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Optimizing Antioxidant and Anti-Hyaluronidase Activities of Mixed *Coffea arabica*, *Centella asiatica*, and *Curcuma longa* Extracts for Cosmetic Application

Natthanan Phupaisan, Chadarat Ampasavate, Surapol Natakankitkul and Kanokwan Kiattisin *

Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand; natthanan_phu@cmu.ac.th (N.P.); chadarat.a@cmu.ac.th (C.A.); surapol.nat@cmu.ac.th (S.N.)

* Correspondence: kanokwan.k@cmu.ac.th

Abstract: *Coffea arabica*, *Centella asiatica*, and *Curcuma longa* extracts have demonstrated significant antioxidant and anti-aging activities. However, research on combining these three extracts in specific proportions to enhance their antioxidant and anti-hyaluronidase effects remains limited. Therefore, this study aimed to determine the optimal proportions of *C. arabica*, *C. asiatica*, and *C. longa* extracts to maximize their combined antioxidant and anti-hyaluronidase activities. A two-level full factorial design was used to identify the optimal concentration ratios of the mixed extracts. The results indicated that all extracts influenced antioxidant activity, with the optimal proportions of *C. arabica*, *C. asiatica*, and *C. longa* extracts being 0.5:6:2 mg/mL, respectively. In addition, all factors affected hyaluronidase enzyme inhibition, with the optimal proportions for *C. arabica*, *C. asiatica*, and *C. longa* extracts being 10:10:5 mg/mL to achieve the best inhibition. In a photostability study on individual extracts, mixed extracts, and mixed extracts combined with sodium metabisulfite and bis-ethylhexyloxyphenol methoxyphenyl triazine, it was observed that preparing the mixed extracts and adding an antioxidant and a sunscreen agent helped reduce the photodegradation of phenolic compounds in the mixed extracts. Consequently, the stabilized mixed extracts could serve as raw materials in cosmetic products.

Keywords: *Centella asiatica*; *Coffea arabica*; *Curcuma longa*; Design of experiments (DOE) experimental design; antioxidant; anti-hyaluronidase; photostability

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1. Introduction

Skin aging is characterized by dry skin, aged spots, wrinkles, and a lack of elasticity. Free radicals play a significant role in skin aging. They can damage cell membranes, lipids, proteins, and DNA. They may arise from normal metabolic processes in the body or from external sources. An imbalance between free radicals and antioxidants can lead to oxidative stress, which activates fibroblast cells and increases matrix metalloproteinase (MMP) levels. MMPs contribute to the accumulation of senescent cells, the degradation of connective tissue, and the breakdown of elastic fibers. A decrease in these proteins reduces the strength and firmness of the skin. Skin aging can be controlled in various ways, and one effective approach to slowing down the aging process is the use of natural substances [1,2]. Nowadays, many cosmetic companies are focused on plant origins because they provide high biological value and are environmentally friendly. The value of plant materials is determined by the content of biological active substances. Therefore, plant extracts are a valuable source of active substances due to the synergy of their biological activities [3]. The antioxidant properties of natural extracts can help shield the skin from both external and internal factors, such as UV radiation, pollutants, and stress, potentially reducing the formation of dark spots and wrinkles [4,5]. Hyaluronidase is an enzyme that breaks down hyaluronic acid in the skin, and its activity increases over time due to environmental stress,

UV exposure, and aging, decreasing hyaluronic acid levels, which are essential for retaining moisture and maintaining hydrated and smooth skin. As a result, increased hyaluronidase activity diminishes the skin's ability to retain moisture, leading to dryness, volume loss, and wrinkle formation [6]. Therefore, natural compounds with anti-hyaluronidase activity are also valuable as moisturizing and anti-aging agents.

Centella asiatica, *Coffea arabica*, and *Curcuma longa* are local medicinal and economic plants in Northern Thailand. *C. asiatica* is a valuable medicinal herb traditionally used for wound healing for centuries. The active substances found in *C. asiatica* are pentacyclic triterpenes such as asiaticoside, asiatic acid, madecassic acid, and madecassoside. These substances play a crucial role in the treatment of skin problems by stimulating collagen synthesis, reducing collagen breakdown, accelerating tissue regeneration, healing scars, reducing inflammation, and providing antioxidant effects [6,7]. Green *C. arabica* beans are a rich source of bioactive compounds, such as chlorogenic acid, caffeic acid, and caffeine [8]. These bioactive compounds exhibit various pharmacological activities, including antioxidant, anti-inflammatory, UV protective, antibacterial, and anti-aging effects [9,10]. *C. longa* has been used as both a spice and a medicinal plant since ancient times. Curcumin, the active compound in *C. longa*, is known for its anti-aging benefits, such as antioxidant and anti-inflammatory activities [11]. Nowadays, *C. asiatica*, *C. arabica*, and *C. longa* extracts are widely used as raw materials or active ingredients in traditional medicine, herbal remedies, and cosmetic products. However, no studies have explored using these three extracts in specific proportions to enhance their antioxidant and anti-hyaluronidase properties. In addition, previous research has reported that curcumin was unstable under UV radiation. Therefore, the objectives of this study were to identify the synergistic proportions of *C. arabica*, *C. longa*, and *C. asiatica* extracts with optimal antioxidant and anti-hyaluronidase activities. Moreover, the study investigated photostability enhancement of the mixed extracts using an antioxidant agent or a sunscreen agent to support their further application as raw materials in the cosmetic industry.

2. Materials and Methods

2.1. Materials

2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), calcium chloride ($CaCl_2$), gallic acid, hyaluronidase, hyaluronic acid, linoleic acid, tricine, and trolox were purchased from Sigma-Aldrich, Steinheim, Germany. Additionally, 95% (*v*/*v*) ethanol, absolute ethanol, and sodium metabisulfite were purchased from United Chemical & Trading Co., Ltd., Bangkok, Thailand. Aluminum chloride ($AlCl_3$), ammonium thiocyanate (NH_4SCN), ascorbic acid, ferric thiocyanate ($Fe(SCN)_3$), ferrous sulfate ($FeSO_4$), Folin–Ciocalteu reagent, sodium acetate, sodium carbonate (Na_2CO_3), and sodium chloride (NaCl) were purchased from LOBA Chemie, Mumbai, India. Asiaticoside, bovine serum albumin, chlorogenic acid, and ferrous chloride ($FeCl_2$) were purchased from Merck, Darmstadt, Germany. Bis-ethylhexyloxyphenol methoxyphenyl triazine (BEMT), butylated hydroxytoluene (BHT), titanium dioxide, and tocopherol acetate were purchased from ChanJao Longevity Co., Ltd., Bangkok, Thailand. Deionized water, di-sodium hydrogen phosphate dihydrate, dimethyl sulfoxide (DMSO), hydrochloric acid (HCl), methanol, and sodium hydroxide (NaOH) were purchased from RCI Labscan, Bangkok, Thailand.

2.2. Plant Collection and Extraction

Green *C. arabica* beans and fresh leaves of *C. asiatica* were collected from Chiang Mai province, Thailand, between April and May 2022. Botanical experts at the Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand, identified the plants by comparing them with reference specimens (voucher specimen numbers 0023340 and 0023341). All plant materials were dried at 45 °C for 72 h in a hot-air oven (BINDER GmbH, Tuttlingen, Germany). The dried plants were then ground and macerated with 95% (*v*/*v*) ethanol at room temperature for 72 h. Each sample was filtered, and the solvent was eliminated

using a rotary evaporator (Rotavapor R-300, Vacuum controller V-300, Buchi Labortechnik AG, Flawil, Switzerland). The crude extracts were stored in an amber bottle at 4 °C. Additionally, *C. longa* extract was purchased from Specialty Natural Products Co. Ltd., Chonburi, Thailand.

2.3. Determination of Antioxidant Activity

2.3.1. DPPH Radical Scavenging Assay

For all crude extracts, free radicals' scavenging activity was determined using a DPPH assay [12]. Various concentrations of each extract were dissolved in absolute ethanol, and 20 µL of each sample was pipetted into a 96-well plate. Then, 120 mM of DPPH in absolute ethanol (180 µL) was added to a 96-well plate. The plate was incubated at room temperature in the dark for 30 min. After incubation, absorbance was measured at 520 nm using a microplate reader (SPECTROstar Nano®, Ortenberg, Germany). Trolox, chlorogenic acid, asiaticoside, and curcumin were used as positive controls. The percentage of inhibition was calculated using Equation (1).

$$\% \text{ DPPH inhibition} = [(Ab_{control} - Ab_{sample})/Ab_{control}] \times 100 \quad (1)$$

where $Ab_{control}$ is an absorbance of the control, and Ab_{sample} is the absorbance of the sample.

The half-maximal inhibitory concentration (IC_{50}) of each sample was calculated.

2.3.2. Lipid Peroxidation Inhibition via Ferric Thiocyanate Assay

All extracts were evaluated for the ability to inhibit lipid peroxidation via a ferric thiocyanate assay [13]. The samples were dissolved in absolute ethanol. DI water (175 µL), 20 mM of phosphate buffer pH 7.0 (350 µL), 1.3% (*v*/*v*) linoleic acid in methanol (350 µL), and an aliquot of each sample (75 µL) were mixed with 46.35 mM of AAPH (50 µL) in a test tube. Then, the mixtures were incubated at 45 °C in a water bath (WNB 14, Schwabach, Germany) for 4 h. Next, 20 mM of $FeCl_2$ solution in 3.5% (*v*/*v*) HCl (50 µL), a 10% NH_4SCN solution (50 µL), and 75% (*v*/*v*) methanol (4.85 mL) were reacted with the sample at room temperature for 3 min. The absorbance of the sample was measured using a UV-visible spectrophotometer (UV-2600i, Kyoto, Japan) at 500 nm. Trolox, chlorogenic acid, asiaticoside, and curcumin were used as positive controls. The percentage of inhibition and IC_{50} were calculated as the same as the DPPH assay.

2.3.3. Ferric Reducing Antioxidant Power (FRAP) Assay

The ability to reduce the ferric of all extracts was evaluated using a FRAP assay [13]. A FRAP reagent was prepared by mixing 300 mM of acetate buffer at pH 3.6, 10 mM of TPTZ in 40 mM of HCl, 20 mM of ferric chloride, and DI water. Then, each sample (20 µL) was mixed with FRAP reagent (180 µL) and kept at 30 °C in the dark for 5 min. The absorbance was measured at 593 nm using a microplate reader (SPECTROstar Nano®, Ortenberg, Germany). The standard curve of ferrous sulfate was prepared, and the FRAP values were calculated. Trolox, chlorogenic acid, asiaticoside, and curcumin were used as positive controls.

2.4. Determination of Hyaluronidase Inhibitory Activity via Turbidimetric Assay

Hyaluronidase inhibitory activity was determined using the turbidimetric method with some modifications [14]. The various concentrations of extract were dissolved with DMSO. The sample (50 µL) and 1 mg/mL of hyaluronidase enzyme in 20 mM of phosphate buffer at pH 7.0 (100 µL) was mixed and incubated in a water bath at 37 °C for 10 min. Then, 0.03% hyaluronic acid in 300 mM of phosphate buffer at pH 5.3 (100 µL) were added to the mixture and incubated in a water bath at 37 °C for 45 min. After incubation, the mixture was blended with acidic albumin in acetate buffer (1000 µL) and incubated for 10 min. Then, the absorbance was measured at 600 nm using a microplate reader (SPECTROstar Nano®,

Ortenberg, Germany). Tannic acid, chlorogenic acid, asiaticoside, and curcumin were used as positive controls. The percentage of inhibition was calculated using Equation (2).

$$\% \text{ Hyaluronidase inhibition} = [(Ab_{sample})/Ab_{control}] \times 100 \quad (2)$$

where $Ab_{control}$ is the absorbance of deionized water, the hyaluronidase enzyme solution, the hyaluronic acid solution, and the acetic albumin acid solution. Ab_{sample} is the absorbance of the sample hyaluronidase enzyme solution, hyaluronic acid solution, and acetic albumin acid solution.

2.5. Determination of Optimal Proportion of Mixed Extracts Using Design of Experiment (DOE)

The optimal proportion of *C. longa*, *C. arabica*, and *C. asiatica* extracts for preparing mixed extracts was determined using a design of experiment program. A two-level factorial design was employed for this experiment. In a full factorial design, each factor can vary at two levels, so the total number of experiments is equal to 2 k, where k represents the number of factors or main effects. Each factor was set to two levels: high and low. All extract ratios determined through the DOE were then evaluated for antioxidant and anti-hyaluronidase activities using the same methods described previously.

2.6. Determination of Total Phenolic Content

The total phenolic content of the single extracts was evaluated both before the design of experiments was applied and after the mixed extracts were selected using the Folin–Ciocalteu assay [14]. Each sample was dissolved in ethanol (1 mg/mL), and 500 µL of the sample was transferred into a test tube and mixed with 2 mL of Folin–Ciocalteu reagent. Then, 75% (*w*/*v*) Na_2CO_3 (4 mL) was added and incubated at 30 °C for 30 min. After incubation, the absorbance was measured at 765 nm using a UV-vis spectrophotometer (UV-2600i, Kyoto, Japan). The total phenolic content of the single extracts and the selected mixed extracts was calculated as the gallic acid equivalent (GAE), expressed in milligrams of gallic acid per gram of sample.

2.7. Photostability Test of Mixed Extracts

The samples were divided into 5 groups as follows: single extract, mixed extracts (combinations of *C. arabica* extract, *C. longa* extract, *C. asiatica* extract in the ratios determined through the DOE), mixed extracts with antioxidant agent (0.1% BHT or 0.1% tocopherol or 0.1% sodium metabisulfite), mixed extracts with a sunscreen agent (0.1% BEMT or 0.1% titanium dioxide), and mixed extracts with both a selected antioxidant and sunscreen agents. All samples were dissolved in DMSO and added to a 6-well plate (Thermo Fisher Scientific Inc., Waltham, MA, USA). The plates were then exposed to a light cabinet equipped with a cool-white fluorescent lamp and a near-UV fluorescent lamp for 8 h [15]. Afterward, the total phenolic content in each sample was analyzed, and the percentage of reduction after 8 h was calculated.

2.8. Statistical Analysis

An analysis of variance (ANOVA) and a post hoc Tukey's range test for multiple comparisons were used for the antioxidant and anti-hyaluronidase assays. Statistical analysis and descriptive statistics, including means and standard deviations (SDs), were conducted using Statistical Package for the Social Sciences (SPSS) version 7.0. The Stat-Ease 360® Trial version was used to evaluate the significance of independent factors using ANOVA and multiple regression approaches. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Preparation of C. longa, C. arabica, and C. asiatica Extracts

C. longa extract from Specialty Natural Product was found to contain curcumin as a chemical marker [16]. Dried green *C. arabica* beans were extracted via maceration using 95% ethanol as the extracting solvent. The *C. arabica* extract was a semi-solid dark brown color extract with a unique odor. The percentage yield of the *C. arabica* extract was 4.68% (%*w*/*w*). Our previous related research showed that *C. arabica* extract contained caffeine and chlorogenic acid as chemical markers [13]. Dried *C. asiatica* leaves were also extracted by maceration using 95% ethanol, yielding a semi-solid, dark green color extract with a unique odor. The percentage yield of the *C. asiatica* extract was 1.30% (%*w*/*w*). Our previous related research showed that *C. asiatica* extract contained asiaticoside as a chemical marker [17].

3.2. Antioxidant Activity of Single Extracts

3.2.1. DPPH Radical Scavenging Assay

The results of the DPPH radical scavenging activity of each extract are shown in Table 1. When the IC_{50} values were compared, it was found that *C. arabica* extract exhibited the highest activity among extracts ($p < 0.05$), with an IC_{50} value of 0.54 ± 0.06 mg/mL. In addition, it showed equal activity to asiaticoside ($p > 0.05$). In contrast, *C. asiatica* and *C. longa* extracts presented IC_{50} values of 2.74 ± 0.04 and 0.92 ± 0.00 mg/mL, respectively. However, all extracts indicated lower radical scavenging activity than trolox, chlorogenic acid, and curcumin.

Table 1. Antioxidant activity of extracts and standards.

Sample	Antioxidant Activity		
	DPPH Assay	Lipid Peroxidation Inhibition Assay	FRAP Assay
	IC_{50} (mg/mL)	IC_{50} (mg/mL)	FRAP Value (mg Fe^{2+}/mg Extract)
C. arabica extract	0.54 ± 0.06 ^{d}	0.17 ± 0.01 ^{c}	3.24 ± 0.14 ^{e}
C. asiatica extract	2.74 ± 0.04 ^{e}	ND	0.77 ± 0.05 ^{b}
C. longa extract	0.92 ± 0.00 ^{c}	0.20 ± 0.01 ^{d}	1.36 ± 0.11 ^{c}
Trolox	0.32 ± 0.01 ^{b}	0.02 ± 0.00 ^{a}	6.51± 0.01 ^{f}
Chlorogenic acid	0.06 ± 0.00 ^{a}	ND	2.64 ± 0.00 ^{d}
Asiaticoside	0.55± 0.16 ^{d}	ND	0.57 ± 0.10 ^{a}
Curcumin	0.06 ± 0.00 ^{a}	0.15 ± 0.03 ^{b}	4.46 ± 0.00 ^{f}

Note: Each value is shown as a mean ± standard deviation (SD) in triplicate (n = 3). Superscript letters indicate significant differences ($p < 0.05$) among samples analyzed through a one-way ANOVA with multiple comparisons using the Tukey test. ND means not detected.

3.2.2. Lipid Peroxidation Inhibition Assay

When the IC_{50} values were compared, it was found that *C. arabica* extract indicated the highest activity when compared to other extracts ($p < 0.05$). As shown in Table 1, the IC_{50} value of *C. arabica* extract was 0.17 ± 0.01 mg/mL, followed by *C. longa* extract with a value of 0.20 ± 0.01 mg/mL. However, both extracts indicated lower lipid peroxidation inhibition activity than trolox and curcumin.

3.2.3. Ferric Reducing Antioxidant Power Assay

The results are shown in Table 1. *C. arabica* extract indicated the highest FRAP value among all extracts at 3.24 ± 0.14 mg Fe^{2+}/mg extract, followed by *C. longa* and *C. asiatica* extracts, which showed FRAP values of 1.36 ± 0.11 and 0.77 ± 0.05 mg Fe^{2+}/mg extract, respectively. Interestingly, *C. arabica* extract possessed a higher ferric-reducing property than chlorogenic acid and asiaticoside.

3.3. Total Phenolic Content of Single Extracts

The total phenolic content of the *C. arabica*, *C. asiatica*, and *C. longa* extracts was found to be 193.20 ± 0.10, 134.82 ± 0.07, and 161.14 ± 0.28 mg gallic acid equivalent (GAE)/g sample, respectively.

3.4. Anti-hyaluronidase Activity of Single Extracts

The results of hyaluronidase inhibitory activity are presented in Table 2. *C. arabica* extract showed the highest inhibitory activity at 37.34 ± 3.55%, which was significantly higher than *C. asiatica* extract, *C. longa* extract, chlorogenic acid, asiaticoside, and curcumin ($p < 0.05$). However, all extracts indicated lower activity than tannic acid.

Table 2. Hyaluronidase inhibitory activity of extracts and standards.

Sample (Conc. 200 µg/mL)	% Hyaluronidase Inhibitory Activity
C. arabica extract	37.34 ± 3.55^{c}
C. asiatica extract	20.33 ± 1.44^{b}
C. longa extract	16.32 ± 7.07^{ab}
Tannic acid	62.51 ± 6.70^{d}
Chlorogenic acid	15.60 ± 1.30^{ab}
Asiaticoside	14.54 ± 0.44^{a}
Curcumin	20.91 ± 4.04^{b}

Note: Each value is shown as a mean ± standard deviation (SD) in triplicate (n = 3). Superscript letters indicate significant differences ($p < 0.05$) among samples analyzed via a one-way ANOVA with multiple comparisons using the Tukey test.

3.5. Optimal Proportion of Mixed Extracts Using Design of Experiment (DOE)

The appropriate proportions of *C. arabica*, *C. asiatica*, and *C. longa* extracts for antioxidant and anti-hyaluronidase activities were studied using a design of experiments (DOE) with the Design Expert program and a two-level full factorial design. Each factor was varied at two levels: the high level was set to twice the IC_{50} value of each extract obtained from the DPPH assay, and the low level was set to the IC_{50} value of the extract used in the DPPH assay, as shown in Table 3. The concentrations of extracts used to determine the anti-hyaluronidase effect were also set to two levels: the high level was set to twice the concentration of the extract obtained from the hyaluronidase inhibition assay, and the low level was set to the concentration of the extract used in the hyaluronidase inhibition assay, as shown in Table 4.

Table 3. Factor levels used in two-level full factorial screening of antioxidant effect.

Factor	Notation	Factor Levels (mg/mL)	
		Low (−)	High (+)
C. arabica extract	A	0.5	1
C. asiatica extract	B	3	6
C. longa extract	C	1	2

Table 4. Factor levels used in two-level full factorial screening of anti-hyaluronidase effect.

Factor	Notation	Factor Levels (mg/mL)	
		Low (−)	High (+)
C. arabica extract	A	5	10
C. asiatica extract	B	5	10
C. longa extract	C	5	10

3.6. Antioxidant Activity of Mixed Extracts by DOE

The extracts were prepared according to the concentration proportions obtained from the experimental model, as shown in Table 5. The antioxidant activity was then tested using the DPPH radical scavenging assay, lipid peroxidation inhibition assay, and FRAP assay. The resulting values were analyzed to determine the effects of the extracts on antioxidant activity using the Design Expert program.

Table 5. Mass of extracts from experimental design and response of antioxidant activity.

Run Order	Factor (mg/mL)			Response		
	A	B	C	DPPH Assay (%inhibition)	FRAP Assay (mg Fe^{2+}/mg Extract)	Lipid Peroxidation Inhibition Assay (%inhibition)
1	0.5	3	1	90.10 ± 0.16	2.16 ± 0.01	60.61 ± 2.34
2	1	3	1	91.69 ± 0.36	3.43 ± 0.30	48.17 ± 1.45
3	0.5	6	1	90.76 ± 0.56	2.96 ± 0.30	54.13 ± 3.22
4	1	6	1	98.47 ± 0.43	3.76 ± 0.12	61.24 ± 3.45
5	0.5	3	2	89.18 ± 1.71	2.98 ± 0.02	64.77 ± 0.06
6	1	3	2	91.12 ± 0.67	3.21 ± 0.10	67.04 ± 0.02
7	0.5	6	2	90.98 ± 0.39	3.62 ± 0.07	67.03 ± 1.24
8	1	6	2	91.34 ± 0.51	3.67 ±0.23	61.67 ± 2.44

Note: A: *C. arabica* extract, B: *C. asiatica* extract, C: *C. longa* extract.

The results are shown in Table 6, Figure 1, and Equation (3). It was found that the concentration of *C. arabica* extract (A), *C. asiatica* extract (B), and *C. longa* extract (C) indicated a statistically significant effect on the reduction in the percentage of DPPH inhibition. However, it was found that the concentration of *C. arabica* and *C. asiatica* extracts (AB), *C. arabica* and *C. longa* extracts (AC), and *C. asiatica* and *C. longa* extracts (BC) had a mutual influence on increasing the percentage DPPH inhibition value. Interaction plots were also obtained to evaluate every two parameters' interaction effects on the percentage of DPPH inhibition. The interaction between two factors was usually confirmed in Figure 1. The effect lines showed little intersection and significant interactions between each pair of factors. However, *C. arabica*, *C. asiatica*, and *C. longa* extracts (ABC) had a mutual influence on the decrease in the percentage of DPPH inhibition values. The coefficient of determination (R-square; R^2) had a value equal to 0.9502, which can explain the change in the dependent variable of 95.02% from the analysis of variance value of the percentage of DPPH inhibition. *p*-values less than 0.05 indicate that model terms are significant.

Table 6. Regression coefficients of extracts and their effects on free radical inhibition when tested using the DPPH method.

Source	Coefficients	F Value	*p* Value
Model	104.01	43.59	<0.0001 *
A—*C. arabica*	−25.09	16.98	0.0008 *
B—*C. asiatica*	−4.75	21.93	0.0002 *
C—*C. longa*	−10.07	10.95	0.0044 *
AB	9.20	51.35	<0.0001 *
AC	16.06	17.41	0.0007 *
BC	2.94	20.97	0.0003 *
ABC	−5.13	39.87	<0.0001 *

Note: * indicates significantly different at $p \leq 0.05$ with a predictive coefficient (R^2) of 0.9502 and adjusted predictive coefficient analysis (Adjust R^2) of 0.9284.

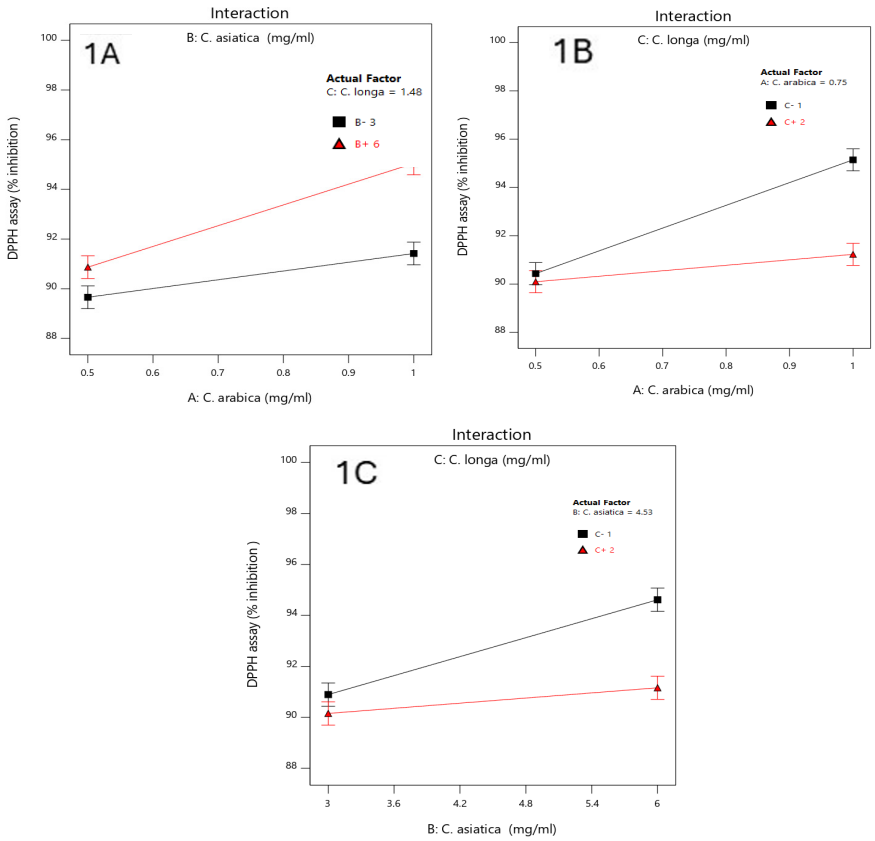


Figure 1. Effect of *C. arabica* extract, *C. asiatica* extract, and *C. longa* extract on free radical scavenging activity through DPPH method. (**1A**) represents interaction between *C. arabica* extract and *C. asiatica* extract. (**1B**) represents interaction between *C. longa* extract and *C. arabica* extract. (**1C**) represents interaction between *C. longa* extract and *C. asiatica* extract.

From the regression coefficients, Equation (3) can be created as follows.

$$\text{DPPH inhibition} = +104.012 - 25.09A - 4.75B - 10.07C + 9.20AB + 16.06AC + 2.94BC - 5.13ABC \quad (3)$$

It was found that the concentration of *C. arabica* extract (A), *C. asiatica* extract (B), and *C. longa* extract (C) affected the increase in FRAP value. In addition, *C. arabica*, *C. asiatica*, and *C. longa* extracts (ABC) had an influence on increasing the FRAP value. In contrast, the concentration of *C. arabica* and *C. asiatica* extracts (AB), *C. arabica* and *C. longa* extracts (AC), and *C. asiatica* and *C. longa* extracts (BC) had an influence on the reduction in the FRAP value, as shown in Table 7. Interaction plots were also obtained to evaluate all two-parameter interaction effects on reducing metal. An interaction between two factors was usually confirmed from Figure 2 and regression coefficients; the equation is shown in Equation (4).

From the regression coefficients, Equation (4) can be created as follows.

$$\text{FRAP value} = 3.23 + 0.2927A + 0.2786 + 0.1458C - 0.0814AB - 0.2231AC - 0.0053BC + 0.0347ABC \quad (4)$$

It was found that the concentration of *C. arabica* extract (A), *C. asiatica* extract (B), *C. longa* extract (C), and mixed extracts (ABC) had a statistically significant effect on the reduction in the percentage lipid peroxidation inhibition value. However, it was found that the concentration of *C. arabica* and *C. asiatica* extracts (AB), *C. arabica* and *C. longa* extracts (AC), and *C. asiatica* and *C. longa* extracts (BC) had a mutual influence on increasing the percentage lipid peroxidation inhibition value, as shown in Table 8 and Equation (5). The interaction between two factors was usually confirmed in Figure 3. The effect lines showed little intersection, with significant interactions between each pair of factors and regression coefficients.

Table 7. Regression coefficients of extracts and their effects on ferric reducing property when tested using the FRAP method.

Source	Coefficients	*p* Value
Model	3.23	<0.0001 *
A—*C. arabica*	0.2927	<0.0001 *
B—*C. asiatica*	0.2786	<0.0001 *
C—*C. longa*	0.1458	<0.0001 *
AB	−0.0814	<0.0001 *
AC	−0.2231	<0.0001 *
BC	−0.0053	<0.0001 *
ABC	0.0347	<0.0001 *

Note: * indicates significantly different at $p \leq 0.05$ with a predictive coefficient (R^2) of 1, and an analysis of the adjusted predictive coefficient (Adjust R^2) of 1.

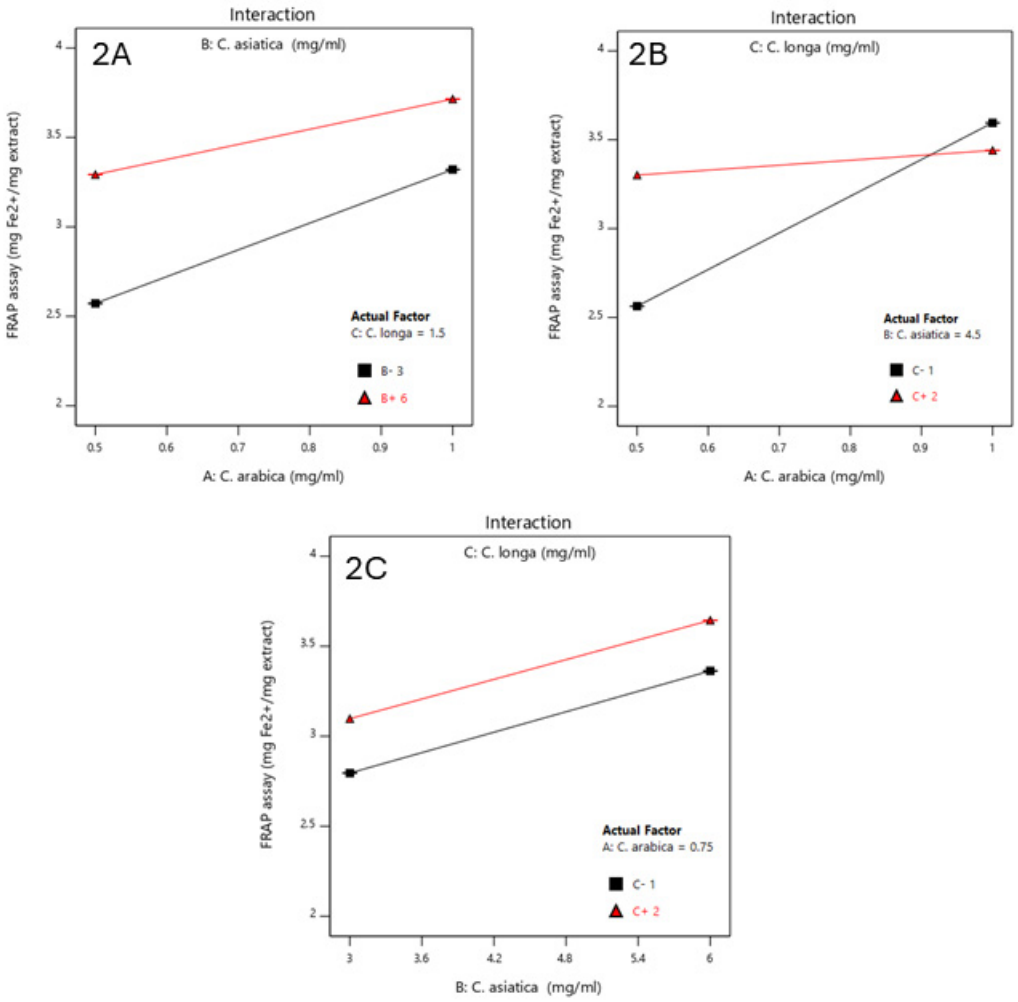


Figure 2. Effect of *C. arabica* extract, *C. asiatica* extract, and *C. longa* extract on ferric reducing property via FRAP method. (**2A**) represents interaction between *C. arabica* extract and *C. asiatica* extract. (**2B**) represents interaction between *C. longa* extract and *C. arabica* extract. (**2C**) represents interaction between *C. longa* extract and *C. asiatica* extract.

Table 8. Regression coefficients of extracts and their effects on lipid peroxidation inhibition when tested through the lipid peroxidation inhibition method.

Source	Coefficients	*p* Value
Model	60.58	<0.0001 *
A—*C. arabica*	−1.05	<0.0001 *
B—*C. asiatica*	0.4347	<0.0001 *
C—*C. longa*	4.55	< 0.0001 *
AB	1.49	<0.0001 *
AC	0.2797	<0.0001 *
BC	−1.21	<0.0001 *
ABC	−3.40	<0.0001 *

Note: * indicates significantly different at $p \leq 0.05$ with a predictive coefficient (R^2) of 1 and an analysis of the adjusted predictive coefficient (Adjust R^2) of 1.

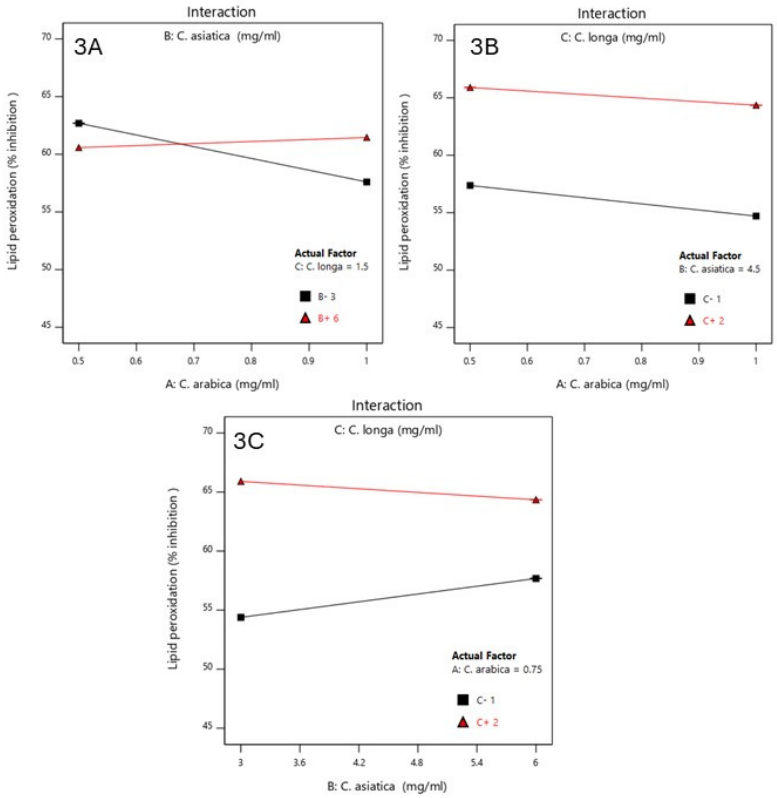


Figure 3. Effect of *C. arabica*, *C. asiatica*, and *C. longa* extracts on lipid peroxidation inhibition activity via lipid peroxidation inhibition method. (**3A**) represents interaction between *C. arabica* extract and *C. asiatica* extract. (**3B**) represents interaction between *C. longa* extract and *C. arabica* extract. (**3C**) represents interaction between *C. longa* extract and *C. asiatica* extract.

From the regression coefficients, Equation (5) can be created as follows.

$$\text{Lipid peroxidation inhibition} = 60.58 - 1.05A - 0.4347B + 4.55C + 1.49AB + 0.2797AC - 1.21BC - 3.40ABC \quad (5)$$

It was found that the actual values obtained from each test and the values from the predictions differed in the range of 1.88–4.7, as shown in Table 9, which was not more than 5%. Therefore, the results from the design of experimental can be used in practice.

Table 9. Predicted antioxidant activity values from DOE and actual antioxidant values.

	C. Arabica Extract	*C. Asiatica* Extract	*C. longa* Extract	DPPH Assay (%inhibition)	FRAP Assay (mg Fe^2+/mg Extract)	Lipid Peroxidation Inhibition Assay (%inhibition)
Model	0.5	6	2	90.98	3.62	67.03
Actual	0.5	6	2	92.86	3.28	62.33
Different				1.88%	0.38%	4.7%

3.7. Anti-hyaluronidase Activity of Mixed Extracts via DOE

Extracts were prepared according to the concentration proportions obtained from the experimental model, as shown in Table 10. It was found that the concentration of *C. arabica* extract (A), *C. asiatica* extract (B), and *C. longa* extract (C) affected the increase in the hyaluronidase inhibition percentage value. In addition, *C. arabica* and *C. longa* extracts (AC) and *C. asiatica* and *C. longa* extracts (BC), shown in Table 11, affected the increase in the percentage of hyaluronidase inhibition. Interaction plots were also obtained to evaluate every two parameters' interaction effects on the percentage of hyaluronidase inhibition that was confirmed in Figure 4. In addition, *C. arabica*, *C. asiatica*, and *C. longa* extracts (ABC) had a mutual influence on the increase in the percentage of hyaluronidase inhibition, but *C. arabica* and *C. asiatica* extracts (AB) had a mutual influence on decreasing the hyaluronidase inhibition percentage value, as shown in Equation (6).

Table 10. Mass of extracts from experimental design and response of anti-hyaluronidase activity.

Run Order	Factor (mg/mL)			Response
	A	B	C	Hyaluronidase Inhibition (%inhibition)
1	5	5	5	11.65 ± 1.97
2	10	5	5	32.29 ± 0.82
3	5	10	5	62.69 ± 9.75
4	10	10	5	32.29 ± 0.82
5	5	5	10	7.48 ± 2.23
6	10	5	10	37.56 ± 5.59
7	5	10	10	79.07 ± 1.72
8	10	10	10	83.00 ± 3.79

Note: A = *C. arabica* extract, B = *C. asiatica* extract, C = *C. longa* extract.

Table 11. Regression coefficients of extracts and their effects on hyaluronidase inhibition when tested through anti-hyaluronidase activity method.

Source	Coefficients	*p* Value
Model	43.25	<0.0001 *
A—*C. arabica*	3.03	0.0038 *
B—*C. asiatica*	21.01	<0.0001 *
C—*C. longa*	8.52	<0.0001 *
AB	−9.65	<0.0001 *
AC	5.47	<0.0001 *
BC	8.25	<0.0001 *
ABC	3.11	0.0031 *

Note: * indicates significantly different at $p \leq 0.05$ with a predictive coefficient (R^2) of 0.9243, and the adjusted predictive coefficient analyzed was (Adjust R^2) 0.8675.

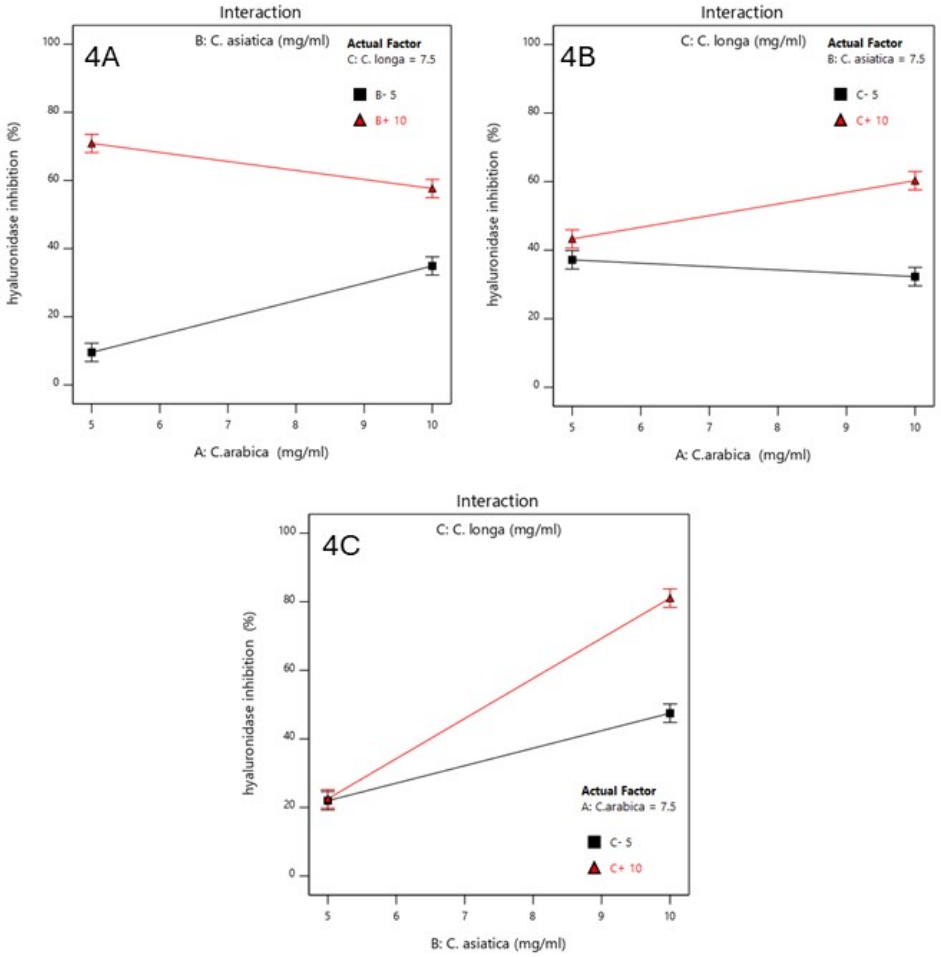


Figure 4. Effect of *C. arabica*, *C. asiatica*, and *C. longa* extract on anti-hyaluronidase activity tested via hyaluronidase inhibition method. (**4A**) represents interaction between *C. arabica* extract and *C. asiatica* extract. (**4B**) represents interaction between *C. longa* extract and *C. arabica* extract. (**4C**) represents interaction between *C. longa* extract and *C. asiatica* extract.

From the regression coefficients, Equation (6) could be created as follows.

$$\text{Hyaluronidase inhibition} = 43.25 + 3.03A + 21.01B + 8.52C \times 9.65AB + 5.47AC + 8.25BC + 3.11ABC \quad (6)$$

It was found that the actual value obtained from the test and the value from the prediction differed by 2.43%, as shown in Table 12, which was not more than 5%. Therefore, the results from this experimental design can be used in practice.

Table 12. Predicted values from hyaluronidase inhibition activity experimental designs.

	C. arabica Extract	*C. Asiatica* Extract	*C. Longa* Extract	Hyaluronidase Inhibition (%)
Model	10	10	5	83.00
Actual	10	10	5	81.04
Different				2.43%

3.8. Determination of Total Phenolic Content of Mixed Extracts

The selected proportion of *C. arabica* extract–*C. asiatica* extract–*C. longa* extract from the DOE with antioxidant activity was 0.5:6:2 mg/mL. The total phenolic content of this proportion was 196.63 ± 0.03 mg gallic acid equivalent (GAE)/g sample, as shown in Table 13. This was significantly higher than the total phenolic content of each extract at the same concentration ($p < 0.05$), which was 39.91 ± 0.03, 93.52 ± 3.55, and

96.78 ± 0.00 mg GAE/g extract, respectively. The selected proportion of *C. arabica* extract–*C. asiatica* extract–*C. longa* extract from the DOE with anti-hyaluronidase activity was 10:10:5 mg/mL. The total phenolic content of this proportion was 270.44 ± 0.24 mg GAE/g sample. This was significantly higher than the total phenolic content of each extract at the same concentration ($p < 0.05$), which was 205.90 ± 0.10, 148.12 ± 0.07, and 153.04 ± 0.08 mg GAE/g
extract, respectively.

Table 13. Total phenolic content of extracts and mixed extracts.

Sample	Total Phenolic Content (mg GAE/g Sample)	
	Concentration of Sample by DOE of Antioxidant Test	Concentration of Sample by DOE of Anti-hyaluronidase Test
C. arabica extract	39.91 ± 0.03 [a]	205.90 ± 0.10 [a]
C. asiatica extract	93.52 ± 3.55 [b]	148.12 ± 0.07 [b]
C. longa extract	96.78 ± 0.00 [c]	153.04 ± 0.08 [c]
Mixed extracts	196.63 ± 0.03 [d]	270.44 ± 0.24 [d]

Note: Each value was shown as a mean ± standard deviation (SD) in triplicate (n = 3). Superscript letters indicate significant differences ($p < 0.05$) among samples analyzed via a one-way ANOVA with multiple comparisons using the Tukey test.

3.9. Photostability Study

The samples were divided into five groups for the photostability test, as follows: single extract, mixed extracts (*C. arabica* extract, *C. longa* extract, and *C. asiatica* extract with the proportions from the DOE), mixed extracts with an antioxidant agent (0.1% BHT or 0.1% tocopherol acetate, or 0.1% sodium metabisulfite), mixed extracts with a sunscreen agent (0.1% BEMT or 0.1% titanium dioxide), and mixed extracts with both selected antioxidant and sunscreen agents.

All samples were evaluated for total phenolic content before and after the photostability study. The results are shown in Table 14. The total phenolic content of *C. longa*, *C. asiatica*, and *C. arabica* extracts at concentrations based on the antioxidant ratio (2 mg/mL, 6 mg/mL, and 0.5 mg/mL, respectively) decreased to 69.07 ± 0.00%, 44.11 ± 0.00%, and 55.58 ± 0.14%, respectively, after the photostability test. When the three extracts were mixed and tested for photostability, the results indicated that the total phenolic content of the mixed extracts was reduced to 41.61 ± 0.1%. The total phenolic content of the mixed extracts after the photostability test was significantly higher than that of the individual *C. longa* and *C. arabica* extracts ($p < 0.05$). Next, the mixed extracts were combined with BHT, tocopherol acetate, or sodium metabisulfite. The results showed that the total phenolic content decreased to 31.64 ± 0.13%, 36.39 ± 0.12%, and 17.91 ± 0.00%, respectively, after the photostability test. This suggests that all antioxidant agents have the potential to protect the degradation of total phenolic compounds in the mixed extracts. Moreover, sodium metabisulfite showed the best photoprotection among all the antioxidant agents. For the mixed extracts combined with BEMT or titanium dioxide, the percentage reduction in the total phenolic content for each combination was 2.84 ± 0.17% and 8.51 ± 0.17%, respectively.

The total phenolic content of *C. longa*, *C. asiatica*, and *C. arabica* extracts (at concentrations based on the anti-hyaluronidase ratio of 5 mg/mL, 10 mg/mL, and 10 mg/mL, respectively) decreased to 51.46 ± 0.00%, 40.45 ± 0.24%, and 48.41 ± 0.07% after the photostability test. When the three extracts were mixed and tested for photostability, the total phenolic content of the mixed extracts was reduced to 66.65 ± 0.04%, which was higher than the reduction observed in the individual extracts. The mixed extracts were then combined with BHT, tocopherol acetate, or sodium metabisulfite. The results showed that the total phenolic content decreased to 63.67 ± 0.02%, 59.02 ± 0.00%, and 58.63 ± 0.00%, respectively, after the photostability test. For the mixed extracts combined with BEMT or

titanium dioxide, the total phenolic content of each combination was 36.27 ± 0.00% and 43.98 ± 0.03%, respectively.

Table 14. Percentage reduction in total phenolic content of samples after photostability test.

Sample	% Reduction
C. longa extract (2 mg/mL)	69.07 ± 0.00 ^{p}
C. asiatica extract (6 mg/mL)	44.11 ± 0.00 ^{i}
C. arabica extract (0.5 mg/mL)	55.58 ± 0.14 ^{q}
Mixed antioxidant ratio	41.61 ± 0.11 ^{i}
Mixed antioxidant ratio with BHT	31.64 ± 0.13 ^{f}
Mixed antioxidant ratio with tocopherol acetate	36.39 ± 0.12 ^{g}
Mixed antioxidant ratio with sodium metabisulfite	17.91 ± 0.00 ^{e}
Mixed antioxidant ratio with BEMT	2.84 ± 0.17 ^{a}
Mixed antioxidant ratio with titanium dioxide	8.51 ± 0.17 ^{b}
C. longa extract (5 mg/mL)	51.46 ± 0.00 ^{k}
C. asiatica extract (10 mg/mL)	40.45 ± 0.24 ^{h}
C. arabica extract (10 mg/mL)	48.41 ± 0.07 ^{j}
Mixed anti-hyaluronidase ratio	66.65 ± 0.04 ^{o}
Mixed anti-hyaluronidase with BHT	63.67 ± 0.02 ^{n}
Mixed anti-hyaluronidase ratio with tocopherol acetate	59.02 ± 0.00 ^{m}
Mixed anti-hyaluronidase ratio with sodium metabisulfite	58.63 ± 0.00 ^{l}
Mixed anti-hyaluronidase ratio with BEMT	36.27 ± 0.00 ^{g}
Mixed anti-hyaluronidase ratio with titanium dioxide	43.98 ± 0.03 ^{i}
Mixed antioxidant ratio with BEMT and sodium metabisulfite	15.33 ± 0.00 ^{d}
Mixed anti-hyaluronidase ratio with BEMT and sodium metabisulfite	12.47 ± 0.00 ^{c}

Note: Each value was shown as a mean ± standard deviation (SD) in triplicate (n = 3). Superscript letters indicate significant differences ($p < 0.05$) among samples analyzed via a one-way ANOVA with multiple comparisons using the Tukey test.

4. Discussion

C. arabica extract, *C. longa* extract, and *C. asiatica* extract are widely used in skincare cosmetic products and are available as commercial extracts for cosmetic formulation. Additionally, various types of extracts are being incorporated into cosmetic products with the aim of providing comprehensive skincare benefits. However, there is limited research on the cosmetic effects of mixed extracts. Therefore, studying the optimal ratio of mixed extracts that provides effective cosmetic benefits is of great interest, as it could serve as valuable information for the development of future cosmetic products. In this study, *C. arabica* beans and *C. asiatica* leaves were extracted using maceration with 95% ethanol as the extracting solvent. Maceration is a conventional extraction method that is both simple and cost-effective, requiring only basic equipment [18]. Ethanol is known to be effective in extracting a wide range of phytochemicals due to its semi-polarity. It can extract high amounts of chlorogenic acid, caffeine, and other beneficial compounds [19]. Previous studies have reported that the yield of *C. arabica* extract using ethanol via maceration ranged from 9% to 16% [20]. In addition, our previous research reported that ethanolic *C. arabica* extract consisted of caffeine and chlorogenic acid [13]. The maceration of *C. asiatica* in 95% ethanol reported a significant extraction of biologically active compounds, with asiaticoside being a major component. Ethanol proved efficient in extracting both moderately polar and nonpolar compounds [21].

Changes in the skin and skin aging are caused by both internal and external factors. One factor that significantly affects skin changes is free radicals. Free radicals are often produced through biological reactions or external factors, primarily from exposure to pollution, cigarette smoke, and ultraviolet radiation, leading to oxidative damage in lipids, proteins, carbohydrates, and DNA [22]. They also stimulate the production of matrix metalloproteinases (MMP-1, MMP-3, MMP-8, and MMP-9) and activate enzymes like collagenase, elastase, tyrosinase, and xanthine oxidase, which contribute to the breakdown of collagen

and elastin, damage the dermal connective tissue, and accelerate premature skin aging. Additionally, free radicals can interact with extracellular matrix lipids, altering the epidermal barrier and leading to transepidermal water loss, resulting in skin dryness [23–25]. Therefore, natural extracts with the ability to inhibit oxidation reactions are highly effective in preventing skin problems. The antioxidant activity of all extracts was evaluated via DPPH, lipid peroxidation inhibition, and FRAP assays. Various methods of antioxidant activity tests are used to reveal the ability of the extracts to inhibit oxidation reactions through different mechanisms. The results showed that C. arabica, C. asiatica, and *C. longa* extracts exhibited good antioxidant activity, which was attributed to the presence of phenolics and flavonoids. Polyphenol compounds are well known for their antioxidant activity, protecting human, animal, and plant cells from the harmful effects of free radicals [26]. All extracts possessed DPPH a free radical-scavenging property. The antioxidant capacity of polyphenols is due to the hydroxyl (-OH) groups in their chemical structure, which can inhibit or prevent target molecules from reactive oxygen species (ROS) such as peroxides, hyperoxides, and lipid peroxyl radicals [27,28]. Additionally, a higher concentration of polyphenolic compounds correlates with increased antioxidant activity [29]. *C. arabica* extract showed outstanding antioxidant activity compared to the other extracts. There were several possible reasons why *C. asiatica* extract showed lower antioxidant activity. It contains compounds like asiaticoside, madecassoside, and other triterpenoids, which may not be as effective in scavenging lipid peroxyl radicals compared to other antioxidants like tocopherol acetate or polyphenols. These specific compounds might lack the necessary redox potential to effectively interrupt the lipid peroxidation chain reaction. Another possibility is that the concentration of bioactive compounds in the extract might be insufficient to produce a strong antioxidative effect in complex biological systems. Additionally, the bioavailability of these compounds could be limited, reducing their efficacy in preventing lipid peroxidation at the site of action [30]. The FRAP method tests antioxidant properties by donating electrons to Fe^{3+}, reducing it to Fe^{2+} [31]. All extracts contain flavonoid compounds that exhibit good ability to reduce metals, but when the effects of the three extracts were compared, *C. arabica* extract showed the highest antioxidant activity, consistent with the results of the DPPH and lipid peroxidation inhibition assays. Phenolic compounds are recognized as effective antioxidants. ROS contribute to various degenerative processes within the body [32]. Certain antioxidants can counteract the damaging effects of free radicals, helping maintain normal production of the skin's structural proteins [23]. The phenolic nucleus acts as an effective sensor for reactive species and also has the ability to reduce and chelate ferric ions, which catalyze lipid peroxidation [25,33].

Skin aging is also associated with a loss of skin moisture. The key molecule involved in maintaining skin moisture is hyaluronic acid (HA). Extracts or compounds with anti-hyaluronidase properties can serve as effective anti-aging and anti-inflammatory agents by enhancing skin elasticity and reducing wrinkle formation [34]. This effect is achieved through the inhibition of the hyaluronidase enzyme, which is linked to inflammation and the breakdown of hyaluronic acid. The primary function of hyaluronic acid is to retain moisture, ensuring that skin tissues remain well-lubricated and hydrated [35]. *C. arabica* extract exhibited higher hyaluronidase inhibition than the other extracts. Hyaluronidase is an enzyme that degrades hyaluronic acid [36], which is crucial for maintaining skin hydration and elasticity. Increased hyaluronidase activity reduces HA levels, leading to the formation of wrinkles. Chlorogenic acids and caffeine in *C. arabica* extract, as well as asiaticoside in *C. asiatica* extract, can inhibit hyaluronidase and promote Type-I collagen synthesis, contributing to improved skin elasticity and reduced signs of aging [37].

C. arabica, *C. asiatica*, and *C. longa* extracts were studied for their optimal proportions for antioxidant and anti-hyaluronidase activities using a design of experiment with a two-level full factorial model in the Design Expert program. The model indicates that DPPH radical scavenging activity was influenced by individual factors; A (*C. arabica* extract), B (*C. asiatica* extract), and C (*C. longa* extract), as well as their pairwise and three-way interactions. Positive coefficients for these interactions indicated that the concentrations

of these extracts had a statistically significant impact on DPPH inhibition. Combining these positive interactions can produce a synergistic effect, where the combined result is greater than the sum of their individual effects, as the different active compounds work together to enhance antioxidant activity [38]. The main active compounds in these extracts, such as chlorogenic acid in *C. arabica* extract, asiaticoside, and other terpenoid compounds in *C. asiatica* extract, and curcumin in *C. longa* extract, contribute to antioxidant activity by reducing free radicals and preventing oxidation [39]. The combination of curcumin from *C. longa* extract and chlorogenic acid from *C. arabica* extract, for example, can lead to a more effective antioxidant response [40]. However, the antioxidant compounds from these extracts may target different types of free radicals, and their combined effect may be distributed across these different radical types, potentially reducing the cumulative effect on DPPH free radical reduction [41]. The model presented in Table 7 indicates the effects on FRAP values. The combination of antioxidants with different chemical structures, such as polyphenols, curcumin, and triterpenoids, enables a synergistic effect in capturing multiple free radicals. This effect increases the efficiency of inhibiting oxidation reactions more than using each extract alone. However, the decrease in FRAP value may result from competition between the antioxidant compounds, leading to less efficient electron donation and a reduced reduction in ferric ions [42]. Table 8 shows the effects of individual and combined extracts on lipid peroxidation inhibition. Positive interaction coefficients (AB and AC) suggest that *C. arabica* extract enhances the effects of *C. asiatica* extract and *C. longa* extract on lipid peroxidation inhibition, likely due to complementary antioxidant mechanisms, where compounds from each extract protect lipids in different ways. For example, chlorogenic acid and curcumin can stabilize lipid bilayers [43]. However, combinations of *C. asiatica* and *C. longa* extracts (BC) and all three extracts (ABC) showed a reduction in inhibition, indicating negative interactions. The negative interaction coefficients (BC and ABC) suggest antagonistic interactions, possibly caused by competition between active compounds, reducing their ability to inhibit lipid peroxidation [44]. The combination of chlorogenic acid and curcumin, as well as asiaticoside and curcumin, can enhance hyaluronidase inhibition activity. This combination could be especially beneficial for preventing tissue degradation and inflammation [45]. Both *C. arabica* and *C. asiatica* extracts contain bioactive compounds that contribute to their ability to inhibit hyaluronidase. However, these extracts may work through different biochemical pathways [46]. The synergistic action of these compounds can result in greater hyaluronidase inhibition compared to using individual extracts.

Previous research has reported that curcumin is unstable under UV radiation [16]. Mixed extracts tend to be unstable when exposed to light. Therefore, a photostability study of mixed extracts with stabilizing agents is essential. The results showed that sunscreen agents effectively protected the mixed extracts from the degradation of total phenolic content. BEMT, in particular, demonstrated better protection compared to titanium dioxide. When the mixed extracts were combined with BEMT and sodium metabisulfite, the reduction in total phenolic content was less than that observed in individual extracts, mixed extracts, or mixed extracts with antioxidants alone. This indicates that the combination of sunscreen and stabilizing agents significantly improved the photostability of the mixed extracts. However, the total phenolic content was reduced more in the mixed extracts with a sunscreen agent alone. From the results mentioned above, it can be concluded that both antioxidants and sunscreen agents effectively help reduce the degradation of total phenolic compounds in the extracts. Total phenolic compounds in each extract showed a greater decrease compared to the other groups. *C. longa* extract exhibited more light degradation than *C. asiatica* extract and *C. arabica* extract because curcumin is sensitive to UV light. The sensitivity of curcumin is related to the presence of aromatic rings on both sides of its structure, which are particularly susceptible to UV degradation [47]. After UV exposure, the structure of curcumin changes into a curcumin free radical. This free radical captures a hydrogen atom from the normal curcumin molecule, which leads to the deterioration of the substance [48]. In contrast, mixed extracts combined with antioxidant or sunscreen agents can maintain higher total phenolic content. Sodium metabisulfite enhances the stability of

active substances by binding and inhibiting the reaction of free radicals. While antioxidants help prevent the formation of curcumin free radicals, they are not strong enough when used alone. Sunscreen agents, particularly BEMT, show a strong effect on improving photostability after UV exposure. BEMT functions primarily as a chemical sunscreen agent, protecting compounds against UVA/UVB rays, especially in the UV-A spectrum (290–370 nm). Additionally, it is highly stable under sunlight and effective over time, preventing the breakdown of phenolic compounds due to UV exposure [49]. Therefore, combining extracts with antioxidants and sunscreen agents can more effectively increase the stability of total phenolic content than using antioxidants alone. This combination provides enhanced protection against degradation caused by UV exposure, ensuring a better preservation of the bioactive compounds in the extracts.

5. Conclusions

This study investigated the optimal proportion of *C. arabica*, *C. asiatica*, and *C. longa* extracts with antioxidant and anti-hyaluronidase effects using two-full factorial levels from the Design Expert program. The results indicated that the proportions of *C. arabica*, *C. asiatica*, and *C. longa* extracts that achieved the highest antioxidant activity was 0.5:6:2 mg/mL. Increasing the concentrations of *C. arabica* and *C. longa* extracts showed the main influence on the free radical scavenging activity via a DPPH assay. *C. arabica* extract had the most significant influence on increasing the FRAP value, followed by *C. longa* extract and *C. asiatica* extract. In terms of inhibiting lipid peroxidation, *C. arabica* and *C. longa* extracts were the main influencing factor for the increase in the percentage of lipid peroxidation inhibition. For the anti-hyaluronidase effect, it was found that the proportion of *C. arabica*, *C. asiatica*, and *C. longa* extracts that achieved the highest hyaluronidase inhibition percentage value was 10:10:5 mg/mL. Increasing the concentration of *C. arabica* extract exhibited the main influence on the higher percentage of enzyme inhibition, followed by the combined influence of *C. asiatica* and *C. longa* extracts. Regarding the photostability test, the results showed that the mixing of sodium metabisulfite and BEMT with the mixed extracts can increase the stability of total phenolic compounds. All results indicate that the mixed extracts exhibited antioxidant activity and hyaluronidase inhibitory activity with good photostability for further use as a raw material in cosmetic products.

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