lotion, facial cream, hand cream, deodorant and hair styling products) (SCCS, 2023) [18].

For E (%), since it is common to add a solvent (water) to a plant at 1:9 or higher for extraction [10], it was assumed to be 10%. DAp (%) was initially set at 100% as a worst-case scenario of Tier 0. The resulting SED values are presented in Table 2.

3.4. The Safety Assessment Using the TTC Approach

Twenty non-food constituents with structure information were classified into Cramer Classes using ChemTunes™ based on the ToxTree 3.1 algorithm. Interestingly, anthocyanins were classified as Cramer Class III due to the presence of phenolic hydroxyl groups and heterocyclic moieties although they are regarded as safe food constituents. There were eight Cramer Class I, one Class II and twelve Class III chemical constituents in ARFE.

The SEDs for most of chemical constituents by their use in leave-on products were below their respective TTC thresholds.

However, naringin and luteolin were estimated to exceed their respective TTC thresholds. For these high-molecular-weight or highly polar constituents, a Tier 0 assumption of 100% Dap is overly conservative. Therefore, we further refined the dermal absorption using in silico prediction tools, EpiSuite™ and the CDC Finite Dose Skin Permeation Calculator (Table 3). By applying Tier 1 in silico refinement, SEDs of naringin (naringin glycoside) and luteolin were estimated to be lower than their TTC thresholds, reflecting that ARFE is safe to use in cosmetics up to 2%.

Table 3. In silico refinement of systemic exposure dose estimation of constituents of *A. rosea* flower extract (μg/kg/day).

Chemical (Formula)	M.W.	LogKow	Melting Point	Boiling Point	Vapor Pressure (mmHg, 25 °C)	Applied Conc. (μg/mL)	Applied Amount μg/cm^2	24 h Cumulative Permeation (%)	Tier 0 SED μg/kg/Day	Tier 2 SED μg/kg/Day
Naringin ($C_{27}H_{32}O_{14}$)	580.5	−0.52	349.84	843.85	7.00×10^{-24}	32	0.435	>1.00	6.681	>0.067
Luteolin ($C_{15}H_{10}O_6$)	286.24	2.36	212.22	499.19	2.19×10^{-13}	14	0.190	50.00	3.003	1.500

3.5. The History of Safe Food Consumption of A. rosea Flower Supporting the Safety of ARFE in Cosmetics

It has been confirmed through the literature that *A. rosea* flower has been consumed as food since ancient times [24,26]. It is recommended to brew tea with 1–2 teaspoons of dried *A. rosea* flower and consume one or four cups a day. Therefore, the tea preparation method (hot water) and manufacture of raw materials for cosmetics (80 °C hot water extraction) are equivalent. For a 60 kg adult, the dietary intake can be converted to mg/kg/day as follows:

- 1 to 2 teaspoons: One teaspoon of dried flowers weighs about 2 to 3 g.

- Daily Intake: Minimum intake = 2 g, maximum intake = 6 g. Thus, the average intake of dried flowers can be estimated as 4 g per a cup and when two cups are consumed daily.

$$\text{Daily Intake of ARFE (mg/kg/day)} = \frac{4000\ \text{mg}}{60\ \text{kg}} \times 2 = 133.34\ \text{mg/kg/day}$$

Assuming ARFE is used in cosmetics at 0.05–2% according to ChinaCosing, the SED of ARFE from its use in leave-on products [207.86 mg/kg (Eproduct) × 2%/100 (C/100) * 10%/100 (Extraction ratio) × 100%/100 (Dap)] would not be more than 0.42 mg/kg bw/d, which is 317-fold lower than the daily food consumption of ARFE, supporting the safety of the use of ARFE in cosmetics.

4. Discussion

Here, we evaluated the systemic safety of ARFE used in cosmetics using the TTC and history of safe food consumption. The TTC approach was particularly useful in assessing the safety of ARFE as botanical extracts are mostly composed of macro- or micronutrients, with only small amounts of bioactive constituents potentially posing a risk to human health. Indeed, 92.6% of ARFE constituted common food components or nutritional ingredients, which are considered safe and assumed to pose no health concerns to consumers, leaving only 12.6% of the constituents of ARFE to be considered safety-wise. Furthermore, botanical extracts used in cosmetics are generally manufactured as diluents, i.e., soaking and stirring solvents with dried plants by a 9:1 ratio or more, substantially lowering the exposure level of chemical constituents by the use of cosmetics. Indeed, by considering the dilution factor, SEDs of ARFE constituents were reduced to 10%, meaning that only two bioactive constituents exceeded TTC thresholds at Tier 0 estimation.

To further refine the SEDs of the two constituents with SEDs exceeding the TTC threshold, we applied an in silico tool, the CDC Finite Dose Skin Permeation Calculator, which estimated the dermal absorption of chemicals using physicochemical properties. Lipophilicity and molecular weight are important parameters determining the dermal absorption of chemicals. Of note, natural bioactive substances occurring in botanical extracts were generally hydrophilic and of high molecular weights, which limits their dermal absorption further. By applying in silico dermal absorption prediction, the SEDs of two constituents were significantly lowered below the TTC, demonstrating the utility of the in silico approach in the safety assessment of botanical extracts.

There are some limitations to consider in the TTC approach we employed. We identified the constituents of ARFE and their content from the literature, which may not fully account for the unknown constituents of ARFE used in cosmetics. It would be more accurate to conduct an instrumental analysis for ARFE used in cosmetics to obtain this information. However, it is often impractical to conduct analysis since the instrumental analysis is expensive and time-consuming. Moreover, a sufficient amount of ARFE must be used, which is often made difficult by the scarcity of plant materials.

To supplement the TTC approach, we also conducted the history of safe food consumption approach to ensure the safety of ARFE used in cosmetics. From tea products, dried herbs can be extracted with hot water, of which the preparation method is similar to the manufacturing process of ARFE for use in cosmetics, providing the rationale for the adoption of this approach. The dietary intake of *A. rosea* flower through teas was estimated to be 133.34 mg/kg/day, whereas the exposure to ARFE through cosmetic use, assuming a worst-case scenario with 2% concentration in all products, was estimated at 0.42 mg/kg/day, indicating that no additional health risk over its food consumption was anticipated. Its long history of food consumption may also support the safety of ARFE

across different batches and harvests of *A. rosea* flower, which can supplement the TTC approach at least in part. However, the history of food consumption approach does not consider individual chemical constituents of a botanical extract, which may be problematic when trace but hazardous constituents exist. By applying two distinct approaches, we can effectively and more credibly ensure the systemic safety of ARFE used in cosmetics.

When considering the safety of ARFE in cosmetic applications, its historical use as a food product suggests a degree of safety. However, the different exposure routes—oral versus dermal—introduce critical uncertainty. Flavonoids, including anthocyanins, are major components of ARFE. When consumed orally, these compounds exhibit poor bioavailability (typically 1–2%) [44] and undergo extensive metabolism in the intestines and liver [45]. This metabolic transformation can significantly alter their intrinsic toxicity profiles. In contrast, while the dermal application of flavonoids in cosmetics also leads to limited absorption [46], they encounter substantially less metabolism compared to oral routes. This difference in metabolic processing means that the safety profile of ingested ARFE may not directly mirror that of topically applied ARFE. Therefore, safety assessors must carefully consider these distinct exposure and metabolic pathways when evaluating the dermal safety of ARFE in cosmetic products.

5. Conclusions

Collectively, we demonstrated that TTC and history of safe food consumption approaches can be useful in ensuring the systemic safety of the botanical extract ARFE in cosmetics. A limitation of this study is the reliance on literature-derived constituent profiles, which may not capture all unknown and trace components. In addition, the history of safe food consumption approach does not account for the difference in metabolic processing for topically applied ARFE. Further case studies and methodologies to resolve the limitations of these two approaches are necessary to advance the safety assessment of botanical extracts.

Author Contributions: Conceptualization, S.G. and K.-M.L.; methodology, S.G. and K.-M.L.; formal analysis, S.G.; investigation, S.G.; resources, K.-M.L.; data curation, S.G.; writing—original draft preparation, S.G.; writing—review and editing, K.-M.L.; supervision, K.-M.L.; project administration, K.-M.L.; funding acquisition, K.-M.L. All authors have read and agreed to the published version of the manuscript.

Funding: This study was part of the cosmetic safety evaluation project carried out by the Korea Cosmetic Industry Institute (KCII) funded by the Ministry of Health and Welfare and National Research Foundation (NRF) of Korea (2021R1A2C2013347 and 2021R1A6C101A442).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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