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Development of mangiferin-loaded anisotropic emulsion for cosmetic applications: A comprehensive study on formulation, antioxidant activity, stability, and skin compatibility

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ABSTRACT

Liquid crystal (LC) emulsions offer a promising system for topical application due to their ability to enhance skin penetration and to improve skin hydration. Mangiferin, a natural polyphenolic compound found in mango leaves, possesses numerous therapeutic properties, including antioxidant and anti-inflammatory effects. This research aimed to develop mangiferin-loaded LC emulsions, focusing on optimizing the formulation, evaluating antioxidation activity, conducting stability tests, and examining skin compatibility. The antioxidant activity was investigated of mangiferin in an aqueous solution and in the developed emulsion against the free radical, 2,2′-diphenyl-2-picrylhydrazyl (DPPH). The developed emulsions containing sorbitan palmitate and sucrose palmitate with a 4:1 ratio displayed an obvious Maltese cross image when observed under polarized light microscopy. The stability results of the developed emulsion containing mangiferin showed that although the mangiferin content, as analyzed by UV absorption, gradually decreased over 90-day study period, its scavenging activity against DPPH radicals remained consistent throughout the study. Furthermore, the mangiferin-loaded emulsion enhanced skin health by increasing water content and reducing transepidermal water loss, without causing any skin irritation.

Keywords: liquid crystal emulsion; mangiferin; antioxidant activity; stability; irritation

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1. INTRODUCTION

Reactive oxygen species (ROS) and other free radicals (oxidants) are typically produced during human metabolism and are neutralized by the endogenous antioxidant system. Besides the formation of free radicals in the body, they can be increased by environmental factor stimulation such as UV radiation, pollution and

cigarette smoking. Oxidative stress describes an excess of oxidants over antioxidants in the body, which results in oxidative damage. Oxidative damage can occur to all types of biomolecules, i.e., proteins, carbohydrates, lipids and nucleic acids. It is associated with the development of several diseases, including cardiovascular diseases, cancers, diabetes, neurodegenerative diseases, immune diseases and eye diseases, which now account for a significant proportion of deaths. Antioxidants are substances that may inhibit some oxidative processes and thereby prevent or delay oxidative stress. Natural resources, especially fruits and vegetables, play an important role in the antioxidant system by providing essential antioxidants, for example, vitamin E, vitamin C, β-carotene, minerals and several polyphenolic compounds (Willcox et al., 2004). Mangiferin ($C_{19}H_{18}O_{11}$), generally called C-glycosyl xanthone, is a xanthone connected to a C-glycoside (structure shown in Figure 1); It is one of the main bioactive compounds in the leaves, barks, and peels of *M. indica* and many other plants (Yehia and Altwaim, 2023). The vast biological actions of mangiferin make it highly used in cosmetic, therapeutic, and pharmaceutical products (Telang et al., 2013). The antioxidant effect of mangiferin is more significant than the effect of vitamin C, E and carotenoid (Masibo and He, 2008), and it also has anticancer, antiviral, antiaging, antidiabetic, hepatoprotective, immunomodulatory, and analgesic effects (Imran et al., 2017).

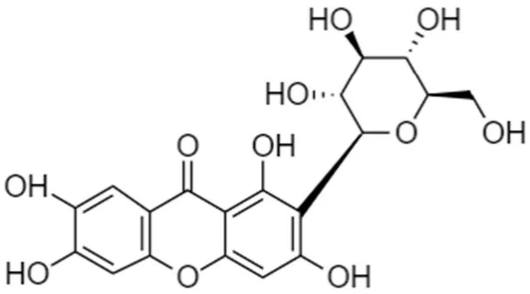

Figure 1. Chemical structure of mangiferin

A current trend in cosmetics is using the products with innovative delivery system, which includes liquid crystal emulsions. This type of emulsion exhibits mechanical properties similar to liquids while displaying optical characteristics similar to solid crystals. Like anisotropic crystalline solids, liquid crystals can display optical and electrical anisotropy and magnetic properties. The liquid crystal emulsion is a novel formula that has surfactant and oil molecules orderly arranged at the oil-water interface, resulting in better stability, better moisturizing effect, and possible control of the release (Liu and Friberg, 2009; Vilasau et al., 2011). Lamellar crystals are the most visually appealing kind of liquid crystals because their structure can mimic that of skin. Lamellar liquid crystal emulsion is the most appealing type of liquid crystal emulsion because the structure resembles the multi-layer skin structure. This emulsion type can entrap both hydrophilic and lipophilic drugs in the aqueous phase and lipid bilayers in the lamellar structure. Moreover, the liquid crystal structure can moisturize the skin (Zhang and Liu, 2013). A lamellar liquid crystal emulsion formulated using a synthetic pseudo-ceramide was found to have better skin permeability and provide more skin hydration and skin occlusion than regular emulsions. Liquid crystal emulsions increase skin hydration by acting as a skin barrier and preventing transepidermal water loss (TEWL) (da Rocha-Filho et al., 2016).

Because of the exceptional properties of liquid crystal emulsion, researchers in the pharmaceutical and cosmetic fields pay much attention to studying this unique emulsion's structure, preparation, and properties. To create liquid crystal-structured emulsions, a variety of ready-to-use surfactants, provided by different suppliers of cosmetic raw materials, are available at a high cost (Zhang and Liu, 2013). These raw materials are composed of mixed surfactants, whose exact formulations are typically not disclosed to customers. Therefore, this research aimed to develop liquid crystal emulsions incorporating mangiferin by using a combination of surfactants and to evaluate their stability and benefits for the skin.

2. MATERIALS AND METHODS

2.1 Materials

Materials used in this study were mangiferin extracted from the leaves of *Mangifera indica* L. as described in our previous study (Tessiri et al., 2021). Other materials used in liquid crystal emulsion formulations were distilled water, capric triglyceride, stearyl alcohol, guar gum, butylene glycol, and Microcare PHC. They were purchased from local distributors. Sorbitan stearate, sucrose stearate, sorbitan palmitate and sucrose palmitate were purchased from Chanjao Longevity Co., Ltd.

2.2 Liquid crystal emulsion formulations

The composition of emulsifiers in the emulsions was varied to investigate the effect of different types and ratios of emulsifiers on the liquid crystal formation. The studied emulsifiers were sorbitan stearate, sorbitan palmitate, sucrose stearate and sucrose palmitate. As our preliminary study, the developed formulations contained the emulsifier mixtures at a concentration of 6% by weight. The specific amounts of each emulsifier incorporated into the formulas were calculated based on the ratios detailed in Table 1, ensuring a total concentration of 6%. The ratios of the emulsifier mixture were systematically varied over a range until the liquid crystal structure could no longer be observed under the polarized light microscopy.

Table 1. Types and ratios of the emulsifier mixtures used in each formulation

Formulation	Type of emulsifier	Ratio
F1	Sorbitan stearate : Sucrose stearate	1:1
F2	Sorbitan stearate : Sucrose palmitate	1:1
F3	Sorbitan palmitate : Sucrose palmitate	1:1
F4	Sorbitan palmitate : Sucrose stearate	1:1
F5	Sorbitan stearate : Sucrose stearate	1:2
F6	Sorbitan stearate : Sucrose stearate	2:1
F7	Sorbitan stearate : Sucrose palmitate	1:2
F8	Sorbitan stearate : Sucrose palmitate	2:1
F9	Sorbitan palmitate : Sucrose palmitate	1:2
F10	Sorbitan palmitate : Sucrose palmitate	2:1
F11	Sorbitan palmitate : Sucrose palmitate	4:1

The liquid crystal emulsion comprises an oil phase and a water phase. The oil phase, capric triglyceride (4%), stearyl alcohol (1%) and lower HLB emulsifier (sorbitan stearate or sorbitan palmitate) were heated to 65°C. In the water phase, mangiferin (0.02%), guar gum (0.6%), butylene glycol (2%), Microcare PHC (0.5%) and emulsifier (sucrose stearate or sucrose palmitate) were dissolved in distilled water and then heated to 68°C. The oil phase was added to the water phase and stirred at an optimum speed to avoid air bubble formation until the emulsion was homogeneous and the temperature reached 35°C. The emulsions were stored in air-tight glass containers and kept at ambient temperature (27±1°C) for further evaluation.

2.3 Evaluation of emulsions

All the developed emulsions (F1–F11) were evaluated for visual appearance and sensory characteristics, including homogeneity, color, odor, ease of spreading, softness to the touch, and any residual sensation after applying to the skin. One gram of the samples was gently applied to the back of the hand, and the sensory experience was continuously evaluated until the rubbed area on the skin was completely dry. These tests aimed to assess the sensory response to the products, facilitating the primary selection of formulations for further study.

The emulsion samples were also examined under a polarized light microscope (ECLIPE 50i, Nikon Corporation, Tokyo, Japan) to investigate the formation of lamellar liquid crystals. The observed sample was prepared by smearing a pin-tip of the emulsion on the glass slide and immediately covered with the cover slip. A 40× objective lens and 10× eyepieces were employed alongside cross-polarizers to detect birefringence. The photomicrograph was captured using a camera attached to the polarized light microscope.

2.4 Stability studies

The emulsion, which displayed a clear liquid crystal structure under polarized light microscopy and exhibited good sensory characteristics, was selected for stability assessment. The stability of the selected emulsion, F11, was evaluated over 90 days at ambient air temperatures. Additionally, an accelerated stability study was performed for six cycles under the temperature cycling condition. This involved alternating storage between a laboratory room and an incubator, with temperatures fluctuating between 27±2°C and 40±2°C every 24 h for each cycle. To assess physical stability, organoleptic tests, including visual inspection for phase separation, color and odor, were conducted. Additionally, the pH values of the emulsions were measured using a pH meter. (F-20 pH meter Mettler Toledo, Switzerland).

To determine the remaining percentage of mangiferin in the studied samples, 1 g of the developed products was dissolved in ethanol and then adjusted to the final volume of 10 mL using the same solvent. The solutions were subsequently subjected to UV-vis spectroscopy analysis at a wavelength of 316 nm.

2.5 *In vitro* antioxidant activity tests

The DPPH scavenging assay of mangiferin containing in aqueous solution and developed emulsion was performed using the method described by Sithisarn et al. (2015). In a 96-well plate, 50 µL of the sample solutions containing mangiferin concentrations ranging from 0.1 to 4 mg% in aqueous solutions or at 2 mg% prepared from LC cream in water, were mixed with 200 µL of freshly prepared methanolic DPPH solution (152 µM). The mixture was incubated in the dark for 30 min at room temperature. The absorbance was measured at 517 nm using a microplate reader (EZ Read 2000 Microplate Reader, Cambridge, UK). The IC value, representing the concentration of the sample required to achieve 50% inhibition of DPPH activity, was calculated from the linear equation derived from the plot of % inhibition versus mangiferin concentration. The activity tests were conducted periodically on two sample sets; mangiferin in aqueous solution and in developed emulsion (F11), both stored at ambient temperature. Each sample was subjected to three replicate assays, and the mean±standard deviation (SD) was reported.

2.6 Skin irritation studies

The irritation assessment of the selected emulsions was carried out with ten healthy volunteers using closed patch tests. First, 0.1 g of the tested samples was applied on the inner forearm of each volunteer, then the applied area was wrapped with a cotton bandage and secured with adhesive tape. After 24 h, the patches were removed, and the skin was carefully cleaned. Visual observation and instrumental measurements were taken an hour later. Irritation score grading was performed based on the COLIPA visual scoring method (CVSM), as detailed in Table 2.

Table 2. Skin irritation assessment using closed patch test (Walker et al., 1996)

Grading	Description of skin response
0	No visible reaction
0.5	Doubtful erythema
+1	Mild erythema
+2	Intense erythema
+3	Intense erythema with edema
+4	Intense erythema with edema and vesicle

2.7 Determination of skin moisture

Ten healthy females with Fitzpatrick skin types II and III, aged 20 to 35, participated in the study. Participants were excluded if they had dermatitis or any other skin or allergic conditions, were smokers, or had previously applied cosmetic products to their forearms' skin. The study was designed as a single-blinded, placebo control to establish comparisons of two emulsion formulations: F11 with and without mangiferin. The corneometer MPA5 (Courage + Khazaka, Germany) was utilized to measure the skin surface's water content and TEWL. These measurements were conducted in a room with controlled humidity at 40% and temperature at 27°C±1°C. The effectiveness of the studied products on the skin was assessed using a sample size of ten volunteers (Septianingsih et al., 2020; Parveen et al., 2014). Approximately 0.1 g of the test product was applied to the marked site (2×2 cm) on the inner forearm using a finger once daily at bedtime for 14 consecutive days. The TEWL and relative water content were measured in triplicate at the applied area after 7 and 14 days of product application. Before each measurement, volunteers were acclimatized to room conditions for at least 30 min. On the first day of the test, baseline values for color, water content, and TEWL were measured at the marked sites on both forearms before the product was applied. After 7 and 14 days, measurements for these parameters were repeated under the same controlled conditions. The statistical level of significance was set at 5%. Statistical analyses were performed using Excel for Microsoft 365. The study protocols were approved by the HCU ethics committee board (HCU-EC1477/2567) and the written informed consent was obtained from all participants.

3. RESULTS AND DISCUSSION

3.1 Evaluation of liquid crystal emulsion

An ideal formulation would be an emulsion displaying clear and abundant Maltese cross images when observed under polarized light microscopy. Moreover, the emulsion should offer a light touch, quick absorption, and leave no greasy residue upon application on the skin. The study explored the different types and proportions of mixed emulsifiers to identify the optimal formulations for liquid crystal emulsions.

The obtained emulsions were also evaluated for their organoleptic properties, including homogeneity, color, odor, ease of spreading, softness to the touch, and any residual feeling after application to the skin. The organoleptic test uses human senses as the primary tool to evaluate product quality (Septianingsih et al., 2020). These tests aimed to assess the sensory response to the products, facilitating the primary selection of formulations for further study. The results are shown in Table 3.

Table 3. Organoleptic tests of the studied emulsions

Formulation	Characteristics					
	Homogeneity	Color	Odor	Easy to spread	Soft to touch	Leave a white cast
F1	H	W	O	E-	S+	C+
F2	H	W	O	E+	S+	C-
F3	H	W	O	E+	S+	C-
F4	H	W	O	E-	S+	C+
F5	H	W	O	E-	S-	C+
F6	H	W	O	E-	S-	C+
F7	H	W	O	E+	S+	C-
F8	H	W	O	E-	S-	C-
F9	H	W	O	E+	S+	C-
F10	H	W	O	E+	S+	C-
F11	H	W	O	E+	S+	C-

Note: H: homogenous, W: white, O: odorless, E+: easily spread, E-: not easily spread, S+: smooth feeling, S-: sticky feeling, C+: leave a white cast, C-: not leave a white cast

All the studied emulsions (F1–F11) demonstrated a homogeneous, opaque, milky-white appearance and were odorless. The F2, F3, F7, F9, F10, and F11 emulsions demonstrated good spreadability upon application to the skin. However, only the F3, F9, F10, and F11 emulsions were quickly absorbed, providing a favorable sensory feeling. They were lightweight and soft, leaving no greasy or white residue on the skin.

3.2 Liquid crystal inspection in emulsion

Polarized light microscopy is an essential tool for investigating liquid crystal formation in samples. This method is particularly valuable because it can identify the presence of liquid crystals by displaying a characteristic Maltese cross pattern on a dark background (Eccleston et al., 2000). All formulations (F1–F11) displayed a consistent distribution of Maltese cross pattern. However, the clarity

and degree of the Maltese cross images varied among the different formulations, suggesting differences in the arrangement of the amphiphilic molecules, including emulsifiers, in the systems, as illustrated in Figure 2. This region of distinct molecular arrangement, known as lamellar structure, is recognized for its ability to effectively solubilize hydrophobic substances and potentially facilitate the retention of water molecules (Jarupinthusophon et al., 2022).

The photomicroscopy revealed that F11 exhibited the clearest and most abundant presence of Maltese cross images. This formulation contained sorbitan palmitate and sucrose palmitate as emulsifiers at a ratio of 4:1. The obvious detection of the lamellar structure in F11 may be attributed to the architectural arrangement of the two emulsifiers with reverse structures at the oil-water interface, thereby creating a mixed critical packing parameter close to that suitable for lamellar formation. Therefore, F11 was chosen for further assessment in stability and skin compatibility studies.

3.3 Stability studies

Stability studies of the selected formulation, F11, containing mangiferin were carried out under temperature cycling and ambient conditions. The stability results, including pH values and the remaining mangiferin content, are presented in Table 4. The results indicate that the pH of the studied emulsion remained consistently within the range of 5.8 to 6.1 throughout the duration of the study. This pH range is compatible with the natural pH of the skin (Huang and Gui, 2018).

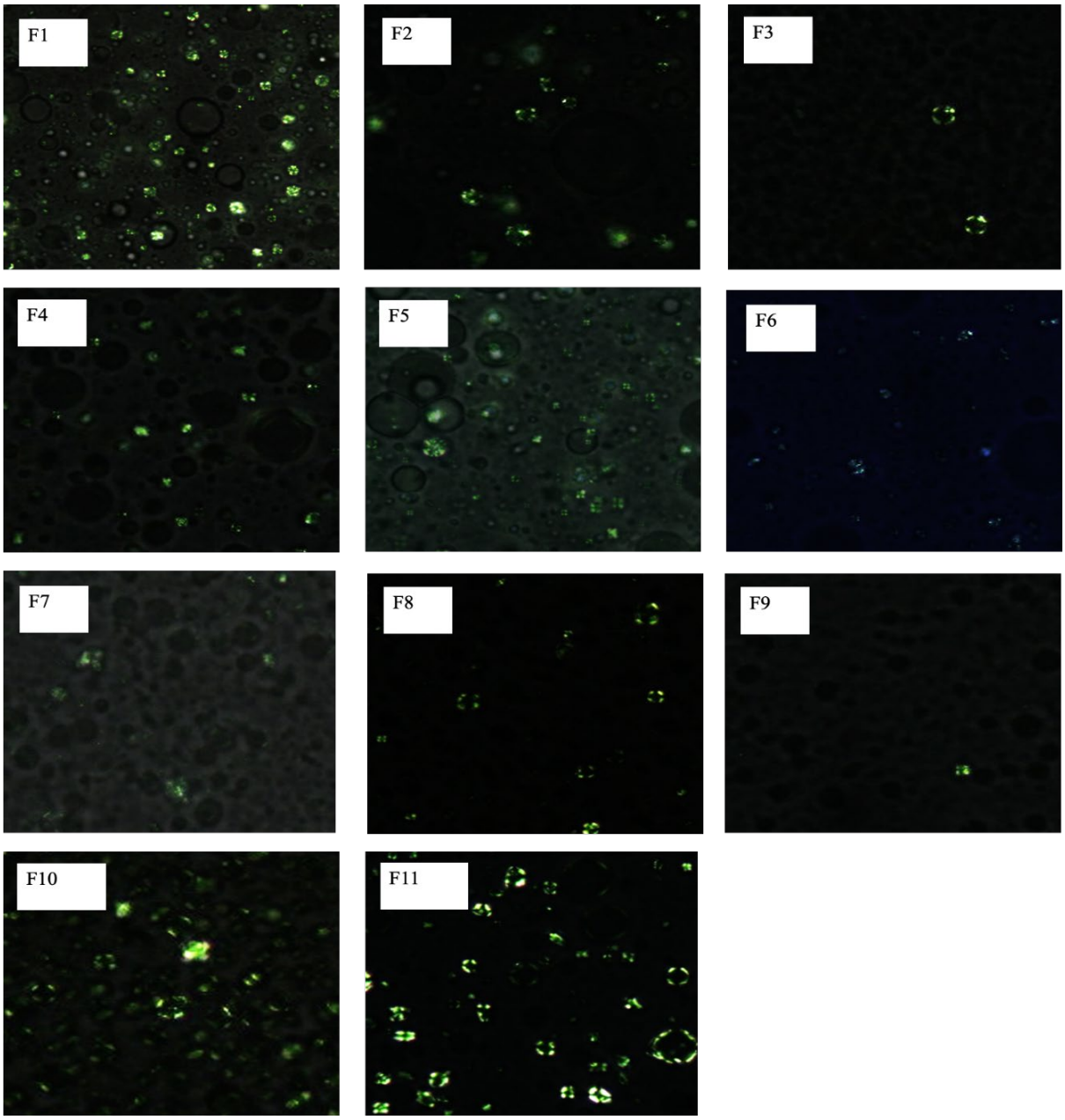

Figure 2. Polarized light photomicrographs (40× magnification) of the studied emulsions

The chemical stability study, conducted by assessing the xanthone skeleton of mangiferin using UV absorption spectroscopy at 316 nm., clearly demonstrated a reduction in mangiferin content over time compared to the freshly prepared samples. The UV absorbance of the samples decreased, leading to a reduction in mangiferin content to 78.38±0.70% after 90 days of storage. Mangiferin is a C-glycosyl xanthone reported to be stable due to the presence of a C-glycosidic bond in its structure. This bond is generally more stable than an O-glycosidic bond and is not easily hydrolyzed (Ehianeta et al., 2016). It has been reported that the optical absorption spectrum of mangiferin's xanthone skeleton undergoes significant changes with varying pH levels in aqueous solution (Mishra et al., 2006). These changes are driven by the redox reactions of the xanthone nucleus, which alter the electronic structure of the molecule in response to the pH environment. This pH-dependent behavior modulated mangiferin optical properties (El-Seedi et al., 2010; Mishra et al., 2006). Additionally, a redox reaction typically occurred when mangiferin, acting as an antioxidant, was exposed to atmospheric oxygen, resulting in the formation of a quinone compound. The changes in functional groups also affected the optical properties of mangiferin, resulting in a decrease in UV absorption at 316 nm and consequently lowering the measured content of mangiferin in the studied samples. To test this hypothesis, samples stored in airtight, unopened glass containers were analyzed for mangiferin content using the same technique. The results indicated that after 90 days of storage in ambient conditions, the percentage of mangiferin remaining was 95.35±0.25%, compared to the initial amount of 99.9±0.14%.

In order to investigate the stability of mangiferin's antioxidant activity, the scavenging activity tests against DPPH radicals were carried out. Mangiferin dissolved in water at concentrations of 0.1, 0.5, 1, 2 and 4 mg% were stored in glass bottle and kept at ambient temperature. Samples were taken periodically and diluted to an appropriate concentration for antioxidant activity testing at different time intervals. The scavenging activity against DPPH radicals of mangiferin, both in aqueous solution and in the studied emulsion (F11), are presented in Table 5. The antioxidant activity of mangiferin in an aqueous solution was assessed to evaluate its effectiveness in neutralizing free radicals, with the results expressed as IC_{50} values. These values were also monitored over time to understand how they changed with prolonged exposure to the aqueous medium. The antioxidant activities remained consistently detectable throughout the stability testing periods, with IC_{50} values ranging from 7.1 to 7.3 µg/mL after 90 days of storage. Meanwhile, the inhibition percentage of DPPH by a specific amount of mangiferin incorporated into the studied emulsion remained stable throughout 90 days of storage. Although changes in the functional groups of mangiferin were observed during storage, indicating an instability, its antioxidant activity derived from the remaining phenolic-containing xanthone moiety remained effective. This suggested that despite the instability, the functional properties associated with the xanthone structure remain intact, thereby enabling mangiferin to maintain its beneficial antioxidant activity (Jutiviboonsuk and Leeprechanon, 2019).

The physical appearance of the studied emulsion (F11) under cycling temperatures and ambient conditions showed no significant changes in odor and no phase separation after 6 cycles and throughout the 90-day storage period. The obtained emulsions containing mangiferin exhibited an opaque, slightly yellowish color, as illustrated in Figure 3.

The organizational stabilities of the liquid crystal structure in the studied emulsion containing mangiferin were examined using a polarized light microscope at different storage times and conditions. The comparison of images between the initial and stored samples was conducted in terms of clarity, size and the degree of the Maltese cross appearance. The results shown in Figure 4 indicate that the liquid crystal structures, characterized by the Maltese cross appearance, remained stable in the studied emulsion containing mangiferin. There were no observed changes in clarity or intensity under different storage conditions over the 90-day period in ambient conditions. Moreover, the droplet size of the particles showed no significant change when observed monthly under optical microscopy.

Table 4. pH values and %LA of mangiferin in the studied emulsion (F11)

Parameter	Storage conditions						
	Cyclic temperature			Ambient temperature			
	1st (2 days)	3rd (6 days)	6th (12 days)	1 day	30 days	45 days	90 days
pH	6.16±0	5.95±0.01	5.97±0.05	6.16±0	6.05±0	5.86±0.01	5.86±0.02
% LA of mangiferin	97.85±0.14	97.07±1.08	93.74±0.44	97.88±0.14	97.5±1.06	92.4±1.55	78.38±0.70

Table 5. Scavenging activity against DPPH radicals of mangiferin in aqueous solution and the studied emulsion (F11)

Formulation	IC_{50} (mg/mL) (Mean*±SD)			% Inhibition (Mean*±SD)		
	1 day	30 days	90 days	1 day	30 days	90 days
Aqueous solution	7.21±0.49	7.21±0.49	7.22±0.31	-	-	-
F11	-	-	-	98.03±0.19	97.97±0.23	97.40±0.30

Note: * n = 3

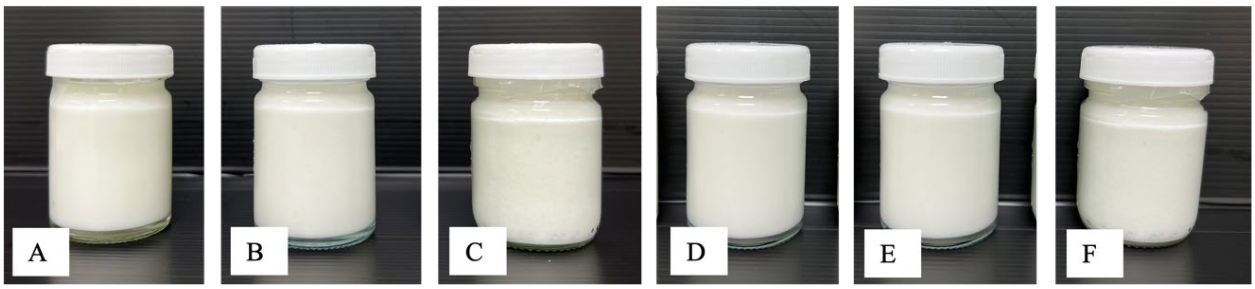

Figure 3. Images of the studied emulsions containing mangiferin in glass bottles after storage at ambient temperature (A;30 days, B; 45 days and C; 90 days) and under temperature cycling conditions (D; 1 cycle, E; 3 cycles and F; 6 cycles)

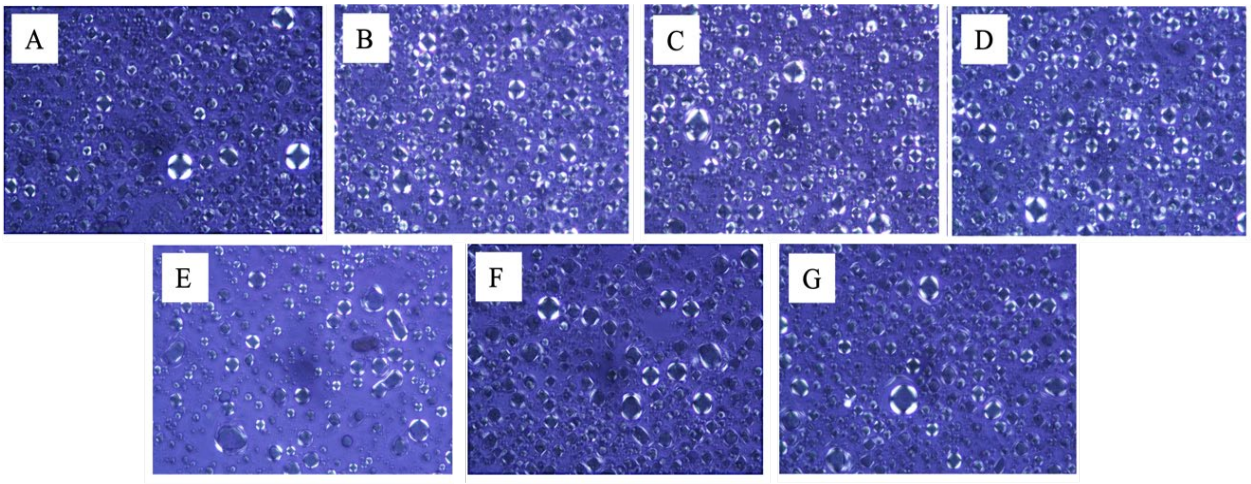

Figure 4. Microscopic images of Maltese cross pattern in F11 containing mangiferin stored at ambient temperature (A: 1 day, B: 30 days, C: 45 days and D: 90 days) and cycling temperature under conditions (E: 1 cycle, F: 3 cycles and G: 6 cycles)

3.4 Skin irritation studies

To ensure the safety of the formulations for skin application, closed patch tests using the visual scoring method according to the COLIPA guidelines were performed. Although the visual scoring method is a subjective evaluation, it is reproducible, sensitive, and reliable. All of the healthy volunteers participating in the study had a zero score, indicating no irritation on the applied area after 24 h of incubation. Therefore, the studied emulsions can be considered a safe formulation, as it caused no erythema, dryness, or edema on the skin under closed patch tests.

3.5 Determination of moisture on the skin

A common expectation for the daily application of skincare products on the skin is to enhance skin hydration. To assess the efficacy of these products in increasing skin hydration levels, specialized equipment was employed to measure two key parameters: the moisture content of the skin and its ability to retain moisture after product application. The technique for determining water content is based on capacitance measurement of a dielectric medium in the skin. Meanwhile, TEWL is assessed to evaluate the skin's barrier function in retaining internal moisture. The results shown in Figure 5 illustrated the electrical conductance of the upper layer of the skin, measured at baseline, and after one and two weeks of applying the studied emulsions, with and without mangiferin. It is clear that the electrical conductance of the stratum corneum, which is directly proportional to the skin's hydration level, gradually increased from the baseline measurement over the weeks following the application of both products. A significant increase in water content was observed after 1 week of applying the emulsion containing mangiferin ($p < 0.01$), whereas it took 2 weeks for this significant increase after the application of the emulsion base ($p < 0.01$), as detailed in Table 6.

TEWL reflects the rate of water evaporation from the skin, quantified as the amount of water (in grams) evaporated per unit area (in square meters) over time (in hours). This measurement directly assesses the skin's barrier function. The results presented in Figure 6 demonstrated notable decreases in water evaporation from the skin after the application of the developed emulsions to the designated skin areas compared to the baseline. This effect was significantly observed after 2 weeks of applying the emulsion containing mangiferin ($p<0.05$).

The skin naturally acts as a barrier to prevent water loss from the body. One of the most effective methods to enhance this barrier function is by applying topical products that provide an additional protective layer on the skin. Skincare products designed to create a water-impermeable occlusive film on the skin surface aim to strengthen the skin's barrier function. The effectiveness of this property depends on the solidity of the film formed after the products are applied to the skin. The developed emulsions contained a liquid crystal structure that was

expected to create a dense film when applied to the skin surface. Moreover, the structure of lamellar liquid crystals closely resembles the arrangement of lipids present in the skin stratum corneum. (Ueoka and Pedriali Moraes, 2018). The creation of a strong film on the skin reduces water evaporation, thereby leading to an increase in the skin's water content over time. This protective barrier helps maintain hydration levels and supports skin barrier function. Mangiferin, being sparingly soluble (Acosta et al., 2016), potentially enhances the integrity of the created film by embedding itself within the hydrophobic region of the lamellar liquid crystal structure. It was hypothesized that the interaction occurring in the studied emulsion containing mangiferin would result in a significant reduction in TEWL and an increase in skin water content.

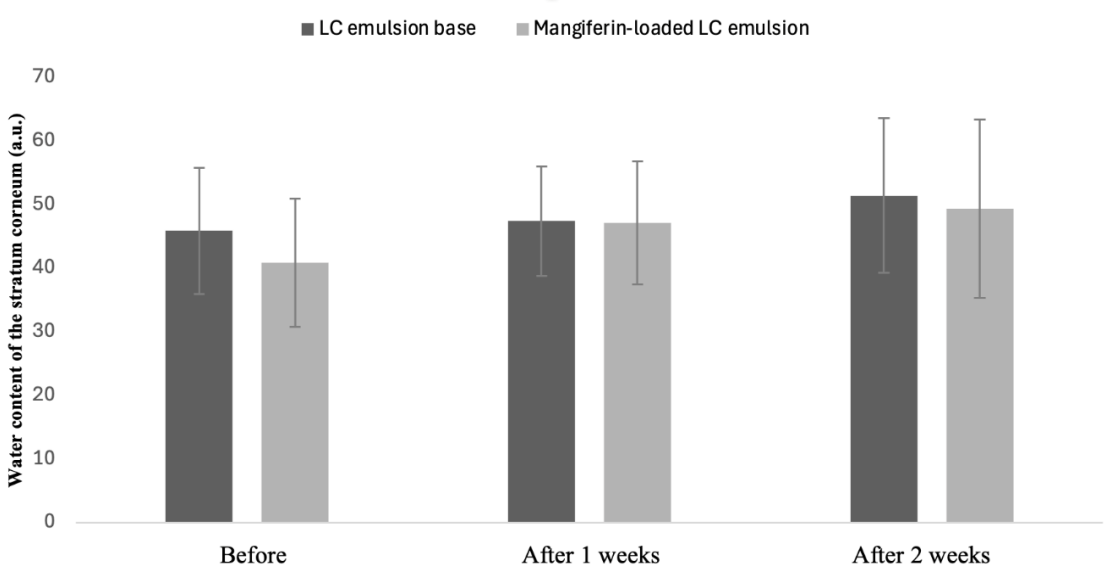

Figure 5. Water content in the stratum corneum measured using a Corneometer MPA5 at baseline and after applying the emulsions for 1 and 2 weeks (n=10)

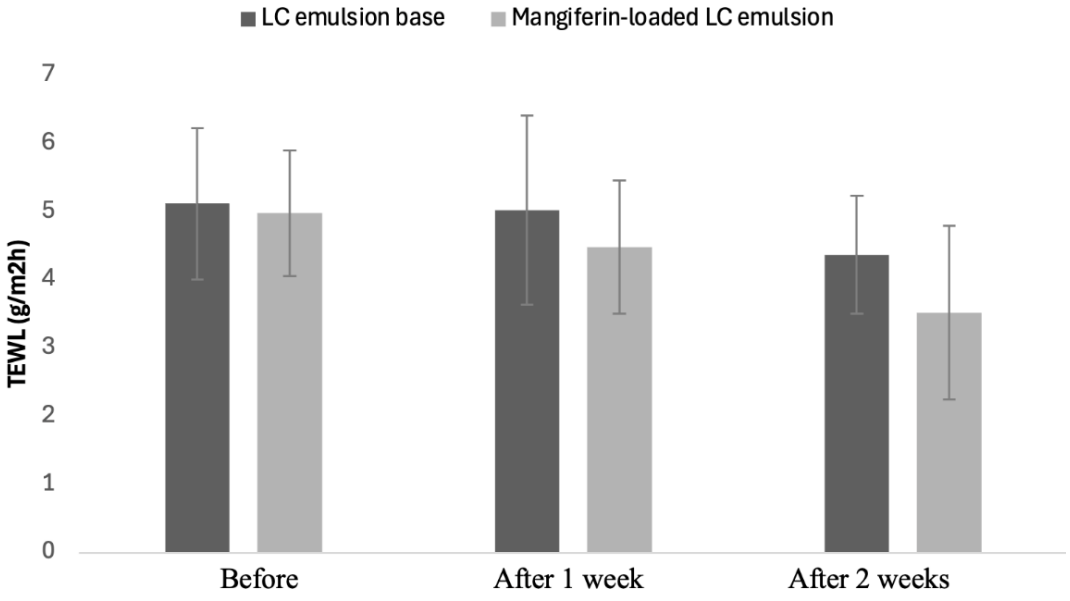

Figure 6. Transepidermal water loss measured using a Corneometer MPA5 at baseline and after applying the emulsions for 1 and 2 weeks (n=10)

Table 6. Statistical analysis of water content in the stratum corneum and transepidermal water loss after applying the emulsions with and without mangiferin for 1 and 2 weeks

	LC emulsion base		Mangiferin- loaded LC emulsion	
	Mean±SD	**p-value**	**Mean±SD**	**p-value**
Water content (a.u.)				
Baseline	45.89±9.94	-	40.83±10.09	-
Week 1	47.40±8.58	0.4908	47.11±9.68	0.0007**
Week 2	51.43±12.20	0.0411*	49.359±14.02	0.0058**
Transepidermal water loss ($g/m^2.h$)				
Baseline	5.12±1.11	-	4.98±0.92	-
Week 1	5.02±1.38	0.8475	4.28±0.97	0.1533
Week 2	4.37±0.86	0.3568	3.52±1.27	0.0288*

Note: Data were analyzed between groups with a paired t-test, *p*-value significant at *p<0.05 and **p<0.01

4. CONCLUSION

The aim of this study was to develop emulsions which contained lamellar liquid crystals by examining the effect of different combinations of emulsifiers in the formulations. The study results reveal a favored emulsion with a combination of sorbitan palmitate and sucrose palmitate with a 4:1 ratio (F11), at a concentration of 6% by weight in the formulation. Although mangiferin appeared to be unstable with age, due to changes in its functional groups, its antioxidant activity remained intact. The assessment of skin hydration levels revealed that the selected emulsion containing mangiferin improved skin health by increasing water content and reducing TEWL, without causing skin irritation.

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