ORIGINAL ARTICLE

Development of Newly Formulated Emulsion Containing PhytoSpherix™ and Actigym™ Marine Ingredient for Skin Protection Potential

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Abstract

Background: PhytoSpherix™ is a nanoparticle derived from non-genetically modified sweet corn, isolated from the plant, and Actigym™ is a marine ingredient created by a Bermudan bacterium (a *Bacillus* sp.). Therefore, our objectives were to (i) formulate an emulsion containing PhytoSpherix™ and Actigym™ marine ingredients, (ii) stabilize the emulsion with Sustoleo™ MCT and SustOleo™ GMS, (iii) determine the sun protection factor (SPF), (iv) analyze ultraviolet A/ultraviolet B (UVA/UVB) protection, and (v) assess *in vivo* skin melanin, erythema, elasticity, and moisture content. **Materials and Methods:** A new oil-in-water emulsion was prepared, incorporating PhytoSpherix™ and Actigym™ marine ingredient, using a mixture of cetyl alcohol, hydrogenated rapeseed oil, triheptanoin, glyceryl stearate, and distilled water. Various tests were conducted, including accelerated thermal stability, pH measurement, color, smell, phase separation, liquefaction, rheology, SPF, UVA/UVB protection, irritation, and *in vivo* effects on skin (melanin, erythema, elasticity, hydration, image visualization, and sensory evaluations). **Results:** The emulsion demonstrated excellent stability in both short and long-term testing, maintained a pH of 5.81, showed no phase separation, and had a viscosity of 1.042 Pa.s with a pseudoplastic trend, indicating shear-thinning behavior. It exhibited satisfactory safety profiles, causing no irritation in rats or human volunteers. *In vitro* testing revealed a SPF value of 23.70 and a UVA/UVB ratio of 0.51, providing effective UVB protection. Furthermore, it improved skin elasticity and moisture retention while reducing melanin and erythema compared to the control. **Conclusion:** The newly formulated emulsion was deemed stable and showed promising effects against UV radiation on the skin, warranting further investigation.

Key words: Actigym™ marine ingredient, PhytoSpherix™, skin protection, ultraviolet rays

INTRODUCTION

The skin is the body's largest organ, covering a surface area of 1.8 m^2.[1-3] Although it is a convenient route for delivering active chemicals, its structure obstructs molecules since it serves as the body's protective barrier.[1] Stable emulsions with suitable emulsifiers are used,[4] to shield the dermis and epidermis from ultraviolet (UV) radiation.[5] The epidermis, dermis, and hypodermis are the three primary layers of the skin.[6] Skin can become damaged for several reasons, both internal and external. Most of these environmental factors up to 80% are caused by UV radiation (UVR). UVR is categorized into three classes: UV-A (320–400 nm), UV-B (280–320 nm), and UV-C (200–280 nm). UVA radiation is divided into two types: UVA radiation is divided into two types: Long-wave UVA (340–400 nm) and short-wave UVA

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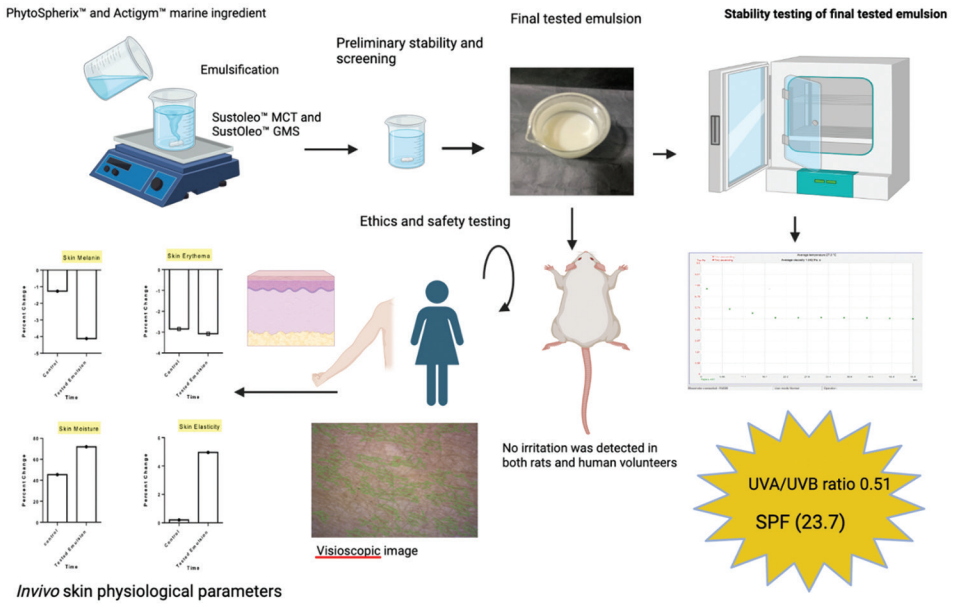
GRAPHICAL ABSTRACT



(320–340 nm), with the former accounting for most UVA rays. Skin physiological parameters are critical for the protection of skin. Melanin of the skin, a part of the skin, absorbs UV radiation and then releases it as heat. Melanin production is controlled by melanocytes within the skin.[7] The melanin that builds up in keratinocytes protects the skin against harmful UV radiation.[8] Skin erythema is the reddening of the skin caused by external stimuli, including sun exposure. Skin erythema is accompanied by signs of dermatological diseases such as acne psoriasis melasma, post-inflammation, and hyperpigmentation.[9] Providing the proper moisturizing and elasticities for the skin, more data on the mechanisms of the effect of different ingredients on the skin are a prerequisite.[10]

PhytoSpherix™ can enhance smoothness, reduce age spots, and reshape the complexion while boosting skin firmness and hydration, aiding in skin rejuvenation.[11] It contains phytoglycogen, a natural substance that has been shown to be effective.[11] The monodisperse, 70 nm-diameter phytoglycogen particles found in non-genetically modified sweet corn are isolated from the plant. Chemically identical to the glycogen found in animal cells, phytoglycogen is produced and retained by plants.[11] It is a type of natural glycogen produced using an advanced, environmentally friendly, water-based method. Like many other natural polysaccharides such as cellulose, dextran, and starches, phytoglycogen has a simple chemical makeup: Monosaccharide units of glucose. These glucose monosaccharide molecules form linear chains with glyosidic linkages acting as branching points, with an average of 11–12 units of glucose per molecule.[12]

In addition, Actigym™, a marine ingredient created by a Bermudan bacterium (*Bacillus* sp.), enhances the benefits of weight loss and metabolism.[13] This component stimulates the release of the adiponectin protein from the skin's fat tissue,[13] reducing matrix metalloproteinase (MMP) expression and promoting skin repair.[13] The topical co-delivery of PhytoSpherix™ and Actigym™ marine ingredient in an emulsion may improve biological and physiological functions affected by UV exposure. This could be due to PhytoSpherix™ enhancing the production of endogenous collagen and HA, two crucial polysaccharides for healthy skin as shown in *in vitro* tests on dermal cells, while Actigym decreases MMP levels and aids in skin repair.[13]

Therefore, our objectives were to (i) formulate an emulsion containing PhytoSpherix™ and Actigym™ marine ingredients, (ii) stabilize the emulsion with Sustoleo™ MCT and SustOleo™ GMS, (iii) determine the sun protection factor (SPF), (iv) analyze UVA/UVB protection, and (v) assess *in vivo* skin melanin, erythema, elasticity, and moisture content.

MATERIALS AND METHODS

Actigym™ marine ingredient (a solution of plankton extract) was generously provided by Lipotec in Barcelona, Spain. PhytoSpherix™ (phytoglcogen nanoparticles) was gifted by Mirexus Inc. in Ontario, Canada. SustOleo™ TSB (hydrogenated rapeseed oil), SustOleo™ MCT (triheptanoin), and SustOleo™ GMS (glyceryl stearate) were provided by Inolex, Inc. in Philadelphia, United States. Valvance® Touch 210 (Silica) was donated by DSM Nutritional Products in the Netherlands. Acacia gum and all other chemicals were of commercial quality, and distilled water was obtained from the laboratory of the Department of Pharmacy at COMSATS Islamabad University, Abbottabad Campus in Pakistan.

Formulation and preparation of emulsions

The emulsions were prepared using different concentrations of cetyl alcohol, hydrogenated rapeseed oil, triheptanoin, glyceryl stearate, and water [Table 1]. Three phases (Phases A, B, and C) were developed. Phase A involved measuring glycerin, xanthan gum, and water in a clean beaker and heating them to 75°C. Separately, PhytoSpherix™ and Actigym™ marine ingredients were moderately dispersed with agitation in Phase A. Phase B was prepared by measuring cetyl alcohol, hydrogenated rapeseed oil, glyceryl stearate, and triheptanoin at 75 °C. Phase B was then mixed into Phase A using a stirrer at 1500 rpm (magnetic stirrer, Velp Scientific, Italy) until completely homogeneous. Once the temperature dropped to 40°C, Phase C (benzoic acid) was added. Homogenization was then performed at 400 rpm until all ingredients were well combined [Figure 1]. A base emulsion was prepared in the same way without PhytoSpherix™ and Actigym™ marine ingredients. Emulsion ingredients and their functions are depicted in Table S1. Xanthan gum was chosen for its excellent properties in creating a suitable environment for emulsions.

Preliminary stability and screening of emulsions

After being incubated at 25°C in a Sanyo MIR-162 incubator from Japan for 4 weeks, samples of 20 emulsions wrapped in aluminum foil were examined for stability. Throughout this period, all emulsions were visually inspected for any signs of deterioration.[14]

Stability testing of final emulsion

Stability testing of the final emulsion was conducted on both a short-term and long-term basis.

Short-term stability testing

The emulsion was transferred into a glass container with the use of a spatula and taped shut. Up to two-thirds of the bottle's volume of emulsion was added before the plug and cap were secured. The full bottle was incubated for 72 h at 45°C with a relative humidity of 75%.[15]

Long-term stability testing

The color, odor, liquefaction, phase separation, and pH of the tested emulsion were visually assessed at storage temperatures of 6°C, 37°C, and 45°C in the dark over a period of 6 months. Assessments were conducted at various time intervals, including the 1st, 2nd, 3rd, 4th, 5th, and 6th months.[16]

Microscopic study

A red dye was used to tint the test emulsion (sample), which was then placed on a glass slide. To determine the type of emulsion, it was examined under a microscope (Optika TCB5.0) (Ali *et al*., 2019).

Rheological study

A rheometer, the RM200 TOUCH (LAMY Rheology 11 A, 69410 France), was connected to a drive, and the data analysis program rheumatic-P was used to analyze the tested emulsion.[14] The measurement was conducted using spindle R-3, with viscosities of the formulation measured at a speed of 100 RPM for 180 s at intervals of 60, 120, and 180 s at a temperature of 20 ± 0.5°C. The measurement was performed as Viscosity = f (Time), allowing for measurement at a fixed shear rate over a predetermined time, or to track the evolution of viscosity with temperature. The following reading protocol was used: Preshearing time: 5 s, Preshearing rate: 10 S¬1, Time: 60 s, Shear rate: 20 S¬1. The measuring system used was 12, and the measurement started immediately.

Determination of SPF

The SPF value of the tested emulsion was calculated using the Masur equation, utilizing a UV spectrophotometer (UV-VIS Spectrophotometer, UVikon XL, Bio-Tek Instruments, Bad Friedrichshall, Germany). To begin, a 100 mL volumetric flask was filled with 1 g of the tested emulsion, following the outlined technique. The emulsion was then diluted with a mixture of ethanol and water (40:60) and subjected to ultrasonic processing for 5 min. The filtrate was collected, discarding the initial 10 mL, and filtered using filter paper. A 5 mL aliquot sample was taken and further diluted with

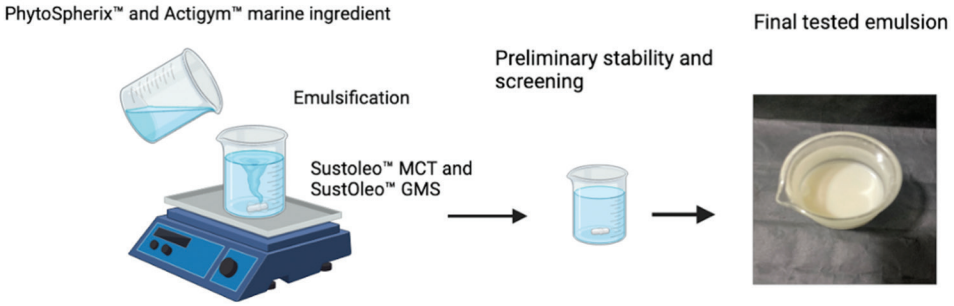


Figure 1: Preparation and appearance of a freshly prepared emulsion containing PhytoSpherix™ and Actigym™

Table 1: Formulations with compositions

No	Emulsion code	Ingredients (%)										
		PhytoSpherix™	Actigym™ marine ingredient	Purified water	Glycerin	SustOleo™ TSB	SustOleo™ MCT	Cetyl alcohol	SustOleo™ GMS	Valvance® Touch 210	Benzoic acid	Gum acacia
1.	C1	0.5	5	Q. R	12	6.4	10	**4**	4	4	4	4
2.	C2	0.5	5	Q. R	12	6.4	10	**3.2**	4	4	4	4
3.	C3	0.5	5	Q. R	12	6.4	10	**2.5**	4	4	4	4
4.	C4	0.5	5	Q. R	12	6.4	10	**2.0**	4	4	4	4
5.	C5	0.5	5	Q. R	12	6.4	10	**1.6**	4	4	4	4
6.	G1	0.5	5	Q. R	12	6.4	10	4	**3.2**	4	4	4
7.	G2	0.5	5	Q. R	12	6.4	10	4	**2.5**	4	4	4
8.	G3	0.5	5	Q. R	12	6.4	10	4	**2.0**	4	4	4
9.	G4	0.5	5	Q. R	12	6.4	10	4	**1.6**	4	4	4
10.	G5	0.5	5	Q. R	12	6.4	10	4	**1.0**	4	4	4
11.	M1	0.5	5	Q. R	12	6.4	**8**	4	4	4	4	4
12.	M2	0.5	5	Q. R	12	6.4	**6.4**	4	4	4	4	4
13.	M3	0.5	5	Q. R	12	6.4	**10.3**	4	4	4	4	4
14.	M4	0.5	5	Q. R	12	6.4	**5.1**	4	4	4	4	4
15.	M5	0.5	5	Q. R	12	6.4	**4.09**	4	4	4	4	4
16.	T1	0.5	5	Q. R	12	**6.2**	10	4	4	4	4	4
17.	T2	0.5	5	Q. R	12	**5.1**	10	4	4	4	4	4
18.	T3	0.5	5	Q. R	12	**4.0**	10	4	4	4	4	4
19.	T4	0.5	5	Q. R	12	**3.2**	10	4	4	4	4	4
20.	T5	0.5	5	Q. R	12	**2.6**	10	4	4	4	4	4

Q. R: Quantity required

ethanol and water in a 50 mL volumetric flask (40:60). From this, another 5 mL aliquot was taken and diluted with ethanol and water in a 25 mL flask (40:60). The absorbance of the aliquot was measured from 290 nm to 320 nm at 5-min intervals, using distilled water as a blank solution. Three readings were taken for each interpretation. To determine the SPF values, the absorbance readings were added together and multiplied by the correction factor of 10, as previously reported.[17]

In vitro analysis of UVA/UVB protection

Each formulation was examined *in vitro* using the UVA/UVB ratio and the critical wavelength. A sample of 1.3 mg/cm^2 was placed in a petri dish. The emulsion was then exposed 3 times to a UV lamp for 30 min each time. After exposure, a spectrophotometer (UV-VIS Spectrophotometer, UVikon XL, Bio-Tek Instruments, Bad Friedrichshall, Germany) was used to take three measurements of transmission on the sample between 290 and 400 nm. The sample was pipetted and dispersed evenly using a pre-soaked fingertip. To ensure the emulsion self-leveled, the sample was allowed to settle for 15 min at room temperature in the dark. Following the completion of all measurements, the UVA/UVB ratio was examined.[18]

Skin irritation test on rats

The laboratory protocols and the use of animals (Rats) in research were performed based on the National Scientific Instructions for animal use.[19] The COMSATS University animal ethics committee reviewed the study and gave approval. The test subjects were healthy rats of similar age, weighing between 180 and 230 g. Before testing, the rats were acclimated for 7 days. They were fed a standard diet and housed in polypropylene cages maintained at a constant temperature of 23 ± 2°C with a 12-h light-dark cycle. Hairs from the rats' backs were removed, and a 1 cm^2 area was marked on each side. One side served as a control, while the other side was treated with the test emulsion. The emulsions (200 mg/rat) were applied, and the sites were covered with bandages. After 24, 48, and 72 h, the emulsions were removed, and the skin was examined for any visible erythema (redness).[20]

Ethical standard

The Registrar Secretariat Academic Unit (PS) of COMSATS University Islamabad, Pakistan, gave its approval for this study (Reference No. CUI-Reg/Notif-4474/21/3140). After receiving their signed informed consent, 11 healthy female volunteers (mean age: 25 ± 6) who had current immunization records and no history of dermatological diseases were enrolled to test the efficacy of the emulsions. The Declaration of Helsinki's guiding principles and best clinical practice were followed throughout the investigation. Each volunteer was evaluated by the investigation team of the job before the study to rule out any skin reactions or sensitivities. Exclusion criteria were any dermatitis, allergic diseases, smoking, and prior use of any cosmetic product. The inclusion criteria were no history of any skin allergies.[21,22]

Safety testing

After obtaining written agreement, safety testing of the emulsions was conducted on the left and right forearms of each participant. A specific location on the forearms was marked, and a bandage disc was used to apply 1 g of each emulsion (control and tested emulsion) to the area. The lead investigator and his team assessed for any skin discomfort in each participant. The erythema, edema, and irritation scores reported for safety were calculated using a numerical scale, and the average irritation index was evaluated using the revised Draize system.[23,24] Instrumental safety testing was performed using a probe from the Multi Skin Test Center MC 1000 and the software "Complete Skin Investigation" (CSI) to measure skin erythema values at the baseline visit and after 48 h (Courage and Khazaka electronic GmbH, Germany).[14]

In vivo effects on skin

A total of 11 female volunteers aged 22–33 were selected for *in vivo* testing. A single-blinded, non-invasive study was conducted. Volunteers used both control and tested emulsions twice daily and spent at least 2 h outdoors each day during the 7-day trial. The control and tested emulsions were applied at a rate of 2 mg/cm^2 on the left and right forearms, respectively. The room maintained a constant temperature of 25 ± 5°C and 50 ± 5% relative humidity for the duration of the measurements. Skin analysis was performed using the multi-skin test center MC 1000 with the software CSI (Courage and Khazaka electronic GmbH, Germany). This included measuring skin melanin and redness (erythema),[25] skin hydration levels, and skin elasticity properties.[22] In addition, the Visioscope PC 35® (Courage and Khazaka electronic GmbH, Germany) was used to capture images at the baseline visit and the 7-day visit.

Panel test

The emulsion was evaluated using a panel test to determine its sensory characteristics. Each participant completed a form with seven criteria to assess sensory values ranging from −5 to +5, indicating extremely detrimental to extremely beneficial. This process was conducted over 7 days.[25,26]

Statistical analysis

The variables of skin melanin, erythema, elasticity, and wetness in both the control and tested emulsion were

compared during the baseline visit and the day 7 visit using paired sample *t*-tests with GraphPad Prism software version 9 to determine if there was a significant difference.

RESULTS

Preliminary stability screening

During the 1st week, 15 out of the 20 topical emulsions (C1-C5, G1-G5, M1-M5, T1-T5) containing PhytoSpherix™ and Actigym™ marine ingredients as active components were stable. Five emulsions showed no stability within the 1st week. By the 2nd week, 11 of the initially stable emulsions remained unchanged. Out of those, 7 emulsions maintained their stability into the 3rd week. After testing storage conditions for a total of 4 weeks, only one emulsion (C1) remained stable, as determined by phase separation, liquefaction, and other physical changes outlined in Table 2. This particular emulsion was identified as C1 in the final test.

Stability testing of final tested emulsion (C1)

Table 3 displays the results for the short-term stability of the final tested emulsion. It shows that the emulsion remained stable for 72 h under storage conditions of 45°C and can be further evaluated over a longer period. Enhanced stability is necessary for topical formulations to ensure their intended efficacy and physical and chemical quality are maintained under specific circumstances for a specific amount of time.[17] Results for the long-term stability of the tested emulsion are provided in Tables 4-6. Evaluation of the emulsion's stability includes monitoring changes in color, odor, liquefaction, phase separation, and pH.[16] The color of the examined emulsion remained white at 6 ± 2°C, 37 ± 2°C, and 45 ± 2.0°C from the day of production up to 6 months in dark storage. No liquefaction, phase separation, or odor changes were observed under various storage conditions during the trial period. The pH of the freshly made emulsion was 5.81 and remained stable at that level for the entire 6 months. The pH of freshly prepared emulsions ranged from 5.5 to 5.9 [Table S2] and remained somewhat acidic, with no significant changes at different temperatures.

Microscopy

Figure 2a shows microscopic images of the tested emulsion. The continuous phase appeared red, but the dispersed globules seemed colorless. The resulting oil-in-water emulsion is depicted in Figure 2b.

Rheology

The tested emulsion had a viscosity of 1.042 Pa.s. In Figure 3, the rheograms are displayed.

Table 2: Preliminary stability screening of emulsions

S. No.	Emulsion code	1st week	2nd week	3rd week	4th week
1.	C1	Stable	Stable	Stable	Stable
2.	C2	Stable	Unstable	Unstable	Unstable
3.	C3	Stable	Stable	Stable	Unstable
4.	C4	Stable	Stable	Unstable	Unstable
5.	C5	Unstable	Unstable	Unstable	Unstable
6.	G1	Unstable	Unstable	Unstable	Unstable
7.	G2	Stable	Stable	Unstable	Unstable
8.	G3	Stable	Stable	Stable	Unstable
9.	G4	Stable	Stable	Stable	Unstable
10.	G5	Stable	Unstable	Unstable	Unstable
11.	M1	Unstable	Unstable	Unstable	Unstable
12.	M2	Stable	Stable	Unstable	Unstable
13.	M3	Stable	Unstable	Unstable	Unstable
14.	M4	Stable	Unstable	Unstable	Unstable
15.	M5	Stable	Stable	Stable	Unstable
16.	T1	Unstable	Unstable	Unstable	Unstable
17.	T2	Stable	Stable	Stable	Unstable
18.	T3	Stable	Stable	Unstable	Unstable
19.	T4	Stable	Stable	Stable	Unstable
20.	T5	Unstable	Unstable	Unstable	Unstable

In vitro SPF

Table 7 displays the *in vitro* SPF results. The tested emulsion's SPF value was found to be 23.7, which is within the recommended SPF range.

In vitro analysis of UVA/UVB protection

Table 8 lists the emulsion UVA/UVB protection factor values. The obtained UVA/UVB ratio was 0.51.

Safety

Visual scoring results from safety testing of the control and tested emulsion revealed no irritation. The final result was zero, indicating that both emulsions were non-irritating and safe for skin contact. We found that skin erythema was slightly reduced after 48 h of patch testing on the forearms of each participant with the application of the control and tested emulsions. Before applying the control and tested emulsions, the mean erythema values were 20.65 and 20.45, respectively. After application, the mean erythema values were 20.35 and 20.05 for the control and test emulsions, respectively.

In vivo effects on skin

Figure 4 illustrates the % change in skin physiological parameters, including skin melanin, skin erythema, skin moisture, and skin elasticity over time on the treated side following application of the control and tested emulsion. Figure 5 displays a visioscopic image of a volunteer's right side at baseline and 7-day visits after the application of the tested emulsion.

Points post-panel test

After the panel test, the findings revealed that both the control and tested emulsions scored above 4 on the measure for volunteer sensory satisfaction. We conducted a paired sample *t*-test and found a negligible difference in pairing between the average points at different time intervals.

Table 3: Short-term stability testing of final emulsion

Time	Temperature (45°C)	Relative humidity (75%)
0 h	No change detected	
24 h	No change detected	
48 h	No change detected	
72 h	No change detected	

Table 4: Long-term stability study of the tested emulsion at 6±2°C

S. No	Parameters	Fresh	D-7	D-15	1st month	2nd month	3rd month	4th month	5th month	6th month
1	Color	W	W	W	W	W	W	W	W	W
2	Odor	-	-	-	-	-	-	-	-	-
3	Liquefaction	-	-	-	-	-	-	-	-	-
4	Phase separation	-	-	-	-	-	-	-	-	-

W: White, Y: Yellow (-): No change, (+): Slight change

Table 5: Long-term stability study of tested emulsion at 37±2.0°C

S. No	Parameters	Fresh	D-7	D-15	1st month	2nd month	3rd month	4th month	5th month	6th month
1	Color	W	W	W	W	W	W	W	W	W
2	Odor	-	-	-	-	-	-	-	-	-
3	Liquefaction	-	-	-	-	-	-	-	-	-
4	Phase separation	-	-	-	-	-	-	-	-	-

W: White, (-): No, (+): Slight

Table 6: Long-term stability study of tested emulsion at 45.0±2.0°C

S. No	Parameters	FresTah	D-7	D-15	1st month	2nd month	3rd month	4th month	5th month	6th month
1	Color	W	W	W	W	W	W	W	W	W
2	Odor	-	-	-	-	-	-	-	-	-
3	Liquefaction	-	-	-	-	-	-	-	-	-
4	Phase Separation	-	-	-	-	-	-	-	-	-

W: White, (-): No, (+): Slight

DISCUSSION

The emulsion containing PhytoSpherix™ and Actigym™ marine ingredients was newly formulated with the emulsifiers SustOleo™ MCT (triheptanoin) and SustOleo™ GMS (glyceryl stearate), and the texturizer SustOleo™ TSB (hydrogenated rapeseed oil). Valvance® Touch 210 (Silica) was used as a sensory modifier. The combination achieved an acceptable and improved emulsion. The emulsion showed no color change, smell change, phase separation, or liquefaction. Furthermore, the outcomes demonstrated stability, which is a crucial requirement for formulations to prevent destabilization.[27] The emulsion containing PhytoSpherix™ and Actigym™ marine ingredients exhibited encouraging long- and short-term stability. These results indicated that the tested emulsion samples had good physical stability. The ideal range for the average pH for aesthetic purposes is between 5.0 and 6.0. The skin has a wide pH range of 4.0 and 7.0.[24]

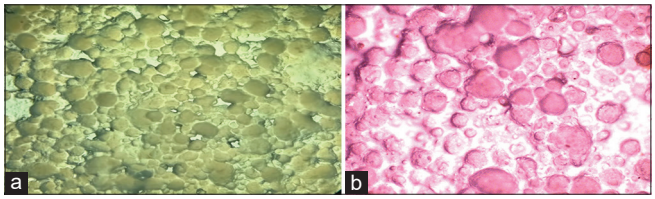


Figure 2: Microscopic images of tested emulsion: (a) Freshly prepared tested emulsion (b) The continuous phase appeared red, but the dispersed globules seemed colorless. The resulting oil-in-water emulsion is depicted

Flow indices <1 were found. Consistency indices (K), shear-thinning factor R, and N values discovered after applying the

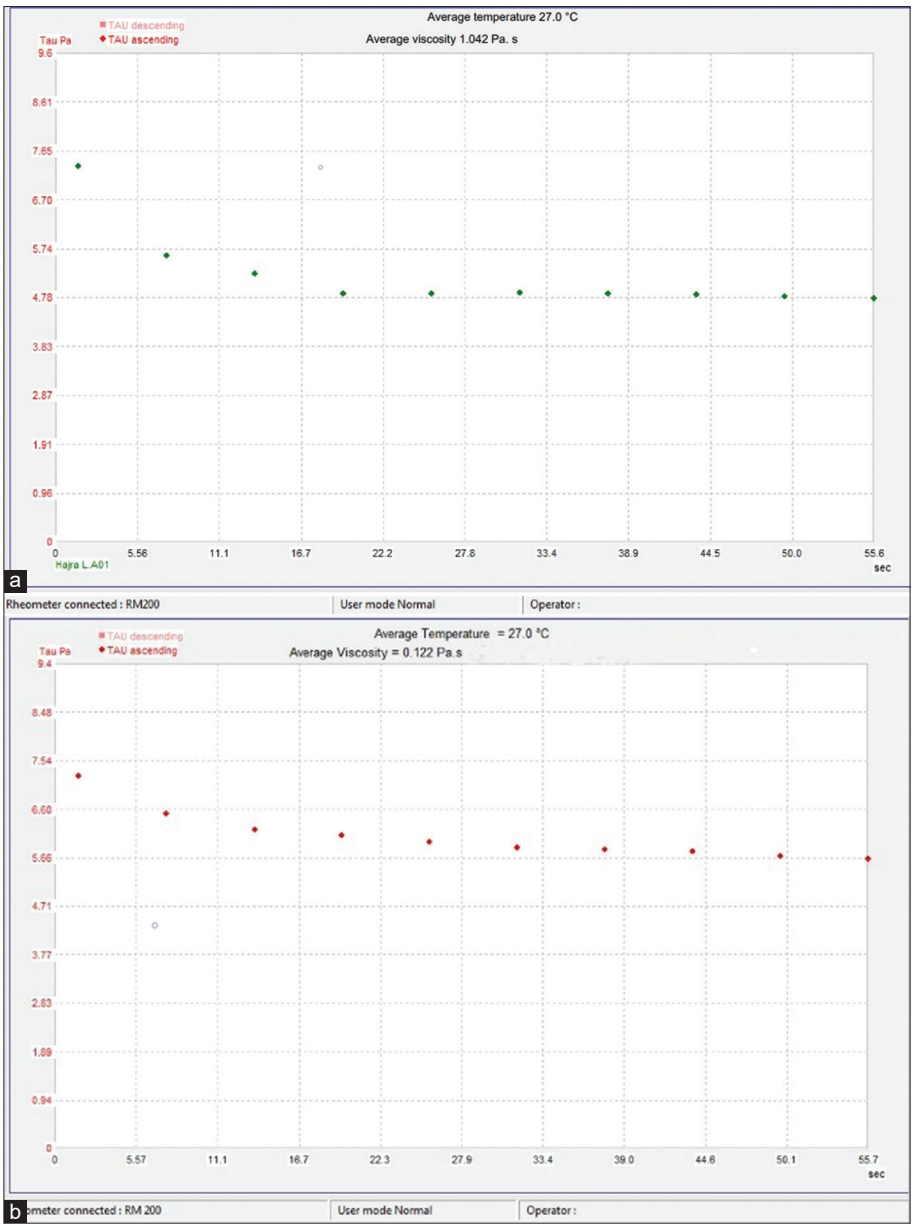


Figure 3: Rheograms of (a) tested emulsion (b) control emulsion

Table 7: SPF of the tested emulsion

Wavelength	EE*1	Absorbance readings				
		1st readings	2nd readings	3rd readings	Avg readings	Standard deviations
290	0.015	0.553	0.554	0.553	0.553	0.0043
295	0.0817	0.58	0.58	0.581	0.58	0.0043
300	0.2874	0.419	0.42	0.419	0.419	0.000
305	0.3278	0.315	0.315	0.315	0.315	0.0043
310	0.1864	0.205	0.206	0.206	0.206	0.0043
315	0.0837	0.158	0.158	0.159	0.158	0.0043
320	0.018	0.138	0.139	0.139	0.139	0.0043
SPF	1				23.7	

SPF: Sun protection factor, *its indicate *P*-value

Table 8: *In vitro* analysis of UVA/UVB protection of tested emulsion

S. No	Wavelength	Values
1	280	0.94
2	285	0.932
3	290	0.928
4	295	0.906
5	300	1.065
6	305	1.049
7	310	1.045
8	315	1.044
9	320	1.047
10	325	1.053
11	330	1.061
12	335	1.07
13	340	1.08
14	345	1.093
15	350	1.104
16	355	1.121
17	360	1.141
18	365	1.206
19	370	1.16
20	375	1.131
21	380	1.096
22	385	1.062
23	390	1.028
24	395	0.996
25	400	0.971
UVA/UVB ratio		0.51

UVA: Ultraviolet A, UVB: Ultraviolet B

Ostwald method were suitable for skin cosmetic formulations. Each Rheograms behavior indicated a flow index of <1, mimicking a pseudo plastic trend with shear-thinning behavior. Similar rheological properties were obtained with the control emulsion. These properties are necessary for the development of a perfect topical formulation. The primary cause of pseudo plastic behavior may be the internal structure of formulations gradually breaking down under increasing shear and then being rebuilt by Brownian movement.[14]

The UV radiation needed to produce one minimum erythema dose (MED) on protected skin following the application of a product at a rate of 2 mg/cm^2 divided by the UV radiation required to produce one MED on unprotected skin is represented by the SPF number.[28] *In vitro* screening techniques may prove to be useful tools for reducing the dangers associated with UV exposure in humans. The UVA/UVB ratio varies from 0 to >0.91, indicating bad to ultra-UVA protection, according to Boot's Star rating system. This shows that the emulsion offers effective UVB protection.[18] The proportion of the transmitter of a sun cream or lotion specimen across the UV spectrum, assessed by erythemal calculation factors at different wavelengths, is one of the calculations for determining UV protection features as indicated in the COLIPA standard and other regulatory authorities. In addition, excipients and other active ingredients can produce UV bands that compete with UV-A and UV-B agents. This outcome is shown in a final emulsion, with an SPF higher than 15. The UV-visible spectroscopy method was used to determine the SPF value for the emulsion that contained PhytoSpherix™ and Actigym™. It is generally believed that more protective effects can be obtained from goods with higher SPFs labels.[17]

Despite being subjective, this kind of evaluation can be a powerful, reliable, and repeatable tool.[25,26] The erythema level after applying the control and tested emulsions decreased after 48 h, according to the results of the instrumental safety testing. The effects of the control and tested emulsion on skin erythema, however, were found to be insignificant using the paired sample *t*-test, even though both emulsions reduced skin erythema. After the application of the control and tested emulsions, there was no erythema, edema, or irritation. The results produced from the paired sample *t*-test were not significant at 0 and after 48 h of control and tested emulsions.

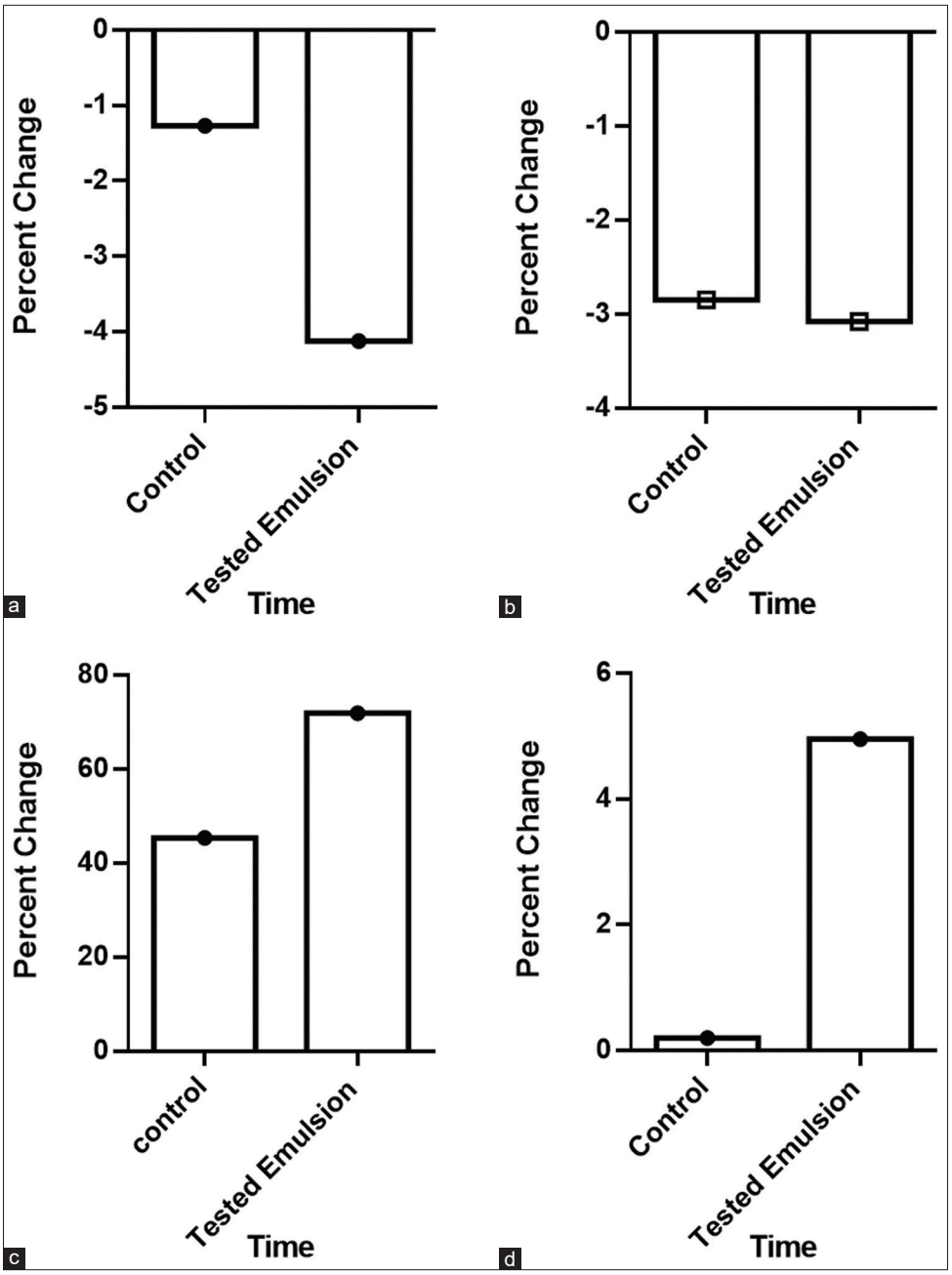


Figure 4: % change on (a) skin melanin, (b) skin erythema, (c) skin moisture, and (d) skin elasticity after application for a period of 7D with respect to baseline visit day of control and tested emulsion. A significant improvement in skin elasticity and moisture contents was found between the tested emulsion-treated side with compared to its control-treated side with respect to time. In addition, skin melanin and erythema contents in the tested emulsion-treated side compared to control treated with respect to time were found to be significantly decreased

Eventually, there was no longer any erythema, edema, or allergic or irritating symptoms. Therefore, emulsions that have been evaluated and established as control can be used securely for future skin research.

UV exposure increases the expression of MMP, inhibits the synthesis of procollagen, and damages the skin. Hyperpigmentation results from incomplete restoration of dermal injury, which compromises the structural and functional integrity of skin physiological activities,[29] skin erythema,[30] water capacity imbalance,[31,32] and collagen impairment.[33] The findings demonstrated that the tested emulsion increased the hydration and suppleness of the skin. A significant improvement in skin elasticity and moisture contents were found between the tested emulsion treated side with compared to its control treated side with respect to time when a paired sample *t*-test was used to determine the significant difference. Mibelle Biochemistry Group claims that PhytoSpherix™ energizes skin cells, encourages the formation of collagen and hyaluronic

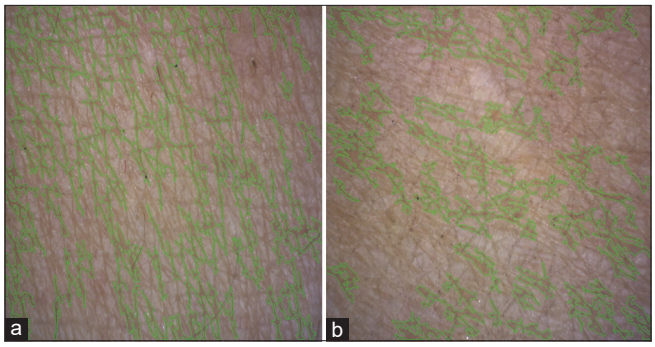


Figure 5: Visioscopic images of the right side of volunteer at (a) baseline visit and (b) 7-D visit after application of tested emulsion

acid, lessens wrinkles and fine lines, and enhances skin moisture. To access the live cells in the epidermis and dermis, PhytoSpherix™ may pierce the intercellular gaps in human skin.[34] In comparison to other moisturizing agents, PhytoSpherix™ can hold onto water for up to 10 days at the upper skin layer level without causing swelling. The result is PhytoSpherix™'s special capacity to bind water which was proved *in vivo* in this study. Phytoglycogen smaller particle size may enable deeper skin penetration and, as a result, greater water retention in the lower layers of the skin. The remarkable water-retention capacity of hyaluronic acid is complemented by the large water-retention capability of phytoglycogen, suggesting that the two polysaccharides may function as complementary humectants in moisturizing applications.[35] Actigym™ marine ingredients can also be applied to the face for lifting and tightening, lowering MMP expression, and repairing skin damage.[36] After a 7D research, it proved that the skin's elasticity and moisture content were improved by the topical co-delivery of PhytoSpherix™ and Actigym™ marine ingredients in the tested emulsion.

The findings demonstrated that the tested emulsion reduced the amount of erythema and pigmentation in the skin. Skin melanin and erythema contents in the tested emulsion-treated side compared to control treated with respect to time were found to be significantly decreased when the paired sample *t*-test was used. According to the website reference for Mibelle Biochemistry Group, PhytoSpherix™, a multifunctional active component, helps to lessen erythema and reduce melanin.[34] Literature alone does not prove that Actigym™ marine ingredient affects skin erythema and melanin; further investigations are required. Therefore, PhytoSpherix™ may be responsible for the effect it had on skin melanin and erythema in our findings. Calculations were made using the average scores donated by volunteers to represent the sensory characteristics.[14] Both showed similar performance after sensorial analysis. However, used as a sensory modifier with surfactants has played well in addition to sensory attributes of emulsions.[14] This test indicates that the prepared emulsion is acceptable and suitable in terms of its sensory qualities. Although similar earlier studies, this study has several limitations. One of the limitations of the study was the small sample size, which consisted of female volunteers, and the determination of UVA photo protection *in vitro*. The UVA/UVB ratio needs to be calculated to assess UVA absorption using persistent pigment darkening.

CONCLUSION

Our findings revealed that an emulsion containing PhytoSpherix™ and Actigym™ marine ingredient exhibits encouraging long- and short-term stability. The emulsion ameliorates effective UVB protection and has enhanced skin elasticity and moisture retention while lowering the amount of melanin and erythema. Additional release studies should be performed in future research to get maximum results from this emulsion.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Registrar Secretariat Academic Unit (PS) of COMSATS University Islamabad gave its approval for this study (Reference No. CUI-Reg/Notif-4474/21/3140). Written informed consent was taken from the volunteers to participate.

CONSENT FOR PUBLICATION

All the authors are agreeing for the publication.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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SUPPLEMENTARY TABLES

Table S1: Emulsion ingredients and functions

Phase	Ingredients	INCI	Function
A		Distilled water	Solvent
	Zemea	Vegetable glycerin	Humectant
	Gum acacia	Acacia senegal gum	Viscosity enhancer
	PhytoSpherix™	Phytoglycogen nanoparticles	Active/Natural re-energizer
	Actigym™ marine ingredient	Glycerin, water, bacillus/soybean ferment extract	Active/skin tightener
	Valvance® Touch 210	Silica	Sensory modifier
B	SustOleo™ MCT	Triheptanoin	Emulsifier
	SustOleo™ TSB	Hydrogenated rapeseed oil	Texturizer
		Cetyl alcohol	Emollient
	SustOleo™ GMS	Glyceryl Stearate	Emulsifier
C		Benzoic acid	Preservative

Table S2: pH of tested emulsion at 8°C, 25°C, and 45°C

Time	8°C	25°C	40°C
0 day	5.81	5.81	5.81
1st day	5.80	5.81	5.80
2nd day	5.80	5.80	5.80
3rd day	5.79	5.77	5.79
7th day	5.73	5.74	5.73
1st month	5.71	5.70	5.71
2nd month	5.69	5.68	5.69
3rd month	5.68	5.65	5.67
4th month	5.61	5.63	5.62
5th month	5.59	5.57	5.58
6th month	5.57	5.56	5.55