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Original Article

FACE MIST FORMULATION OF *CHRYSANTHEMUM INDICUM* L. FLOWER ETHANOLIC EXTRACT, ANTIOXIDANT ASSAY, AND *IN SILICO* TOXICITY PREDICTION

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ABSTRACT

Objective: *Chrysanthemum indicum* Linné (L.) flower is one of the popular ornamental plants in Indonesia. It is known to contain antioxidants that are essential for protecting our skin from free radicals. The objective of this study is to perform an *in silico* toxicity prediction of *Chrysanthemum indicum* L. metabolites, to formulate the *Chrysanthemum indicum* L. flower ethanolic extract into a face mist preparation, and to analyze the effect of extract variations on the physical and chemical stability and antioxidant activity of face mist preparations.

Methods: The antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay; toxicity prediction was performed with the pkCSM Website. The extract was formulated into face mist preparations with 0.4, 0.6, and 0.8% concentrations. Furthermore, the stability of the face mist preparations was assessed through the cycling test. Finally, the data results were analyzed using a two-way Analysis of Variance (ANOVA).

Results: The majority of the metabolites derived from *Chrysanthemum indicum* L. were found to be non-toxic. All formulations of the face mist preparation were liquid, exhibiting a color range from yellowish-brown to dark yellowish-brown, and had a rose-like scent. The formulations met the requisite specifications of specific gravity, clarity, spray dispersibility, drying time, and pH. The IC_{50} values of face mist preparations were 87.75±0.02 ppm (F1), 77.57±0.02 ppm (F2), and 53.51±0.03 ppm (F3). After completing the cycling test, a rise in IC_{50} values was observed, with the values increasing to 88.28±0.04 ppm (F1), 79.74±0.009 ppm (F2), and 56.94±0.008 ppm (F3).

Conclusion: The extract concentration variation significantly affected the face mist preparation's clarity, pH, and antioxidant stability.

Keywords: Face mist formulation, Chrysanthemum, Toxicity prediction, *In silico*, Antioxidant

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INTRODUCTION

The current lifestyle of metropolitan cities is associated with an elevated risk of free radical exposure, which is caused by various environmental factors such as Ultraviolet (UV) rays, pollution, and smoke, thus antioxidants are essential for the human body to help neutralize free radicals [1]. Although the human body is capable of producing antioxidants, external sources of antioxidants are still necessary when the body is exposed to excessive free radicals [2]. Generally, there are synthetic and natural antioxidants. Synthetic antioxidants such as Butylated Hydroxy Anisole (BHA) and Butylated Hydroxy Toluene (BHT) are commonly utilized, yet they are associated with potential health risks. BHA is suspected to be a human carcinogen, while the metabolites of BHT have been linked to oxidative Deoxyribonucleic Acid (DNA) damage [3]. Therefore, natural antioxidants can be a safer alternative [4].

Chrysanthemum indicum L., commonly known as Chrysanthemum, is one of Indonesia's most popular ornamental plants and has the potential to be a source of antioxidants [5]. The popularity of this plant has reached a high economic value, as indicated by the fact that 394,502,208 stems were produced in 2022 [5]. Its potential as a source of antioxidants is based on the presence of flavonoids and triterpenoids. Research by Shin Youn Joo (2013) revealed that the *Chrysanthemum indicum* L. flower ethanolic extract has a very strong ability to inhibit or suppress the activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals with an IC_{50} value of 43.34 ppm. This inhibitory capability is attributed to the presence of phenolic and flavonoid content, which is effectively extracted in 70% ethanol solvent [4, 6, 7].

In the fast-paced modern lifestyle, there is a high demand for skin care products that offer convenience and versatility, such as face mists. Due to its spray bottle packaging, the face mist is a convenient product that can be easily carried and used anytime. Besides refreshing the skin amidst pollution and sun exposure, face mist can also act as a mild disinfectant and help minimize skin pores [8].

The development of new skin care products from natural ingredients requires consideration of the safety of the active substances. One method for doing so is to perform an *in silico* toxicity prediction of *Chrysanthemum indicum* L. flower metabolite compounds. The *in silico* method has several advantages over *in vivo* and *in vitro* experimental methods. It is more environmentally friendly, relatively more affordable, simple yet faster, and can be performed before compound synthesis [9].

Although previous studies have examined the antioxidant properties of *Chrysanthemum indicum* L. extract, its formulation in face mist preparation and comprehensive evaluation still needs to be explored. This study introduces an approach to utilize the potential antioxidant properties of *Chrysanthemum indicum* L. flower ethanolic extract. By varying the extract concentration, the study aims to optimize the formulation for maximum antioxidant activity while maintaining its physical and chemical stability. Additionally, *in silico* toxicity prediction will be conducted to evaluate the safety of the metabolite compounds that may be present within the extract, mainly focusing on potential skin sensitization due to the limited available information. This assessment will contribute to developing a promising natural skin care product and offer insights into the possible benefits and risks of using *Chrysanthemum indicum* L. extract for skin care products.

MATERIALS AND METHODS

Materials

The primary material was the *Chrysanthemum indicum* L. flower, collected from Cipanas-West Java, identified by the Herbarium Depokensis (UIDEP), and extracted with 70% ethanol solvent. Additional ingredients were PVP (Polyvinyl Pyrrolidone) K-30 (MP Biomedicals, USA), phenoxyethanol, glycerin (Wilmar, Indonesia), rose oil (Fagron, Belgium), vitamin C (Merck, Germany), DPPH powder (Sigma Aldrich, USA), methanol pro analysis (Merck, Germany), quercetin powder (Sigma Aldrich, USA), aluminum (III) chloride powder (Xilong Scientific, China), sodium acetate powder (Merck, Germany), and pure water.

Tools

Instruments and other equipment used in this study include a laptop (ASUS X412FL), UV-Vis Spectrophotometer (Shimadzu 1900), a

microbalance (Mettler MT5), a Karl Fischer (Metrohm 870KF), a pH meter (HANNA HI 221), a stirrer (Thermolyne), an analytical balance (KERN ABT 100-5M), and some glassware (Pyrex and Iwaki).

Extract evaluation

Organoleptic

The organoleptic properties of the extract, including texture, color, and aroma, were observed using the human senses [10].

Miscibility

One g of viscous extract was mixed with pure water and glycerin separately until the extract was mixed entirely. The ratio of extract to solvent was recorded, and the miscibility category was determined [11].

pH value

Ten grams of extract were dissolved in 100 ml of pure water. Then, its pH was tested using a calibrated pH meter [12].

Water content

An extract of 30 to 50 mg extract was prepared, and its water content was determined using the Karl Fischer instrument [12].

Residual solvent

Two grams of extract were dissolved in 25 ml of pure water. This solution was then distilled at 78.5 °C temperature for around 2 h or until the dripping stopped. The water was added to the obtained distillate (approximately 23 ml) until 25 ml. The density of the obtained distillate solution was determined at a temperature of 25 °C [10].

Phytochemical screening

Flavonoid

One g of extract was weighed, and 10 ml of hot water was added. The mixture was then boiled for 5 min, filtered, shaken, and observed until the separation formed. A positive sample formed a red, yellow, or orange color in the amyl alcohol layer [13].

Saponin

A total of 0.5 g of extract were weighed, and 10 ml of hot water was added afterward. The solution was then poured into a testing tube and vigorously shaken for 10 sec. If a foam was formed and persisted for at least 10 min with a height of approximately 1-10 cm, a drop of 2N hydrochloric acid was added. The presence of stable foam indicates the presence of saponins [13].

Tannin

Five grams of extract were weighed, then dissolved in 10 ml of water, filtered, and the filtrate was diluted until it became colorless. Two milliliters of the solution were taken, and 1-2 drops of iron (III) chloride reagent were added. A positive sample formed a dark green, dark blue, or black color solution [13].

Alkaloid

Two milliliters of extract were evaporated in a porcelain dish, and the residue was dissolved in 5 ml of 2N hydrochloric acid. The solution was then divided into three tubes, with tube 1 used as a control, while tubes 2 and 3 for the test solution. Three drops of Dragendorff's reagent were added to tube 2, and three drops of Mayer's reagent were added to tube 3. A positive sample formed an orange precipitate in tube 2 and a yellow precipitate in tube 3 [14].

Steroid and triterpenoid

Two milliliters of extract were evaporated in a porcelain dish, and the residue was then dissolved in 0.5 ml of chloroform, and 0.5 ml of acetic anhydride was added. Then, 2 ml of concentrated sulfuric acid was added down the side of the tube. A positive sample containing triterpenoid formed a brownish or violet ring, while the positive steroids formed a blue-green ring [14].

Total flavonoid content

The total flavonoid content of the extract was quantified through a colorimetric assay. A solution of 0.5 ml of the extract at a concentration of 5000 ppm was added to a solution of 0.1 ml of 10% aluminum (III) chloride, 0.01 ml of 1 M sodium acetate, and 2.8 ml of pure water in a volumetric flask. The flask volume was adjusted with methanol to a total volume of 5 ml. The mixture was then incubated for 25 min, and its absorbance was measured at 433 nm wavelength versus a blank. A quercetin solution was used as a standard for the calibration curve. The total flavonoid content of the extract was expressed as milligrams of quercetin equivalents per g of sample (mgEQ/g) [15].

Antioxidant activity

The antioxidant activity was measured using a DPPH assay. The extract sample solution concentration was serialized by pipetting 0.1, 0.15, 0.2, 0.25, and 0.3 ml of 1000 ppm extract solution into a volumetric flask, thus resulting in 20, 30, 40, 50, and 60 ppm of sample solution, respectively. One milliliter of DPPH solution was added into each volumetric flask, and methanol pro analysis was added up to 5 ml. The mixture was then incubated for 20 min in a dark place, and the absorbance was measured at a wavelength of 516 nm versus a blank. Vitamin C solution was used as a standard for the calibration curve. Then, the percentage of free radical inhibition was calculated with the following formula:

$$\text{Inhibition (\%)} = (\text{Blank absorbance} - \text{Sample absorbance})/(\text{Blank absorbance}) \times 100\%$$

Following that, the IC_{50} value, which is the concentration of the extract that can scavenge free radicals by 50%, was calculated using the linear equation y = a+bx, with the IC_{50} value defined by x value [16].

***In silico* toxicity prediction**

The PubChem database was utilized to obtain canonical Simplified Molecular Input Line Entry System (SMILES) strings for metabolite compounds isolated from *Chrysanthemum indicum* L. flower, as previously retrieved [6]. The SMILES were then entered into the pkCSM tool to predict potential toxicity parameters, including Ames-toxicity, Lethal Dose $(LD)_{50}$, Lowest Observed Adverse Effect Level (LOAEL), hepatotoxicity, and skin sensitization. This *in silico* approach provided an initial assessment of the safety of these compounds for use in skin care applications [17, 18].

Face mist formulation

The face mist was formulated with concentration variations, as seen in the table below:

Table 1: Face mist formulation

Material	Formula (%)			
	F0	F1	F2	F3
Ethanolic extract of *Chrysanthemum indicum* L. flower	-	0.4	0.6	0.8
Glycerin	15	15	15	15
PVP K-30	0.1	0.1	0.1	0.1
Phenoxyethanol	0.5	0.5	0.5	0.5
Rose oil	0.05	0.05	0.05	0.05
Pure water	Ad 100	Ad 100	Ad 100	Ad 100

The concentration variations were chosen based on a preliminary optimization process to obtain face mist preparations with strong antioxidant activity while meeting the requirements. A series of extract concentrations, ranging from 0.1% to 1%, were initially evaluated to obtain a narrower range of promising concentrations that exhibited strong antioxidant activity while meeting the desired

formulation characteristics. Glycerin, a humectant, was added to enhance moisture retention and facilitate the dispersion of the extract. PVP K-30, a stabilizing agent, was added to prevent particle aggregation and maintain the face mist's consistency. Phenoxyethanol, a broad-spectrum antimicrobial preservative, was added to inhibit microbial growth in this aqueous formulation while remaining safe for skin application due to its minimal impact on the skin's normal flora. Rose oil, as a fragrance, was added in a deficient concentration to provide a pleasant scent in this formulation [8, 19, 20].

The face mist was formulated by dissolving the *Chrysanthemum indicum* L. flower ethanolic extract and phenoxyethanol in glycerin. Then, PVP K-30, which had been previously dissolved in hot water before, was added, followed by rose oil. The final mixture was poured into the spray bottle, and then purified water was added until the calibration mark of 100 ml volume.

Face mist evaluation

Organoleptic

The organoleptic properties of the face mist preparation, including texture, color, and aroma, were observed using the human senses [8].

Specific gravity

The specific gravity was determined using a pycnometer. An ideal face mist should have a density similar to that of water (1 g/ml) [8].

Clarity

The clarity of each formula was determined by measuring the absorbance at a wavelength of 640 nm using a UV-Vis spectrophotometer, with pure water as a control. A face mist is considered transparent if it has a percent of transmittance ranging from 90-100% [21].

Spray coverage

The spray coverage was determined by spraying the face mist preparation onto a sheet of clear plastic at 5 cm. The spray coverage pattern was then measured using a ruler. A face mist preparation is considered to have good spray coverage if it has a diameter of 5-7 cm spraying pattern [22].

Drying time

The drying time was determined by spraying the face mist preparation onto the forearm and measuring the time required for the mist to dry completely. The optimal drying time was found to be no more than 5 min [22].

pH

The pH value was measured using a calibrated pH meter. A face mist preparation should have a pH of 4.5-6.5 [8].

Antioxidant activity

The antioxidant activity was determined using a DPPH assay. Each formula of the face mist preparation was serialized by pipetting a particular volume of sample solution, resulting in a serial concentration of each formula's sample solution. One milliliter of DPPH solution was added into each volumetric flask, and methanol pro analysis was added up to 5 ml. The mixture was then incubated for 20 min in a dark place, and the absorbance was measured at a wavelength of 516 nm versus a blank. Vitamin C solution was used as a standard for the calibration curve. The percentage of free radical inhibition was calculated with the following formula:

$$\text{Inhibition (\%)} = (\text{Blank absorbance} - \text{Sample absorbance})/(\text{Blank absorbance}) \times 100\%$$

Following that, the IC_{50} value, which is the concentration of the extract that can scavenge free radicals by 50%, was calculated using the linear equation $y = a+bx$, with the IC_{50} value defined by x value [16].

Cycling test

A stability test was conducted using the cycling test method throughout six cycles. The face mist preparation was stored at 4±2 °C for 24 h, then transferred to a storage location at 40±2 °C for 24 h, allowing one cycle to occur over two days. Following the completion of six cycles, changes in organoleptic properties, specific gravity, clarity, spray coverage, drying time, pH, and antioxidant activity of each formula were observed [23]. The obtained data was then analyzed using a two-way Analysis of Variance (ANOVA) method to determine the influence of the cycling test and extract concentration variation on the face mist preparation's physical and chemical properties and antioxidant activity.

RESULTS AND DISCUSSION

Extract evaluation

The results of the extract evaluation indicated that the substance appeared in a dark yellowish-brown viscous form with a distinctive odor. The extract was found to be miscible with pure water and glycerin, as the solvent in the face mist formulation. The pH value of the extract was determined to be 5.19, which is characteristic of an acidic solution. This occurs due to the presence of flavonoids and tannins, which are acidic, as evidenced by the phytochemical screening result [6].

The water content in the extract was 7.51%, meeting the requirement of less than 11.1%. This evaluation was intended to give information on the extract's water content, as the presence of water could serve as a suitable medium for bacterial and mold growth, decreasing the extract quality. The residual solvent content in the extract was 0.82%, meeting the requirement of less than 1%. This evaluation is crucial because the residual solvent within the extract may have an unfavorable impact on dermal conditions. Furthermore, if it enters the body, it will also affect the body [12].

The metabolite compounds of the extract were evaluated both qualitatively and quantitatively. The initial qualitative assessment was conducted through phytochemical screening, while the later quantitative analysis was performed to determine the total flavonoid content. The phytochemical screening results indicated the presence of saponins, alkaloids, flavonoids, tannins, and triterpenoids in the extract. In addition, the total flavonoid content of the extract was 11.6667 mgQE/g extract.

Antioxidant activity of the extract

The DPPH assay is a simple yet sensitive method for determining antioxidant activity. The assay is based on the concept that antioxidants function as hydrogen donors. The sample solution will be added to DPPH, a free radical, which will result in a shift from purple to yellow or pale yellow, indicating the loss or absence of DPPH in the compound [1].

The initial step of the antioxidant assay was to determine the maximum wavelength and operating time of the DPPH. The maximum wavelength of DPPH, as determined by measuring within the 400-600 nm range, was found to be 516 nm. The operating time, as measured at a wavelength of 516 nm, was found to be 20 min. Measurement of maximum wavelength was performed to determine at which wavelength the DPPH shows optimum absorbance. This information will be applied in subsequent antioxidant assay procedures. Additionally, the operating time measurement determines the optimal time to measure the sample solution that reacts to the free radicals.

Based on this study, the IC_{50} value of vitamin C was 3.60 ppm, while the IC_{50} value of the *Chrysanthemum indicum* L. flower extract was 40.88 ppm. Both are categorized as very strong antioxidants. However, there are notable differences between the two. Vitamin C is a single compound, whereas the *Chrysanthemum indicum* L. flower extract contains a diverse range of metabolites, including flavonoids and phenolic acids [6, 24].

Regarding the IC_{50} value of the *Chrysanthemum indicum* L. flower ethanolic extract, the result in this study is generally comparable to that of a previous study conducted in 2013, which reported an IC_{50} value of 43.34 ppm [7]. The difference can be attributed to several factors, including the source of the plant, analysis methods, and so on. However, the overall results of this study are in line with previous findings regarding the very strong antioxidant potential of *Chrysanthemum indicum* L. ethanolic extract. The results are shown in the following table.

Table 2: The antioxidant activity result of the extract

Sample	IC_{50} (ppm)*
Vitamin C	3.60±0.05
Ethanolic extract of *Chrysanthemum indicum L.* flower	40.88±0.01

*Data presented is mean±SD for n=3 measurements

Table 3: *In silico* toxicity prediction of metabolite compounds derived from *Chrysanthemum indicum* L. flower

Compounds	Ames-toxic?	Max. human dose (log/mg/kg BW/d)	LD_{50} (mol/kg)	LOAEL (log/mg/kgBW/d)	Hepatotoxic?	Skin sensitizer?
Acacetin	No	0.090	2.220	1.259	No	No
Acacetin-7-O-rutinoside	No	0.532	2.510	3.319	No	No
Apigenin	No	0.328	2.450	2.298	No	No
Apigenin-7-O-glucoside	No	0.515	2.595	4.359	No	No
Apigenin-7-O-rutinoside	No	0.533	2.520	3.338	No	No
Diosmetin	No	0.420	2.338	2.271	No	No
Diosmetin-7-O-glucoside	No	0.583	2.570	4.302	No	No
Eupatilin	No	0.328	2.113	2.112	No	No
(2S)-Hesperetin	No	0.250	2.042	2.605	No	No
Linarin	No	0.532	2.521	3.319	No	No
Luteolin	No	0.499	2.455	2.409	No	No
Luteolin-7-O-glucoside	No	0.584	2.547	4.279	No	No
Luteolin-7-O-glucuronide	No	0.547	2.560	4.304	No	No
Tricin	No	0.351	2.229	1.82	No	No
5,3',4'-Trihydroxy-6,7-dimethoxyflavone	No	0.379	2.387	2.399	No	No
Kaempferol	No	0.531	2.449	2.505	No	No
Quercetin	No	0.499	2.471	2.612	No	No
Sudachitin	No	0.484	2.336	2.016	No	No
Isoquercitrin	No	0.569	2.541	4.417	No	No
Isorhamnetin	No	0.576	2.407	2.499	No	No
Eriodictyol	No	0.014	2.030	2.475	No	No
Eriodictyol-7-O-ß-D-glucuronide	No	0.429	2.631	4.481	No	No
5,7,3',5'-tetrahydroxyflavanone-7-O-ß-D-glucopyranoside	No	0.369	2.729	4.319	No	No
Arteglasin A	Yes	0.085	2.690	1.511	No	No
Chrysanthemol	No	0.632	1.655	1.849	No	Yes
Chrysantherol	No	0.378	1.930	1.909	No	Yes
Clovanediol	No	0.073	1.696	1.893	No	Yes
Cumambrin A	Yes	0.208	2.420	1.581	No	No
Indicumenone	No	0.763	1.692	1.879	No	Yes
Intermedeol	No	0.290	1.721	1.280	No	Yes
Kikkanol A	No	1.204	1.511	2.739	No	No
Kikkanol B	No	0.764	1.621	2.132	No	Yes
Kikkanol C	No	0.790	1.887	2.686	No	No
Kikkanol D	No	1.063	1.731	2.773	No	No
Kikkanol E	No	0.996	1.930	1.959	No	No
Kikkanol F	No	1.063	1.731	2.773	No	No
Ligucyperonol	No	0.179	1.820	1.993	No	Yes
Matricarin	Yes	0.195	1.989	1.448	No	No
Oplopanone	No	0.005	1.638	2.170	No	Yes
Yejuhua Lactone	No	-1.001	3.194	1.879	No	No
11,13-dehydrodesacetylmatricarin	Yes	0.305	2.112	2.476	No	No
Caffeic acid	No	1.145	2.383	2.092	No	No
Chlorogenic acid	No	-0.134	1.973	2.982	No	No
Chlorogenic acid methyl ester	No	-0.312	1.844	2.403	No	No
Cryptochlorogenic acid methyl ester	No	-0.445	1.727	2.168	No	No
Chrysophanol	Yes	-0.256	2.275	2.057	No	No
Syringin	No	0.890	1.830	3.718	No	No
Dihydrosyringin	No	0.924	1.802	3.717	No	No
Syringaresinol	No	-0.404	2.019	1.510	No	No
Vanillic acid	No	0.719	2.454	2.032	No	No
Zhebeiresinol	No	-0.130	2.098	2.030	Yes	No
p-Hydroxybenzoic acid	No	0.846	2.255	2.483	No	No
1,3-dicaffeoylquinic acid	No	0.367	2.567	3.459	No	No
1,5-dicaffeoylquinic acid	No	0.367	2.567	3.459	No	No
3,5-dicaffeoylquinic acid	No	0.393	2.643	4.374	No	No
3,5-dicaffeoylquinic acid methyl ester	No	0.435	2.475	3.568	No	No
4-O-caffeoylquinic acid	No	-0.002	2.073	3.643	No	No
5-O-caffeoylquinic acid	No	-0.134	1.973	2.982	No	No
Z-1,6-dioxaspiro[4,4]non-3-ene	No	0.162	1.464	1.723	No	Yes
E-1,6-dioxaspiro[4,4]non-3-ene	No	0.162	1.464	1.723	No	Yes

Compounds	Ames-toxic?	Max. human dose (log/mg/kg BW/d)	LD_{50} (mol/kg)	LOAEL (log/mg/kgBW/d)	Hepatotoxic?	Skin sensitizer?
Lupeol	No	-0.502	2.563	0.890	No	No
α-Terpineol	No	0.886	1.923	1.945	No	Yes
Borneol	No	0.577	1.707	1.877	No	Yes
Bornylacetate	No	1.080	2.463	1.617	No	No
Camphor	No	0.473	1.653	1.981	No	Yes
Cis-sabinol	No	0.429	1.565	1.817	No	Yes
Thujone	No	0.493	1.618	1.934	No	Yes
Thymol	No	1.007	2.074	2.212	Yes	Yes
1.8-Cineole	No	0.553	2.010	2.029	No	Yes
Uracil	No	1.247	1.837	2.572	No	No
Uridine	No	1.251	1.904	3.246	No	No
Thymidine	No	1.079	2.054	2.762	Yes	No
Guanosine	No	0.198	2.375	3.006	Yes	No
Adenosine	No	0.848	1.864	3.366	No	No
Bis(2-ethylhexyl)phthalate	No	1.393	1.451	2.535	No	No

***In silico* toxicity prediction of metabolites compound derived from *Chrysanthemum indicum* L. flower**

An i*n silico* toxicity prediction was conducted using the pkCSM website to assess the potential toxicity profile of the compounds that may be present within the *Chrysanthemum indicum* L. flower ethanolic extract. This analysis provided several parameters: Ames-toxicity to evaluate mutagenic potential, maximum human dose to estimate the maximum tolerable dose, LD_{50} to determine acute toxicity in rats, LOAEL to determine acute toxicity in rats, hepatotoxicity to predict liver damage, and skin sensitization to assess skin allergenic potential. Skin sensitization represents the most critical toxicity parameter for topical applications, as it directly assesses the potential for a compound to induce allergic reactions on the skin. While parameters such as Ames toxicity, maximum human dose, LD_{50}, LOAEL, and hepatotoxicity are essential for assessing overall chemical safety, their direct relevance to topical use is less considered. However, it is crucial to recognize that factors like cumulative exposure, individual skin permeability variations, and formulation properties can influence systemic absorption, even if it is generally limited. Therefore, a comprehensive safety assessment should consider all relevant toxicity parameters to ensure the long-term safety of topical products.

The findings of this study indicate that the majority of metabolite compounds derived from *Chrysanthemum indicum* L. flower were non-toxic. Nevertheless, 5 out of 76 compounds were predicted Ames-toxic (Arteglasin A, Cumambrin A, Matricarin, 11,13-dehydrodesacetylmatricarin, Chrysophanol). 4 out of 76 compounds were predicted hepatotoxic (Zhebeiresinol, Thymol, Thymidine, Guanosine), and 17 out of 76 compounds were expected to be skin sensitizer (Chrysanthemol, Chrysantherol, Clovanediol, Indicumenone, Intermedeol, Ligucyperonol, Oplopanone, Z-1,6-dioxaspiro[4,4]non-3-ene, E-1,6-dioxaspiro[4,4]non-3-ene, α-Terpineol, Borneol, Camphor, Cis-sabinol, Thujone, Thymol, 1,8-Cineole). On the other hand, the maximum human dose ranged from-1.001 to 1.393 log/mg/kg BW/day, LD_{50} ranged from 1.451 to 3.194 mol/kg (Class 4 to 5 toxicity), and LOAEL ranged from 1.259 to 4.481 log/mg/kg BW/day [17, 18]. However, further research is required to confirm the *in silico* prediction result and to determine the amount of each metabolite compound within the extract that causes allergic reactions and toxic effects on humans. The results are shown in the following table.

Face mist evaluation

Each face mist evaluation was conducted before and after six cycles of the cycling test, which was performed by storing the face mist at 4±2 °C/50-60% RH for 24 h and then moving it to a storage place at 40±2 °C/50-60% RH for 24 h, thus one cycle occurred over two days.

Table 4: Organoleptic of the face mist preparations

Formula	Parameter	Result	
		Before cycling test	After cycling test
F0	Texture	Liquid	Liquid
	Color	Colorless	Colorless
	Aroma	Rose-scented	Rose-scented
F1	Texture	Liquid	Liquid
	Color	Yellowish-brown colored	Yellowish-brown colored
	Aroma	Rose-like scented	Rose-like scented
F2	Texture	Liquid	Liquid
	Color	Dark yellowish-brown colored	Dark yellowish-brown colored
	Aroma	Rose-scented	Rose-scented
F3	Texture	Liquid	Liquid
	Color	Dark yellowish-brown colored	Dark yellowish-brown colored
	Aroma	Rose-scented	Rose-scented

Table 5: Specific gravity of the face mist preparations

Formula	Specific gravity (g/ml)	
	Before cycling test*	After cycling test*
F0	1.0163±0.0005	1.0163±0.0005
F1	1.0229±0.0005	1.0228±0.0005
F2	1.0244±0.0005	1.0245±0.0005
F3	1.0261±0.0003	1.0261±0.0007

*Data presented is mean±SD (SD) for n=3 measurements

Organoleptic

The results revealed that all formulas were liquid and rose-scented due to the addition of the rose oil. Significant color differences were observed due to variations in concentration; the higher extract concentration resulted in a darker face mist color. No notable changes were evident following the cycling test cycles, indicating that the face mist preparations were stable during storage.

Specific gravity

An evaluation of the specific gravity of the face mist preparation is necessary to ensure its consistency, which is essential for its spray ability and may also influence the consumer experience. The specific gravity requirement for face mist is close to water's (approximately 1 g/ml). Based on the results, all formulas met the specific gravity requirement, which was higher yet similar to that of water as the solvent. However, variations were observed due to extract concentration differences. A higher extract concentration resulted in a higher specific gravity of the face mist preparation, as evidenced by the two-way ANOVA results (p-value ≤ 0.05). Furthermore, no significant changes were noted after the cycling test cycles (p-value ≥ 0.05), indicating that the specific gravity of face mist preparations remained stable during storage.

Clarity

Given its solution form, clarity is a critical factor for face mist. It serves to ensure that the ingredients are homogeneously distributed and indicate stability. The results demonstrated that all formulas met the requisite clarity criteria, demonstrating a transmittance of 90-100%. This clear appearance could be achieved due to the addition of glycerin, which facilitated the dissolving of the extract during the formulation process. The clarity of the face mist contributes to the visual appeal, indicating that this face mist preparations are well-mixed and have a homogenous mixture so that each spray will deliver a consistent amount of active ingredients to the skin. Extract concentration variations resulted in significant differences in transmittance percentage, as demonstrated by two-way ANOVA results (p-value ≤ 0.05). After the cycling test cycles, significant changes were observed (p-value ≥ 0.05), with the face mist preparations appearing slightly more transparent due to the addition of PVP K-30, which helped maintain particle dispersibility in the solution.

Table 6: Clarity of the face mist preparations

Formula	Transmittance (%)	
	Before cycling test*	After cycling test*
F0	99.67±0.02	99.70±0.01
F1	96.30±0.00	96.63±0.01
F2	94.66±0.02	94.92±0.01
F3	93.45±0.02	93.75±0.01

*Data presented is mean±SD for n=3 measurements

Table 7: Spray coverage of the face mist preparations

Formula	Spray coverage (cm)	
	Before cycling test*	After cycling test*
F0	6.80±0.10	6.77±0.12
F1	6.37±0.15	6.33±0.12
F2	6.27±0.15	6.23±0.21
F3	6.13±0.21	6.07±0.15

*Data presented is mean±SD for n=3 measurements

Spray coverage

Given the spraying method of application, it is necessary to assess the spray coverage of the facial mist preparations. The results demonstrated that all formulas met the specified spray coverage requirement, with a range of 5 to 7 cm. The results demonstrated that extract concentration variations resulted in differences in spray coverage. This was due to the increased consistency of the face mist, as evidenced by the two-way ANOVA results (p-value ≤ 0.05). The spray coverage of the face mist preparations remained stable during the storage period, as evidenced by the absence of significant changes after the cycling test cycles (p-value ≥ 0.05). Adequate spray coverage ensures that the mist is evenly distributed across the facial skin, which maximizes the product's benefits. In addition, a well-dispersed mist improves the overall consumer experience since they prefer light and refreshing products.

Table 8: Drying time of the face mist preparations

Formula	Spray coverage (cm)	
	Before cycling test*	After cycling test*
F0	6.80±0.10	6.77±0.12
F1	6.37±0.15	6.33±0.12
F2	6.27±0.15	6.23±0.21
F3	6.13±0.21	6.07±0.15

*Data presented is mean±SD for n=3 measurements

Drying time

The drying time of face mist preparations was evaluated to ensure consumer satisfaction while using the product. Face mist preparation is intended to provide instant hydration at any time. Therefore, a face mist that dries rapidly while maintaining optimal skin hydration is more likely to be perceived favorably. It should be noted that the drying time may vary depending on the individual's skin type and external factors such as temperature. In this study, drying time was tested on the author's skin with the following conditions: dry skin type at room temperature (25 °C/50-60%RH). The results demonstrated that all formulas met the requisite drying

time of less than five minutes. Extract concentration variations resulted in significant differences in drying time. The higher the extract concentration, the longer the drying time, due to the increased water content within the face mist preparation. This finding was supported by the two-way ANOVA results (p-value ≤ 0.05). No significant changes were observed following the completion of the cycling test cycles (p-value ≥ 0.05), indicating that the drying time of the face mist preparations remained consistent throughout the storage period. This finding suggests that the face mist drying time could positively impact the consumer experience.

Table 9: pH of the face mist preparations

Formula	pH	
	Before cycling test*	After cycling test*
F0	6.96±0.01	6.83±0.02
F1	5.42±0.02	5.23±0.02
F2	5.34±0.02	5.17±0.02
F3	5.09±0.02	5.03±0.02

*Data presented is mean±SD for n=3 measurements

pH

As a topical product, pH evaluation is a critical factor. The pH of any topical product must be closely aligned with the natural pH of human skin for the product to be deemed suitable for use on the skin. The results demonstrated that all formulas met the requisite pH range for safe topical application on the skin, falling within the range of 4.5 to 6.5. Extract concentration variations resulted in significant differences in pH value, with higher concentrations leading to a reduction in the pH of the face mist preparation. The results of the two-way ANOVA demonstrated that a greater number of metabolite compounds present in the extract, which exhibited acidic characteristics, influenced the pH level (p-value ≤ 0.05). Significant changes were observed following the completion of the cycling test cycles (p-value ≥ 0.05). Reduction in pH value of the face mist preparations may occur due to the possibility of degradation during storage in extreme temperatures. However, the pH of face mist preparations remained within the acceptable range for topical use. Topical products with a pH above 6.5 are considered too alkaline and have the potential to disrupt the skin barrier, leading to higher risk of acne development and bacterial growth. In contrast, products with a pH below 4.5 are too acidic potentially initiate irritation and cause skin sensitivity [25].

Table 10: Antioxidant activity of the face mist preparations

Formula		IC_{50} (ppm)	
		Before cycling test*	After cycling test*
F1		87.75±0.02	88.28±0.04
	IC_{50} increased (%)		0.60
F2		77.57±0.02	79.74±0.01
	IC_{50} increased (%)		2.80
F3		53.51±0.03	56.94±0.01
	IC_{50} increased (%)		6.41

*Data presented is mean±SD for n=3 measurements

Antioxidant activity

The results demonstrated that all formulas exhibited strong antioxidant activity and an elevated IC_{50} percentage following the cycling test. An elevated IC_{50} value signifies a decrease in the capacity of the facial mist to scavenge free radicals. However, the elevated IC_{50} value was still considered a strong antioxidant. The results demonstrated that variations in extract concentration resulted in differing outcomes. A higher extract concentration led to a reduction in the IC_{50} value of the face mist preparation, indicating that a greater number of metabolite compounds could scavenge free radicals. This finding was supported by the two-way ANOVA results, which revealed a statistically significant difference (p-value ≤ 0.05). Significant differences were observed following the completion of the cycling test cycles (p-value ≥ 0.05). The IC_{50} value of the face mist preparation was elevated due to the absence of an additional antioxidant agent. Furthermore, storage conditions, including temperature, humidity, and air, can also influence the reduction in antioxidant activity of the formulation. Such changes may be attributed to the sensitivity of the metabolite compounds in the *Chrysanthemum indicum* L. flower extract, which act as antioxidants, to environmental factors. Flavonoids, for instance, are particularly thermolabile, making them susceptible to temperature fluctuations.

This face mist preparation provided a unique skincare solution, combining the benefits of natural ingredients with a sustainability commitment compared to market products. This face mist can be marketed as a natural option by formulating *Chrysanthemum indicum* L. ethanolic extract as the main ingredient, appealing to customers seeking more natural and environmentally conscious skincare alternatives. The utilization of the extract's antioxidant properties, as indicated by the IC50 value, provides the face mist with the ability to protect the skin from environmental damage and maintain skin health. Although an *in vitro* assay, specifically a DPPH assay, was performed in this study to determine the IC_{50} value of the face mist, further *in vivo* assay is needed to evaluate its efficacy in addressing specific skin types and skin concerns. Furthermore, *in silico* prediction, as a preliminary approach, can offer preliminary insights into the potential toxicity of metabolite compounds that may be present. However, it is still important to complement this prediction with further *in vitro* and *in vivo* testing to ensure the product's safety and efficacy.

CONCLUSION

This study has demonstrated that the majority of metabolite compounds derived from *Chrysanthemum indicum* L. flower are non-toxic and may be safely utilized as active ingredients in skincare products. Nevertheless, further studies, including an in vivo study to confirm the prediction and an irritation test to ensure product safety are necessary. Additionally, the *Chrysanthemum indicum* L. flower ethanolic extract can be formulated into a face mist preparation that meets the requirements and has strong antioxidant activity. As indicated by the IC_{50} value of 87.75±0.02 ppm (F1), 77.57±0.02 ppm (F2), and 53.51±0.03 ppm (F3), variations in extract concentration influence the clarity, pH, and antioxidant activity stability of the face mist preparations.

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AUTHORS CONTRIBUTIONS

YN: conception and design of the work; AH: collecting data; EM: review of the literature and the interpretation of data. All authors discussed the results and contributed to the manuscript.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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