https://doi.org/10.48048/tis.2025.9261

Mimusops elengi Flower Hydroethanolic Extract as A Safe Antioxidant Ingredient for Cosmeceutical Applications

Wannisa Keawbankrud[1], Surachai Techaoei[2], Suradwadee Thungmungmee[2], Khemjira Jamkom[2], Siriphatr Chamutpong[2], Patamaporn Rachawat[2], Phitchan Sricharoen[3], Nattharika Pheedee[4], Saowanee Phetkeereerat[4], Siriwimon Seekhaw[4], Rawisara Pittayanon[2] and Wongnapa Nakyai[2,*]

[1]Health Science and Aesthetic Program, Department of Science, Faculty of Science and Technology, Rajamangala University of Technology Krungthep, Bangkok 10120, Thailand
[2]Faculty of Integrative Medicine, Rajamangala University of Technology Thanyaburi, Pathum Thani 12130, Thailand
[3]Division of Health, Cosmetic and Anti-Aging Technology, Faculty of Science and Technology, Rajamangala University of Technology Phra Nakhon, Bangkok 10800, Thailand
[4]Department of Chemistry, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand

([*]Corresponding author's e-mail: wongnapa_n@rmutt.ac.th)

Received: 2 November 2024, Revised: 18 December 2024, Accepted: 25 December 2024, Published: 20 January 2025

Abstract

Natural phenolic antioxidants have gained increasing attention for their safety and effectiveness in cosmeceutical products. Among these, the flowers of *Mimusops elengi* (*M. elengi*), traditionally used in Thai medicine, are known for their rich content of phenolic and terpene compounds, which contribute to their therapeutic potential. This study investigates the antioxidant activity and skin safety of *M. elengi* flower hydroethanolic extract (MEFE) for cosmeceutical applications. The MEFE was prepared through maceration, and the total phenolic content was measured using the Folin-Ciocalteu method. FT-IR and UV-VIS absorption spectroscopy were employed to confirm the presence of phenolic functional groups. Antioxidant activities were tested using DPPH, FRAP, ABTS, and NO assays to assess the MEFE's radical scavenging efficiency. In addition, cytotoxicity assays on human skin cells, including keratinocytes (HaCaT), fibroblasts, and melanocytes (B16F1), were conducted to ensure safety. A body serum lotion containing the 2.00 % w/w MEFE was formulated and evaluated for stability. Results showed that the extract contained phenolics 77.02 ± 0.01 mg GAE/g and exhibited strong antioxidant activities with IC_{50} values of 33.16 ± 1.04, 4.16 ± 1.06, 132.84 ± 1.05, and 293.10 ± 1.03 µg/mL for DPPH, FRAP, ABTS, and NO assays, respectively. Cytotoxicity testing confirmed low toxicity, with IC_{50} values of 483.28 ± 31.56 µg/mL for keratinocytes, >1,000.00 µg/mL for fibroblasts, and 630.51 ± 17.59 µg/mL for melanocytes. The 2.00 % MEFE body serum lotion demonstrated good physical stability, suggesting that the MEFE is a promising ingredient for skincare products. Therefore, further studies are needed to identify the phytoconstituents in the MEFE, and explore the molecular mechanisms by which the MEFE inhibits skin aging from oxidative stress. Additionally, clinical trials are needed to evaluate its overall safety through a skin irritation test and to assess its efficacy in the 2.00 % MEFE body serum lotion.

Keywords: *Mimusops elengi*, Phenolics, Free radical scavenging, Skin cells, Cytotoxicity, Cosmeceuticals, Stability test

Introduction

Endogenous reactive species, consisting of reactive oxygen species (ROS) and reactive nitrogen species (RNS), are produced by the mitochondria of skin cells, while exogenous free radicals can be generated by external factors such as UV radiation, atmospheric pollution, and smoking. These reactive molecules can be

neutralized by an antioxidant defense system composed of detoxifying enzymes such as the 1st-line group superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), as well as compounds like tocopherols, ascorbic acid, and glutathione (GSH). An overproduction of ROS/RNS disrupts the homeostatic balance, leading to oxidative stress in the skin [1]. In order to promote redox homeostasis of the skin, phytoconstituents, especially phenolic compounds, can quench ROS/RNS. These compounds are useful for regulating anti-aging and anti-melanogenesis processes, which involve the ROS/MAPK/AP-1 cascade. This cascade ultimately leads to the production of melanin pigment and the enzyme matrix metalloproteinases (MMPs) [2-4]. These enzymes destroy the extracellular matrix proteins, which causes a phenotype of skin aging.

M. elengi dried flowers have been documented to be a rich source of natural phytoactive compounds, including phenolics such as gallic acid, chlorogenic acid, protocatechuic acid, and rutin, as well as triterpenoids like β-sitosterol [5-7]. These bioactive compounds have been found to decrease nitrative and oxidative stress levels, which are associated with their neuroprotective properties [5,6]. Additionally, *M. elengi* flowers exhibit various pharmacological properties, including wound healing, cognitive enhancement, and anti-tyrosinase activity [7-9].

Many studies have revealed the phytochemical profile and diverse pharmacological activities of *M. elengi* extract. However, this current study aimed to evaluate the antioxidant activities and cytotoxic concentration of the MEFE and develop a stable body serum lotion containing the MEFE. The total phenolic content of the MEFE was quantified using the Folin-Ciocalteu method and the presence of phenolic compounds was further analyzed through FT-IR and UV-VIS absorption spectroscopy. Free radical scavenging abilities were evaluated using various *in vitro* assays, including DPPH, FRAP, ABTS, and NO assays. The cytotoxic effects of the extract on skin cells, including keratinocytes, fibroblasts, and melanocyte B16F1, were assessed. Additionally, the developed cosmeceutical body serum lotion product containing the 2.00 % MEFE was tested under accelerated condition. The findings highlight the potential of the MEFE as a source of natural phenolic antioxidants and its possible applications in cosmeceutical products.

Materials and methods

Preparation and extraction of plant material

M. elengi flowers were obtained from Chaokrompoe herbal store in Bangkok, Thailand, in October 2020. This shop is registered under Thai FDA, department of public health. The flowers of this plant were identified by Assist. Prof. Dr. Pranee Nangngam, an expert in plant botany, Department of Biology, Faculty of Science, Naresuan University. A voucher specimen (PNU 6010) was deposited at the PNU herbarium, Department of Biology, Faculty of Science, Naresuan University, Thailand. The air-dried flowers were ground into a powder and then extracted using the maceration method with 95 % (v/v) ethanol at a ratio of 1:10 for 72 h at room temperature. The extraction process was repeated for 3 cycles. The collected extract solution was then filtered to remove the marc. The extract solution was evaporated using a rotary evaporator under vacuum at 45 °C. The concentrated extract was lyophilized to eliminate any residual solvent, and the crude extract was kept in the freezer at −20 °C for further studies.

Phytochemical screening

Evaluation of total phenolic content

The total phenolic content of the MEFE was determined using the Folin-Ciocalteu method according to a previous study [10]. A combined mixture of deionized water, the MEFE solution and Folin-Ciocalteu reagent at ratio 130:10:10 was mixed. A neutralization step was then performed by adding 100 µL of a 7 % (w/v) sodium carbonate solution to the mixture. The absorbance was measured at 734 nm using a microplate spectrophotometer (Sunrise; Tecan, Mannendorf, Switzerland) after incubating the reaction mixture in the darkness at room temperature for 30 min. Total phenolic content was quantified as milligram gallic acid equivalent per gram of extract (mg GAE/g dry weight) using a standard curve.

Spectroscopic studies of the MEFE

IR spectrum of the MEFE was studied using the PerkinElmer Frontier FT-IR Spectrometer with UATR (PerkinElmer, Canada). The MEFE was placed directly onto the surface of the UATR crystal then the spectrum was collected from 4,000 to 650 nm^{-1} with resolution of 4 cm^{-1}.

UV-Visible absorption study of the MEFE was conducted using the Shimadzu UV-1800 UV Spectrophotometer (Shimadzu, Japan). The MEFE was dissolved in ethanol and filtered through 0.45 µM nylon membrane. The absorption spectrum of the MEFE solution was scanned over a wavelength range of 200 - 800 nm using ethanol as a blank.

Evaluation of antioxidant activities

DPPH radical scavenging assay

The antioxidant potential of the MEFE was tested using the DPPH free radical-scavenging assay [10]. Various concentrations of the MEFE were dissolved in ethanol, and a 0.2 mM DPPH solution was prepared. Then, 75 µL of the MEFE solution was mixed with 150 µL of the DPPH solution. The reaction was conducted in the dark for 30 min at room temperature. Subsequently, the absorbance of the mixture was measured at 517 nm using a microplate spectrophotometer. Ethanol was served as the blank and L-ascorbic acid was used as the positive control. The DPPH radical-scavenging activity was then calculated using the following equation:

$$\% \text{ free radical scavenging activity} = [1 - (A/B)] \times 100 \quad (1)$$

where A is the absorbance of the DPPH solution mixed with the extract or L-ascorbic acid, and B is the absorbance of the DPPH solution mixed with ethanol (blank).

ABTS radical cation decolorization assay (ABTS assay)

The ability to scavenge ABTS radical cations ($ABTS^{\bullet+}$) of the MEFE was assessed following the method described by a previous study [10]. Firstly, 2.45 mM potassium persulfate was mixed with 7 mM ABTS solution in the ratio of 1:1. This mixture was incubated in the dark at room temperature for 18 h to generate free radical $ABTS^{\bullet+}$. The $ABTS^{\bullet+}$ free radical solution was then diluted with ethanol to achieve an absorbance of 0.70 ± 0.02 at 750 nm. For the ABTS assay, 200 µL of $ABTS^{\bullet+}$ solution was mixed with 20 µL of the MEFE. The reaction mixture was kept in the dark at room temperature for 10 min, and the absorbance was measured at 734 nm using a microplate reader. L-ascorbic acid was used as a positive control. The percentage of $ABTS^{\bullet+}$ decolorization activity was calculated as described for the DPPH assay.

Ferric reducing antioxidant power assay (FRAP assay)

The FRAP assay was performed in accordance with the standard method outlined by a previous study [10]. The FRAP solution was prepared in a 10:1:1 ratio, comprising 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) solution, and 20 mM ferric chloride. Subsequently, 200 µL of the FRAP solution was mixed with 20 µL of the MEFE. The reaction mixture was incubated at 37 °C for 30 min and the absorbance was immediately measured at 570 nm. L-ascorbic acid was used as a positive control. The FRAP value was then calculated as described for the DPPH assay.

Nitric Oxide (NO) radical scavenging assay

The NO scavenging effects of the extract were determined using a method described in a previous study [10]. Briefly, 60 µL of the MEFE at the various concentration was combined with 60 µL of 10 mM sodium nitroprusside (SNP) in phosphate-buffered saline buffer. The mixture was left in the dark for 120 min at room temperature. After incubation, 120 µL of Griess reagent was added and further incubated at room temperature for an additional 10 min. Then the absorbance was measured at 550 nm. L-ascorbic acid was used as a positive control. The percentage of NO radical scavenging activity was calculated as described for the DPPH assay.

Evaluation of cytotoxicity to skin cells

Cell culture

Keratinocytes (HaCaT) and melanocytes (B16F1) were maintained in DMEM-high glucose, while primary fibroblasts were maintained in DMEM-low glucose. Both media contained 10 % fetal bovine serum, 1 % pen-strep and 0.4 % fungizone. The cells were incubated at 37 °C in a humidified atmosphere with 5 % CO_2. When the cells reached approximately 80 % confluence, the medium was discarded, and the cells were rinsed with PBS. Cells were then trypsinized with 0.25 % Trypsin/EDTA, resuspended in fresh medium, and transferred to a 96-well plate.

Cell viability assay

Skin cells were seeded into 96-well plates at a density of 1×10^4 cells/well. After a 24-h incubation, the medium was replaced with the MEFE at concentrations ranging from 10 - 1,000 µg/mL and incubated for another 24 h. The control group consisted of cells treated with 0.1 % DMSO. The cells were rinsed with PBS, and then 200 µL of MTS solution was added to each well. After incubation for 4 h, cell viability was assessed by measuring the absorbance at 493 nm using a UV/Vis microplate spectrophotometer. Each extract concentration was tested in triplicate, and results were reported as a percentage of the control values [11].

Formulation of body serum lotion

The base formulation for the body serum lotion was prepared using a cool process. Ingredients including deionized water, sodium polyacrylate, jojoba oil, capric/caprylic triglyceride, butylene glycol, isopropyl myristate, isododecane, glyceryl citrate/lactate/linoleate/oleate, disodium EDTA, rice bran oil, propylene glycol, caprylhydroxamic acid, 1,2-hexanediol, and tocopheryl acetate were combined and homogenized until a uniform lotion was achieved. The homogeneity of the lotion base was evaluated by centrifugation at 3,500 rpm for 30 min. The stable formulation was then mixed with the 2.00 % MEFE. The physicochemical properties of the body serum lotion containing the MEFE and the lotion base were characterized, including color, odor, homogeneity, texture, pH, and viscosity. The specified characteristics of the final formulation include a slightly brown, homogeneous appearance, a viscosity range of 3,000.00 to 5,000.00 cP, and a pH between 5.00 - 6.00.

Stability study

The stability of body serum lotion containing the 2 % MEFE was conducted under accelerated condition using the heating/cooling cycling method (45 °C for 24 h alternated with 4 °C for 24 h, 1 cycle) for 6 cycles. The physicochemical properties were evaluated [12].

Statistical analysis

Data values are presented as means ± SD. Statistical significance was determined by Student's unpaired *t*-test, with $p < 0.05$ analyzed as significant.

Results and discussion

Extraction

The powdered *M. elengi* flowers underwent hydro-ethanolic maceration, yielding a crude extract with a 15.59 % yield. The MEFE appeared as a viscous dark brown liquid with a characteristic herbal scent. Ethanol, used as the solvent, effectively extracts a wide range of plant metabolites, encompassing both polar and non-polar compounds. Additionally, it is recognized for its low toxicity and minimal environmental impact, making it a green extraction method ideal for cosmetic applications and supporting the trend towards eco-friendly and natural ingredients. This approach reflects the principles of sustainable extraction, which prioritize safety and efficacy [13].

Identification of the phenolic bioactive compounds and antioxidant activities

Free radicals, including ROS and RNS, such as singlet oxygen ($^1O_2$), superoxide anion ($^{\bullet}O_2^-$), hydroxyl radical ($^{\bullet}HO$), peroxyl radicals ($ROO^{\bullet}$), and nitric oxide (NO), are subsequently generated from the oxidation of the skin's extracellular matrix (ECM), lipids, and proteins [14]. These free radicals can contribute to skin aging and hyperpigmentation by initiating oxidative stress pathways that alter cellular structures and functions [14,15]. Antioxidants are critical in counteracting this oxidative damage, and their effectiveness can be measured through multiple *in vitro* assays. The DPPH and ABTS assays evaluate antioxidant potency via a mixed mode involving both hydrogen atom and electron transfer mechanisms, while the FRAP assay measures the 1-electron reduction of the ferric iron (Fe^{3+}) complex to the more stable ferrous iron (Fe^{2+}) form in the presence of antioxidants to analyze the total antioxidant capacity of the plant extracts [16]. Additionally, NO can react with $^{\bullet}O_2^-$ to form the highly reactive peroxynitrite ($ONOO^-$) molecule, which leads to harmful effects in the skin, such as DNA damage, lipid peroxidation, and protein nitration [17]. The Griess reagent can be used to detect and quantify the levels of nitric oxide, providing insights into potential oxidative stress and inflammatory conditions [18]. The total phenolic content and the antioxidant activities of the MEFE are shown in **Table 1**. This study demonstrated that the MEFE had a total phenolic content of 77.02 ± 0.01 mg GAE/g of extract. Previous studies have

identified several phenolic compounds in the MEFE, including gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, rutin, luteolin 7-O-glucoside, and undatuside C. These compounds significantly contribute to its antioxidant and hyaluronidase inhibitory properties [6,19,20]. Furthermore, aromatic phenolic esters such as 2-phenyl ethyl acetate, methyl-E-cinnamate, and methyl salicylate, recognized as safe flavoring agents and widely used in the fragrance industry, have also been reported in the flowers of this plant and may enhance its antioxidant capacity [21]. The spectroscopic studies of the MEFE, including FT-IR and UV-Visible spectroscopy, were performed to support the presence of phenolic compounds in the MEFE. The obtained FT-IR spectrum presented significant absorption bands at 3307.98 cm^{-1} corresponding to the stretching vibrations of hydroxyl (-OH) groups, 2927.61 cm^{-1} relating to the stretching vibration of carbon-hydrogen (C-H) bonds, 1635.55 cm^{-1} involving carbonyl group (C=O) stretching vibration and 1036.044 cm^{-1} presenting the ether (C-O) stretching vibration band (**Figure 1**). Besides, the UV-Visible absorption spectrum of the extract showed the intense absorption band with the highest absorption at 260 nm (λ_{max}) which represented UV absorption due to a conjugated π electron system of aromatic rings (**Figure 2**). Thus, the results of both spectroscopic studies along with antioxidant activities indicated the presence of phenolic compounds in the MEFE.

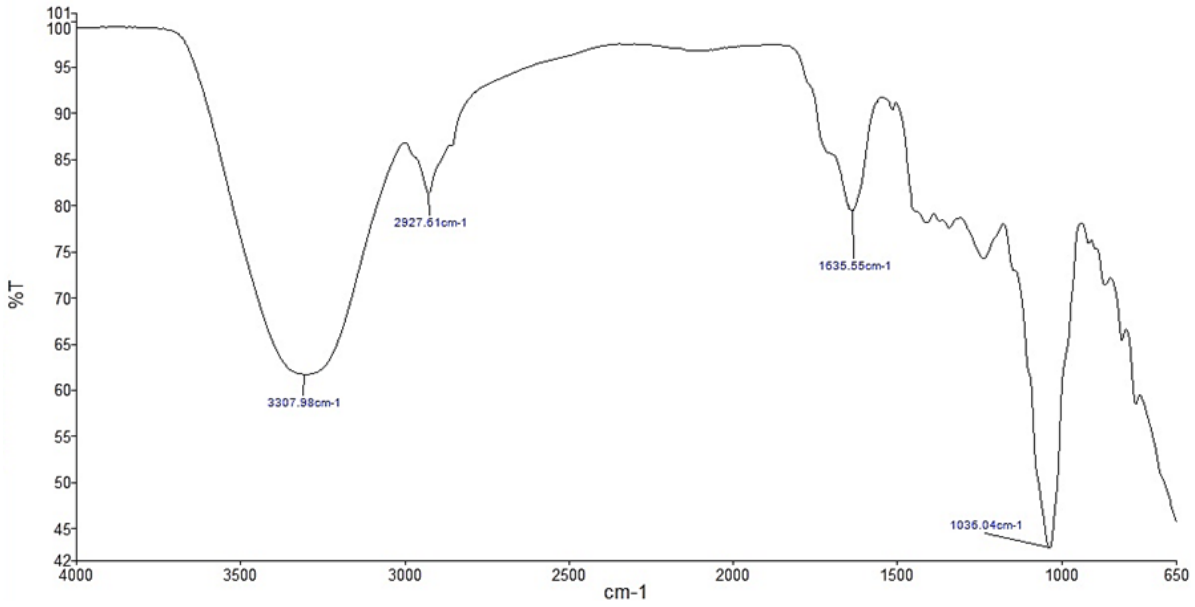

Figure 1 FT-IR spectrum of the MEFE.

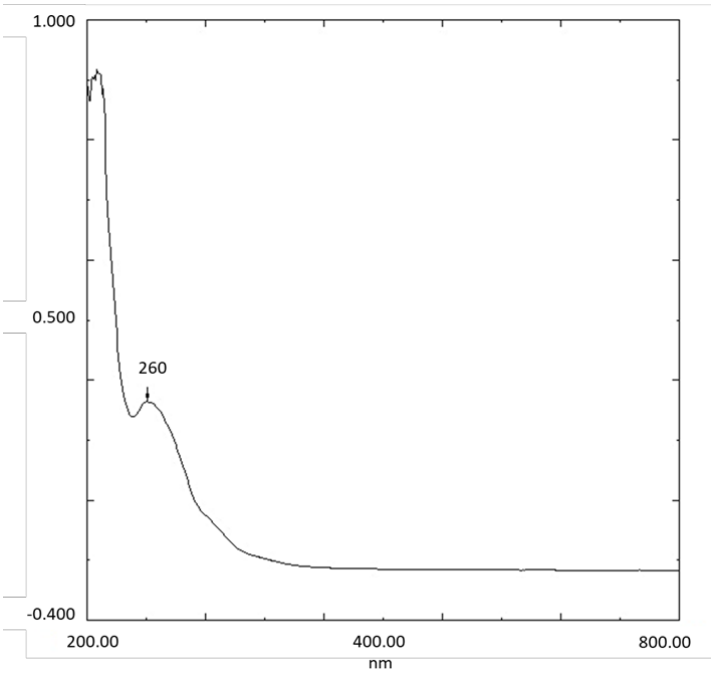

Figure 2 UV-Visible absorption spectrum of the MEFE.

The MEFE exhibited effective antioxidant activity across multiple assays, showing notable effectiveness in the ABTS assay (IC_{50} 4.16 ± 1.06 µg/mL), along with measurable activity in the DPPH (IC_{50} 33.16 ± 1.04 µg/mL), FRAP (IC_{50} 132.84 ± 1.05 µg/mL), and NO (IC_{50} 293.10 ± 1.03 µg/mL) activities. These findings indicate that the MEFE has broad-spectrum antioxidant potential, demonstrating both proton-donating and electron-transfer mechanisms [22], which could effectively neutralize free radicals and reduce oxidative stress. Nevertheless, its antioxidant potency was lower than that of the standard compound L-ascorbic acid, known for its strong antioxidant properties. In this study, the MEFE exhibited notable antioxidant properties, suggesting it could be an alternative agent to phytopolyphenol-rich plants such as mulberry (*Morus alba*), cica (*Centella asiatica*), and rosemary (*Rosmarinus officinalis*) [23-25]. The antioxidant capacity of the MEFE supports its potential use for innovative skincare applications by neutralizing free radicals, which play a major role in skin aging and pigmentation signaling cascades.

In this study, compounds belonging to the phenolic group were identified. Future research should focus on the isolation and precise characterization of these compounds. LC-MS can be utilized for both the identification and quantification of phenolic compounds in the crude extract. Additionally, NMR spectroscopy could further confirm the molecular structures of these compounds. This integrative approach would enhance our understanding of the phytochemical composition and provide valuable insights into the bioactive properties of the identified compounds, facilitating more accurate evaluations of their potential applications.

Table 1 Total phenolic content (TPC) and antioxidant activities (DPPH, ABTS, FRAP, and NO assay) of the MEFE.

Sample	TPC (mg GAE/g extract)	Antioxidant activities (IC_{50} ± SD; µg/mL)			
		DPPH	ABTS	FRAP	NO
MEFE	77.02 ± 0.01	33.16 ± 1.04	4.16 ± 1.06	132.84 ± 1.05	293.10 ± 1.03
L-ascorbic acid	-	4.51 ± 0.13	0.86 ± 1.03	2.06 ± 1.56	0.04 ± 1.04

Remark: The data values are reported as the mean ± SD. All experiments were performed in triplicate. L-ascorbic acid was employed as the positive control.

Cytotoxic effects of the MEFE on different skin cells

To evaluate the safety of the MEFE for cosmeceutical applications, its cytotoxic effects were tested on 3 key skin cell types. Keratinocytes (HaCaT) and fibroblasts, both commonly used for studies in skin aging processes, while melanocytes (B16F1**)** used for melanogenesis mechanisms, were exposed to concentrations ranging from 10.00 to 1,000.00 µg/mL of the MEFE for 24 h to assess cytotoxic effects. The results indicated that the MEFE exhibited cytotoxic effects on various skin cell types at specific concentrations. The IC_{50} values of the MEFE were 483.28 ± 31.56 µg/mL, >1,000.00 µg/mL, and 630.51 ± 17.59 µg/mL for keratinocytes, fibroblasts, and melanocytes, respectively (**Table 2**). According to the ISO 10993-5 standard, cell viability above 70 % compared to non-treated cells is considered non-cytotoxic [26]. Based on this criterion, the MEFE exhibited cytotoxicity towards keratinocytes, fibroblasts, and melanocytes at concentrations above 100.00, 500.00, and 500.00 µg/mL, respectively, as the percentages of cell viability were below 70 %. The results for skin fibroblast cytotoxicity are consistent with previously published studies, demonstrating a similar trend. The 50 % ethanol extract of *M. elengi* at 100 µg/mL exhibited cell viability of 73.23 ± 16.91 % [27]. In this study, the cell viability was 80.39 ± 6.85 %. This difference in cytotoxicity is not statistically significant. The methanol extract of *M. elengi* sepals demonstrated a dose-dependent effect, with approximately 60 % cell viability at 1,000 µg/mL [28]. The results are consistent with our data. In another study, the MEFE was incorporated into a semi-solid cream base at concentrations of 2.5, 5.0, and 20.0 %. Safety evaluations, following OECD guidelines No. 404 (skin irritation test) and No. 420 (acute oral toxicity study), showed no dermal irritation and no significant toxic effects in animal tests. The skin irritation test on female healthy rabbits using a 20 % of the MEFE showed no signs of irritation after topical application, confirming its suitability for topical use [7].

In future studies, the MEFE will be selected at the highest concentrations that do not affect cell viability to investigate its anti-aging and melanogenesis activities. The study will focus on the molecular mechanisms of the MEFE's effects on skin cells, emphasizing its ability to mitigate oxidative stress, enhance ECM integrity, and regulate pigmentation. In keratinocytes, the MEFE's modulation of ROS-driven MAPK signaling and transcription factors like AP-1 and NF-κB will be studied to clarify its role in suppressing MMPs [29]. In fibroblasts, the MEFE's potential to boost ECM protein synthesis such as collagen, elastin, and hyaluronic acid through the TGF-β/Smad pathway and its interaction with MAPK will be explored to reveal its function in preventing ECM degradation [30]. In melanocytes, the effects of the MEFE on melanogenesis will be investigated by examining its role in the α-MSH/MC1R pathway, which activates the cAMP-PKA-CREB signaling cascade. This pathway regulates microphthalmia-associated transcription factor (MITF), a key transcription factor that controls the expression of melanogenic enzymes tyrosinase (TYR), tyrosinase-related protein 1 (TRP-1), and tyrosinase-related protein 2 (TRP-2) [31]. These studies will provide valuable insights into the MEFE's potential as a multifunctional agent for diminishing oxidative damage, maintaining skin structure, and alleviating pigmentation problems.

Table 2 Skin cell viability after 24 h treatment with various concentrations of the MEFE.

Concentration (µg/mL)	% cell viability		
	HaCaT	Fibroblasts	B16F1
Untreated cell	99.31 ± 6.00	98.17 ± 4.20	116.78 ± 4.62
0.1 % DMSO	100.00 ± 14.99	100.00 ± 7.65	100.00 ± 5.09
10.00	104.45 ± 5.15	93.43 ± 8.15	125.09 ± 15.22
25.00	90.23 ± 2.21	85.07 ± 4.94*	111.16 ± 6.13
50.00	85.94 ± 1.07	82.81 ± 5.74*	113.11 ± 10.20
100.00	70.41 ± 5.46*	80.39 ± 6.85*	115.26 ± 8.75

Concentration (µg/mL)	% cell viability		
	HaCaT	Fibroblasts	B16F1
250.00	56.93 ± 8.13*	77.88 ± 5.07*	95.48 ± 5.39
500.00	43.77 ± 6.03**	73.15 ± 5.98**	69.32 ± 3.36**
1,000.00	12.71 ± 1.35***	58.92 ± 2.17***	5.29 ± 1.30***
IC_{50} (µg/mL)	483.28 ± 31.56	>1,000.00	630.51 ± 17.59

Remark: Data values are expressed as the means ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to the 0.1 % DMSO group (control).

Formulation and stability of the body serum lotion

Many studies have shown that the MEFE is compatible with both skin and gingival fibroblasts [32,33], making it suitable for use in cosmetic skincare, oral care products, and fragrance applications [34-37]. In oral care applications, extracts from the bark and leaves of *M. elengi* have demonstrated antioxidant properties and cytocompatibility with normal gingival fibroblasts [33]. A clinical study has shown that mouthwash containing aqueous extract of *M. elengi* bark acts as a plaque inhibitor and also exhibits anti-inflammatory effects against gingivitis caused by bacterial infections [35]. Additionally, another clinical study found that an 8 % *M. elengi* leaf extract incorporated into chip film is effective as an adjunct to non-surgical periodontal therapy in chronic periodontitis, particularly in reducing probing depth [36]. In topical skincare products, encapsulating the MEFE in niosomes has been shown to reduce cytotoxicity to fibroblasts while enhancing the inhibition of MMP-2 [32], as well as providing antioxidant and anti-tyrosinase activities [9,38]. Moreover, the previous study also reported its antimicrobial properties and the development of a hand cream formulation [37]. Therefore, these findings support the potential of the MEFE as a safe and effective ingredient for use in skin anti-aging and whitening products.

The use of the 2.00 % w/w MEFE in a topical emulsion dosage form provides an optimal balance between safety and efficacy. At this concentration, the lotion delivers 40 µg of the MEFE/cm^2 when applied topically at 2 mg/cm² dose, as recommended by OECD guidelines for topical products [39]. The phenolic compounds, such as gallic acid, are active components in the MEFE that act as powerful antioxidants [7,28], neutralizing free radicals on the skin's surface [40-42]. Cytotoxicity assays have demonstrated that the MEFE has an IC_{50} of 483.28 µg/mL for keratinocytes and more than 1,000.00 µg/mL for fibroblasts, ensuring that the 40 µg/cm^2 applied is far below toxic thresholds, making it safe for use. This concentration effectively provides protection against oxidative stress, which plays a significant role in skin aging. The 2.00 % w/w MEFE formulation offers enhanced antioxidant benefits while posing a low risk of irritation or adverse reactions. Further clinical testing on volunteers is recommended to confirm the product's safety and efficacy over long-term use.

The body serum lotion containing the 2.00 % MEFE was successfully formulated with a slightly brown color and a unique scent. The texture was smooth with no phase separation observed, and the formulation exhibited good homogeneity. The initial pH was 5.60 ± 0.00, and the viscosity was 2,111.50 ± 3.50 cP, both of which are suitable for skin application. The stability study was conducted under accelerated conditions using 6 heating/cooling cycles (45 °C for 24 h, alternating with 4 °C for 24 h per cycle). The physicochemical properties of the body serum lotion after the stability test were compared with the initial measurements (**Table 3**). The results indicated that the lotion maintained its slightly brown color, unique odor, and smooth texture throughout the stability test. No phase separation was observed. The pH decreased slightly from 5.60 ± 0.00 to 5.50 ± 0.00, and the viscosity showed a minor reduction from 2,111.50 ± 3.50 cP to 2,024.50 ± 2.10 cP. These minor changes in pH and viscosity remained within acceptable limits, confirming the stability of the formulation containing the 2.00 % MEFE.

A future study will conduct a clinical trial to evaluate the efficacy and safety of the 2.00 % MEFE body serum lotion. The study will employ a randomized,

double-blind, split-body, placebo-controlled design over a duration of 12 weeks [43,44]. First, a 4-h human patch test will be conducted to determine that the 2.00 % MEFE body serum lotion does not induce skin irritation. Participants will subsequently use the product in accordance with the study protocol, and its efficacy will be assessed. Skin elasticity and firmness, indicative of elastin fiber strength and collagen integrity, will be assessed using a cutometer. The effectiveness of whitening and wrinkle reduction will be assessed using Antera 3D, which utilizes high-resolution imaging and 3D modeling to investigate pigmentation distribution, wrinkle depth, and skin surface texture. Skin hydration levels and transepidermal water loss (TEWL) will be assessed to determine the lotion's impact on moisture retention and skin barrier function with a corneometer and a Tewameter, respectively. These thorough assessments will offer a deeper understanding of the lotion's potential to enhance the texture, firmness, hydration, and overall appearance.

Table 3 Physicochemical properties of body serum lotion with the 2.00 % MEFE.

Parameters	Initiation	6 Heating/Cooling cycles
Color	Slightly brown	Slightly brown
Odor	Unique scent	Unique scent
Homogeneity	Good	Good
Texture	Smooth	Smooth
pH	5.60 ± 0.00	5.50 ± 0.00
Viscosity (cP)	2,111.50 ± 3.50	2,024.50 ± 2.10

Conclusions

This study demonstrated that the MEFE, obtained through hydroethanolic maceration, contains bioactive phenolic compounds with significant antioxidant properties. The MEFE exhibited low cytotoxicity on skin cells, making it a promising candidate for cosmeceutical formulations targeting antioxidant, anti-aging, and skin-whitening effects. Additionally, the stability of the body serum lotion under stress conditions supports the feasibility of incorporating the MEFE into skincare products. Future studies should focus on conducting *in vivo* evaluations to confirm the efficacy and safety of the MEFE on human skin. Investigations into the mechanism of action underlying its anti-aging and melanogenesis activities, as well as long-term stability studies under real-world conditions, are also recommended to ensure product effectiveness and market viability.

Acknowledgements

This research was funded by the faculty of science, Ramkhamhaeng University, Thailand (Fiscal year 2021). The authors thank the faculty of science, Ramkhamhaeng University, Thailand, Health Science and Aesthetic Program, Department of Science, Faculty of Science and Technology, Rajamangala University of Technology Krungthep, Thailand and Faculty of Integrative Medicine, Rajamangala University of Technology Thanyaburi, Thailand on facility support. In addition, we would like to express our gratitude to Dr. Prapha Arnnok for kindly assisting with the FT-IR and UV-VIS spectroscopic studies, as well as for the helpful discussions.

References

[1] N Leerach, S Yakaew, P Phimnuan, W Soimee, W Nakyai, W Luangbudnark and J Viyoch. Effect of Thai banana (*Musa* AA group) in reducing the accumulation of oxidation end products in UVB-irradiated mouse skin. *Journal of Photochemistry and Photobiology B: Biology* 2017; **168**, 50-58.

[2] A Merecz-Sadowska, P Sitarek, E Kucharska, T Kowalczyk, K Zajdel, T Cegliński and R Zajdel. Antioxidant properties of plant-derived phenolic compounds and their effect on skin fibroblast cells. *Antioxidants* 2021; **10(5)**, 726.

[3] RU Nisa, AU Nisa, AY Tantray, AH Shah, AT Jan, AA Shah and IA Wani. Plant phenolics with promising therapeutic applications against skin disorders: A mechanistic review. *Journal of Agricultural and Food Research* 2024; **16**, 101090.

[4] KC Wu, YC Hseu, YC Shih, G Sivakumar, JT Syu, GL Chen, MT Lu and PC Chu. Calycosin, a common dietary isoflavonoid, suppresses melanogenesis through the downregulation of PKA/CREB and p38 MAPK signaling pathways. *International Journal of Molecular Sciences* 2022; **23(3)**, 1358.

[5] P Nagakannan, BD Shivasharan, BS Thippeswamy and VP Veerapur. Restoration of brain antioxidant status by hydroalcoholic extract of *Mimusops elengi* flowers in rats treated with monosodium glutamate. *Journal of Environmental Pathology, Toxicology and Oncology* 2012; **31(3)**, 213-221.

[6] P Nagakannan, BD Shivasharan, BS Thippeswamy, VP Veerapur and P Bansal. Protective effect of hydroalcoholic extract of *Mimusops elengi* Linn. flowers against middle cerebral artery occlusion induced brain injury in rats. *Journal of Ethnopharmacology* 2012; **140(2)**, 247-254.

[7] S Shailajan and D Gurjar. Wound healing efficacy of *Mimusops elengi* L. flowers in albino Wistar rats using excision wound model. *Pharmacognosy Communications* 2016; **6(2)**, 72-79.

[8] RV Hadaginhal, VP Tikare, KS Patil, MD Bhanushali, NS Desai and A Karigar. Evaluation of cognitive enhancing activity of *Mimusops elengi* Linn on albino rats. *International Journal of Research in Ayurveda and Pharmacy* 2010; **1(2)**, 484-492.

[9] N Narayanaswamy, S Rohini, A Duraisamy and KP Balakrishnan. Antityrosinase and antioxidant activities of various parts of *Mimusops elengi*: A comparative study. *International Journal of Research in Cosmetic Science* 2011; **1(1)**, 17-22.

[10] W Nakyai, W Pabuprapap, W Sroimee, V Ajavakom, B Yingyongnarongkul and A Suksamrarn. Anti-acne vulgaris potential of the ethanolic extract of *Mesua ferrea* L. flowers. *Cosmetics* 2021; **8(4)**, 107.

[11] S Yakaew, P Phimnuan, K Tiensomjitr, W Nakyai, N Nuengchamnong, G Ross, M Ungsurungsei and J Viyoch. Hom-Kularb-Dang rice bran extract for the prevention of UVB-damage against human skin fibroblast. *Chiang Mai University Journal of Natural Sciences* 2020; **19(1)**, 34-51.

[12] L Kirkbride, L Humphries, P Kozielska and H Curtis. Designing a suitable stability protocol in the face of a changing retail landscape. *Cosmetics* 2021; **8(3)**, 64.

[13] F Chemat, MA Vian and G Cravotto. Green extraction of natural products: Concept and principles. *International Journal of Molecular Sciences* 2012; **13(7)**, 8615-8627.

[14] F Papaccio, AD Arino, S Caputo and B Bellei. Focus on the contribution of oxidative stress in skin aging. *Antioxidants* 2022; **11(6)**, 1121.

[15] C Li, L Kuai, R Cui and X Miao. Melanogenesis and the targeted therapy of melanoma. *Biomolecules* 2022; **12(12)**, 1874.

[16] IG Munteanu and C Apetrei. Analytical methods used in determining antioxidant activity: A review. *International Journal of Molecular Sciences* 2021; **22(7)**, 3380.

[17] R Radi. Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. *Proceedings of the National Academy of Sciences of the United States of America* 2018; **115(23)**, 5839-5848.

[18] CV Suschek, T Schewe, H Sies and KD Kröncke. Nitrite a naturally occurring precursor of nitric oxide that acts like a 'prodrug'. *Biological Chemistry* 2006; **387(5)**, 499-506.

[19] S Shailajan and D Gurjar. Evaluation of *Mimusops elengi* L. flowers using pharmacognostic approach. *Pharmacognosy Communications* 2015; **5(1)**, 83-92.

[20] T Morikawa, Y Manse, M Koda, S Chaipech, Y Pongpiriyadacha, O Muraoka and K Ninomiya. Two new aromatic glycosides, elengiosides A and B, from the flowers of *Mimusops elengi*. *Journal of Natural Medicines* 2018; **72(2)**, 542-550.

[21] PK Rout, D Sahoo and LN Misra. Comparison of extraction methods of *Mimusops elengi* L. flowers. *Industrial Crops and Products* 2010; **32(3)**, 678-680.

[22] J Rumpf, R Burger and M Schulze. Statistical evaluation of DPPH, ABTS, FRAP, and Folin-Ciocalteu assays to assess the antioxidant capacity of lignins. *International Journal of Biological Macromolecules* 2023; **233**, 123470.

[23] JH Hsu, CS Yang and JJ Chen. Antioxidant, anti-α-glucosidase, antityrosinase, and anti-

inflammatory activities of bioactive components from *Morus alba*. *Antioxidants* 2022; **11(11)**, 2222.

[24] A Kandasamy, K Aruchamy, P Rangasamy, D Varadhaiyan, C Gowri, TH Oh, S Ramasundaram and B Athinarayanan. Phytochemical analysis and antioxidant activity of *Centella asiatica* extracts: An experimental and theoretical investigation of flavonoids. *Plants* 2023; **12(20)**, 3547.

[25] N Bai, K He, M Roller, CS Lai, X Shao, MH Pan and CT Ho. Flavonoids and phenolic compounds from *Rosmarinus officinalis*. *Journal of Agricultural and Food Chemistry* 2010; **58(9)**, 5363-5367.

[26] P Pimton, P Ratphibun, N Tassaneesuwan, R Chiangnoon and P Uttayarat. Cytotoxicity evaluation of hydrogel sheet dressings fabricated by gamma irradiation: Extract and semi-direct contact tests. *Trends in Sciences* 2022; **19(13)**, 4583.

[27] B Kietthanakorn, W Ruksiriwanich, W Manosroi, J Manosroi and A Manosroi. Biological activities of supercritical carbon dioxide fluid ($scCO_2$) extracts from medicinal flowers. *Chiang Mai Journal of Science* 2012; **39(1)**, 84-96.

[28] K Natungnuy and S Poeaim. Antioxidant and cytotoxic activities of methanolic extracts from *Mimusops elengi* flowers. *International Journal of Agricultural Technology* 2018; **14(5)**, 731-740.

[29] A Merecz-Sadowska, P Sitarek, K Zajdel, E Kucharska, T Kowalczyk and R Zajdel. The modulatory influence of plant-derived compounds on human keratinocyte function. *International Journal of Molecular Sciences* 2021; **22(22)**, 12488.

[30] J Zhang, H Yu, MQ Man and L Hu. Aging in the dermis: Fibroblast senescence and its significance. *Aging Cell* 2024; **23(2)**, e14054.

[31] SH Naikoo, H Rashid, S Kumar, S Archoo, UA Sheikh, NA Lone, PP Singh and SA Tasduq. Inhibition of melanogenesis by 3-(1′-methyltetrahydropyridinyl)-2,4-6-trihydroxy acetophenone via suppressing the activity of cAMP response element-binding protein (CREB) and nuclear exclusion of CREB-regulated transcription coactivator 1 (CRTC1). *European Journal of Pharmacology* 2023; **952**, 175734.

[32] A Manosroi, B Kietthanakorn, C Chankhampan, N Khositsuntiwo and W Manosroi. Physical characteristics and biological activities of Thai flower extracts loaded in niosomes. *Chiang Mai Journal of Science* 2013; **40(4)**, 603-617.

[33] SK Bhavikatti, MI Karobari, SLA Zainuddin, A Marya, SJ Nadaf, VJ Sawant, SB Patil, A Venugopal, P Messina and GA Scardina. Investigating the antioxidant and cytocompatibility of *Mimusops elengi* Linn extract over human gingival fibroblast cells. *International Journal of Environmental Research and Public Health* 2021; **18(13)**, 7162.

[34] B Arifin, R Nasution, N Desrianti, M Marianne and H Helwati. Antimicrobial activity of hand lotion of flower *Mimusops elengi*. *Open Access Macedonian Journal of Medical Sciences* 2019; **7(22)**, 3748-3756.

[35] A Choudhary, CN Smitha, DK Suresh and SK Basu. Clinical evaluation of efficacy of *Quercus infectoria* and *Mimusops elengi* Linn. herbal preparation in inhibition of gingivitis. *Advances in Human Biology* 2015; **5(3)**, 68-76.

[36] AS Udhya, G Vivekanandan, GP Elangovan, S Saranya, D Surya and KK Munagala. Efficacy of locally delivered herbal chip containing (8 % *Mimusops elengi*) as an adjunct to scaling and root planing in chronic periodontitis: A randomised split-mouth clinical study. *Advances in Human Biology* 2024; **14(4)**, 357-361.

[37] R Nasution, AI Azwar, H Helwati and Marianne. Antibacterial activities of perfume: Combination flower *Magnolia alba*, *Cananga odorata* and *Mimusops elengi* L, fixed with *Pogostemon cablin* oil. *Indonesian Journal of Pharmaceutical and Clinical Research* 2019; **2(1)**, 19-23.

[38] N Koomklang, P Tangsombatvichit, S Tumthong, K Kongsong, T Nawatnatee and J Khongdetch. Antioxidant and tyrosinase inhibition activities from ethanolic extracts of *Mimusops elengi* and *Hibiscus sabdariffa* flowers and *Dillenia indica* and *Abelmoschus esculentus* fruits. *Advanced Science Journal* 2024; **24(1)**, 127-142.

[39] U Bernauer, L Bodin, Q Chaudhry, P Coenraads, M Dusinska, J Ezendam, E Gaffet, CL Galli, B Granum, E Panteri, V Rogiers, C Rousselle, M Stepnik, T Vanhaecke, S Wijnhoven, A

Koutsodimou, W Uter and NV Goetz. The SCCS Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation, 11th revision, 30 - 31 March 2021, SCCS/1628/21. *Regulatory Toxicology and Pharmacology* 2021; **127**, 105052.

[40] C Alonso, R Lucas, C Barba, M Marti, L Rubio, F Comelles, JC Morales, L Coderch and JL Parra. Skin delivery of antioxidant surfactants based on gallic acid and hydroxytyrosol. *Journal of Pharmacy and Pharmacology* 2015; **67(7)**, 900-908.

[41] RK Harwansh, R Deshmukh, VP Shukla, D Khunt, BG Prajapati, S Rashid, N Ali, GM Elossaily, VK Suryawanshi and A Kumar. Recent advancements in gallic acid-based drug delivery: Applications, clinical trials, and future directions. *Pharmaceutics* 2024; **16(9)**, 1202.

[42] A Butkeviciute, K Ramanauskiene, V Kurapkiene and V Janulis. Dermal penetration studies of potential phenolic compounds *ex vivo* and their antioxidant activity *in vitro*. *Plants* 2022; **11(15)**, 1901.

[43] CM Kavali, TQ Nguyen, AS Zahr, LI Jiang and T Kononov. A randomized, a randomized, double-blind, split-body, placebo-controlled clinical study to evaluate the efficacy and tolerability of a topical body firming moisturizer for upper arm rejuvenation. *Aesthetic Surgery Journal* 2021; **41(6)**, NP472-NP483.

[44] HS Kwon, JH Lee, GM Kim and JM Bae. Efficacy and safety of retinaldehyde 0.1 % and 0.05 % creams used to treat photoaged skin: A randomized double-blind controlled trial. *Journal of Cosmetic Dermatology* 2018; **17(3)**, 471-476.