IJSCIA

International Journal of Scientific Advances
ISSN: 2708-7972
Volume: 5 | Issue: 1 | Jan – Feb 2024 Available Online: www.ijscia.com
DOI: 10.51542/ijscia.v5i1.26

Natural Anti-aging formulations with UV/Photo-protective potential containing Plant Derived Oils

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ABSTRACT

Topical natural oil-based anti-aging o/w emulsions with UV protection capability were developed with the help of sesame (F1), black cumin (F2), flax (F3), and poppy seed oil (F4) and their combination(F5) having comparable SPF and antioxidant activities and their values were calculated using UV spectrophotometer and DPPH scavenging test respectively. Five separate formulations of all oils were made and stored at various storage temperatures, including 8°C, 25°C, 40°C and 40°C ± 75% RH. These formulations were then examined for stability at their storage parameters which involved changes in color, odor, phase separation, pH, conductivity, centrifugation as well as spreadability for two months, with tests being conducted after a particular duration. According to the experimental findings, the antioxidant activity of sesame, black cumin, flax, and poppy seed oil is 63%, 59%, 72%, and 47%, respectively while the combination formulation showed 76% activity. On day 60th at 40°C ± 75%RH, the SPF values of F1, F2, F3, F4 & F5 were 2.53, 6.26, 8.11, 6.09, and 12.01 respectively. F1 and F2 displayed a white-to-yellowish color change on the 60th day at 40°C ± 75%RH. No formulations showed odor change. Phase separation was seen in F1 and F2 after two months at 40°C, while F3, F4, and F5 were stable and their spreadability also increased with time at an elevated condition while the pH of all five formulations tended to decrease. The study results provided evidence that combining essential oils produces effective outcomes without affecting the emulsion system stability.

Keywords: sesame; black cumin; poppy seed; flex seed; antioxidants; sunscreen

INTRODUCTION

The pursuit of everlasting youth and other innovative methods that could turn back the hands of time have grown rapidly. Skin aging can occur over time; nobody is ever going to be young forever [1]. According to a 2015 survey, drooping skin disturbed 67% of respondents, whereas skin surface imperfections and discoloration bothered 72% of respondents[2].

Rhytids, age spots, laxity, and dryness are among the most visible signs of aging and both intrinsic (cellular metabolism, gene mutation, as well as a hormonal factor) along with extrinsic (UV-rays, chemicals, pollutants, and toxins) factors contribute to specific alteration in the skin[3]. Epithelial cell membranes alter as a result of intrinsic aging. In contrast, extrinsic aging results in an enormous bulk of elastic fibers building up inside the dermis [4]. The goal of cosmetic science is to find substances that can mitigate these effects.

When UV rays penetrate the dermis deeply, they also stimulate the formation of metalloproteinases of the matrix as well as free radicals, which further leads to DNA mutation as well as starts the oxidative cascade [5]. This makes it easier for collagen to break down, which eventually causes wrinkles as well as fine lines to appear. Because they mitigate the consequences of ROS, antioxidant products are gaining popularity and admiration.

The aging process of the skin is significantly influenced by both endogenous and external causes. Epithelial cell membranes alter as a result of intrinsic aging. In contrast, extrinsic aging results in an enormous bulk of elastic fibers building up inside the dermis [4].

During the aging process, the dermis is seen to undergo significant changes. Collagen is broken down into smaller pieces, and its volume is significantly reduced. Matrix metalloproteinases along with enhanced ROS generation, which changes the signaling pathways, are the main causes of this process. Reduced collagen levels prevent fibroblasts and ECM from interacting, further impairing fibroblast function as well as lowering collagen levels[6].

Recent studies have demonstrated the effectiveness of herbal medicines, herbal products, and certain phytonutrients in slowing the aging process[7].

Sesamumindicum is indeed the source of sesame oil. The additive effect of tocopherol along with lignan contents in sesame contributes to the antioxidant properties. Sesame seeds are rich in lignans such as sesamolin, sesamin, and sesamol, all of which have antioxidant properties. It was also widely believed that sesamol is a potent antioxidative component found in sesame oil [8].

Black cumin seeds are sometimes referred to as black caraway, black onion, as well as black kalonji. They originate from N. Sativa. The thymoquinone, t-anethole, carvacrol, and 4-terpineol components of black cumin seeds and their essential oil all indicated noteworthy radical scavenging properties [9]. High levels of unsaturation, natural phenolic compounds, as well as tocopherols all contributed to BCSO's relative oxidative stability improvement [10].

The ripened, dried seeds of the flax plant are used to prepare the transparent to pale yellow oil known as flaxseed oil. Linoleic acid, alpha-linolenic acid, and beta-linolenic acid, as well as lignin, carotenoids, phenolics, and tocopherols, are examples of antioxidants that are all present in flaxseed [11].

The seeds of the plant papaver somniferous, which produces poppies, are used to form poppyseed oil. Along with other antioxidants like phenolics, tocopherols, and tocotrienols play a crucial role in maintaining unsaturated fats in foods and also offer the body excellent protective effects toward anti-oxidative stress [12].

The study's specific goals and objectives were:

- Prepare four plant oils containing O/W emulsions with compositions such as sesame seed oil, black cumin seed oil, flaxseed oil, and poppy seed oil.
- Evaluation of combined effect and productivity of natural oil-containing cream to ascertain their anti-oxidant activity and UV-ray protection factor.
- Identify the cream's in-vitro stability under varied storage parameters.
- Examine the degree of protection provided by cream without synthetic UV filters against UV radiation which, in turn, is effective in preventing the photo-aging process.

MATERIAL & METHODS

Material

Chemicals and Materials

All materials used in formulation and evaluation were of analytical grade.

Methods

Formulation of Anti-Aging Cream

2% concentration of sesame, black cumin, flax, and poppy seed oils was used to develop an o/w (oil-in-water) type emulsion with UV protection capabilities. A combination formulation was developed to assess the SPF, anti-aging properties & various physicochemical parameters.

Phase	Ingredients	F1	F2	F3	F4	F5
Oil phase	Oils	2% Sesame seed oil(SSO)	2% Black cumin seed oil (BCSO)	2% Flax seed oil(FSO)	2% Poppy seed oil (PSO)	2% Combination of all oils
	Span 60	2%	2%	2%	2%	2%
	Cetyl alcohol	3%	3%	3%	3%	3%
	Steric acid	6%	6%	6%	6%	6%
	Liquid paraffin	10%	10%	10%	10%	10%
Water phase	Tween 80	2%	2%	2%	2%	2%
	Glycerin	5%	5%	5%	5%	5%
	Water	q. s	q.s	q. s	q. s	q. s

Weigh every ingredient in a different beaker, giving it a unique label. The internal (oily) as well as external (aqueous) phases were heated to 75°C with the help of water bath. The oily phase then slowly incorporated into the aqueous phase while homogenizing continuously for about 10 minutes at 2000 rpm. After the liquid had cooled, the homogenizer's rotating speed was decreased gradually to 1500 rpm, 1000 rpm and 500 rpm respectively, and homogenized for 10 minutes at each speed.

Each formulation's entire process took 30 to 40 minutes to complete. The emulsions were then left to cool at room temperature and then divided into four equal portions and stored at 8°C, 25°C, 40°C, and 40°C ± 75% Rh to conduct the stability test.

In-vitro Characterization of Anti-Aging Cream

- **Determination of Antioxidant Properties:** A DPPH free radical test was used to assess anti-oxidant properties of tested samples. To accurately measure antioxidant activity, weigh 24 mg of DPPH and mix it with 100 mL of methanol to make a DPPH stock solution, which was then frozen. The absorbance of DPPH stock solution was in the region of (0.9-1.1). Set the absorbance in the appropriate range after diluting stock solution of 10ml using 65ml of methanol. Antioxidant activity of all oils was measured at 517nm with the help of spectrophotometer using the mixture of 0.15 ml of oils and 2.85 ml of solution DPPH. This solution then kept in the dark about 20-30 minutes till the reaction was complete before screening for free radical scavenging activity.

The following formula was used to assess this activity: % Inhibition= [absorbance of control – absorbance of test solution /absorbance of control] ×100

Where,
Control absorbance = blank solution absorbance.
Test solution absorbance = absorbance of test solutions (All Oils + Solution of DPPH).

- **Determination of Factor of Sun Protection:** The same approach as previously described was used to assess the SPF of the anti-aging cream. 1.0g of cream was precisely weighed and put in volumetric flask of 100ml, and the total volume was made up with 100ml of ethanol. This dilution was ultra-sonicated for 10-15 minutes using an ultra Sonicator and purified with No. 1 Whatman's filter paper, after discarding the first 10ml. The SPF of all formulations was determined using the same procedure. After that absorbance spectrum of all formulation solutions were measured using a UV-spectrophotometer. At intervals of 5nm, absorption measurements were obtained (in triplicate) at wavelengths ranging from 290nm to 320nm. SPF was measured using the Mansur equation.

$$SPF_{spectrophotometric} = CF \times {}^{320}\sum_{290} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

CF = Correction Factor which is 10
EE (λ) = Erythemal Effect of radiation at wavelength (λ)
I (λ) = Spectrum of solar light intensity
Abs (λ) = Sample/sunscreen absorbance value at wavelength (λ)

The total of the absorbance measurements was multiplied by the radiation's EE (erythemal effect) as well as with correction factor. "EE x I is a normalized product function with constant values as shown in the table. The formula EE x I=1 was employed to calculate the SPF in this test.

TABLE 1: Normalized product function used in the calculation of SPF.

Wavelength (λ max)	EE x I (Normalized)
290 nm	0.051
295 nm	0.0817
300 nm	0.2874
305 nm	0.5378
310 nm	0.1864
315 nm	0.0839
320 nm	0.018
Total	**1**

- **Organoleptic Analysis:** Organoleptic qualities (Texture, Color, Thickness, overall appearances) and physical characteristics including phase separation were assessed in all prepared formulations. Over the course of three months, stability studies were performed on samples stored at 80°C, 250°C, 400°C, and 40°C + RH 75%. Freshly prepared formulations were tested, followed by formulations that had been exposed to various temperatures for 15, 30, 45, 60, 75, and 90 days.

- **Electrical Conductivity Analysis**: Electrical conductivity tests were performed on all prepared formulations in order to establish the type of emulsion. A digital conductivity meter was used to achieve this goal. Observations were kept over the duration of the study to see whether the formulations had changed.

- **Centrifugation Analysis**: The centrifugation analysis was done by centrifuging 10 g of all prepared samples for 10 minutes at 5000 rpm in centrifugation tubes. After each 15 days, the test should be repeated for every formulation, which is held at varied temperatures (8°C, 25°C, 40°C, and 40°C ±75 %RH) for three months.

- **pH Analysis:** Digital pH-Meter was used to analyzed, the pH reading of the all formulation at different temperatures (8°C, 25°C, 40°C, and 40°C±75% RH). Measurements were obtained immediately after the creams were prepared, and then every 15 days for the next three months.

- **Spreadability Analysis:**0.5 g of cream was weighed and dispersed it over a circle of 1cm diameter which is marked on a glass plate, then using the second glass plate for evaluation of the spreadability. After that, for the interval of 5 mins, 500g of load was put on the outermost glass plate. The circular diameter was then measured once the cream had dispersed. This test was performed every 15 days for 3 months for each formulation.

RESULTS

Antioxidant test

The anti-oxidant potential of SSO, BCSO, FSO and PSO was assessed using the DPPH technique in comparison to the benchmark vitamin C. (Ascorbic Acid). The results are tabulated in table 4.1 and fig 4.1 presents a pictorial comparison.

TABLE 2: DPPH Analysis of Vit.C, SSO, BCSO, FSO and PSO.

Serial No.	Name	DPPH value
1	Vitamin C	96%
2	SSO	63%
3	BCSO	59%
4	FSO	72%
5	PSO	47%
6	Combination of oils	76%

SSO= Sesame Seed Oil
BCSO= Black Cumin Seed Oil
FSO= Flax Seed Oil
PSO= Poppy Seed Oil

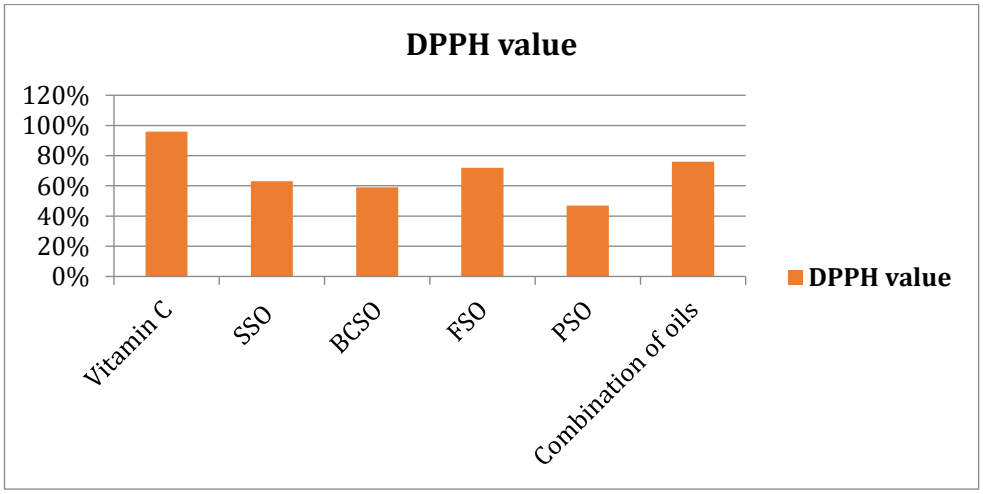

FIGURE 1: Antioxydant test.

SPF ANALYSIS

The SPF values of all the five formulations was determined at various temperatures (8 C, 25 C, 40 C, and 40°C ± 75% RH) at periods of 1, 7, 14, 28, 42, as well as 60 days by applying the Mansur's equation.

TABLE 3: SPF analysis at different temperatures.

Parameter	Time	Formulations				
		F1	**F2**	**F3**	**F4**	**F5**
SPF analysis at 8˚C	**Day 1**	2.85	6.93	8.78	6.64	12.75
	Day 7	2.83	6.84	8.62	6.52	12.61
	Day 14	2.78	6.69	8.51	6.48	12.47
	Day 28	2.76	6.51	8.49	6.31	12.38
	Day 45	2.71	6.42	8.23	6.29	12.12
	Day 60	2.62	6.28	8.17	6.15	12.09
SPF analysisat 25˚C	**Day 1**	2.98	6.95	8.79	6.63	12.74
	Day 7	2.85	6.88	8.63	6.51	12.60
	Day 14	2.78	6.68	8.50	6.46	12.46
	Day 28	2.77	6.52	8.48	6.33	12.35
	Day 45	2.72	6.43	8.22	6.27	12.15
	Day 60	2.61	6.29	8.16	6.12	12.08
SPF analysis at 40˚C	**Day 1**	2.84	6.91	8.76	6.61	12.73
	Day 7	2.79	6.83	8.59	6.49	12.58
	Day 14	2.76	6.65	8.48	6.43	12.43
	Day 28	2.73	6.49	8.44	6.28	12.31
	Day 45	2.68	6.41	8.20	6.25	12.11
	Day 60	2.55	6.27	8.12	6.09	12.02
SPF analysis 40˚C± 75%RH	**Day 1**	2.82	6.90	8.74	6.60	12.72
	Day 7	2.80	6.82	8.57	6.48	12.56
	Day 14	2.75	6.61	8.45	6.42	12.42
	Day 28	2.73	6.47	8.45	6.27	12.30
	Day 45	2.67	6.39	8.19	6.24	12.08
	Day 60	2.53	6.26	8.11	6.09	12.01

F1= Sesame seed oil formulation (SSO).
F2= Black Cumin seed oil Formulation (BCSO).
F3= Flax seed oil formulation (FSO).
F4= Poppy seed oil Formulation (PSO).
F5= Formulation containing combination of oils (SSO, BCSO, FSO, PSO).

Parameters	F1	F2	F3	F4	F5
Appearance	Smooth, Opaque	Smooth, Opaque	Smooth, Opaque	Smooth, Opaque	Smooth, Opaque
Color	Yellowish	Yellowish	White	White	White
Odor	Not Offensive	Not Offensive	Not Offensive	Not Offensive	Not Offensive
Texture	Smooth	Smooth	Smooth	Smooth	Smooth
Consistency	Good	Good	Good	Good	Good
Irritation	Non-irritant	Non-irritant	Non-irritant	Non-irritant	Non-irritant

pH Analysis

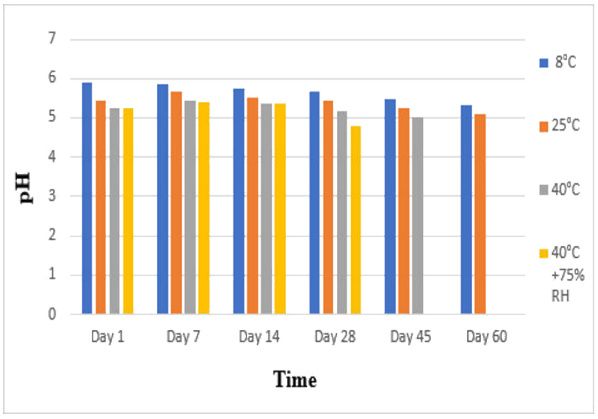

FIGURE 2: pH Analysis of F1 (SSO).

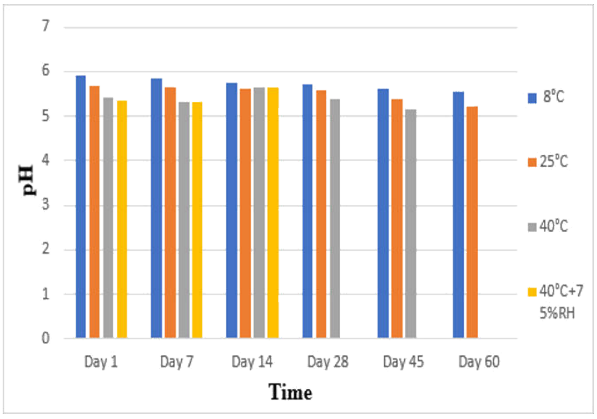

FIGURE 3: pH Analysis of F2 (BCSO).

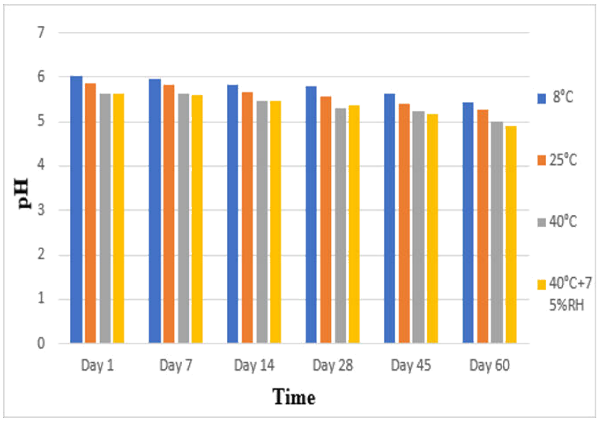

FIGURE 4: pH Analysis of F3 (FSO).

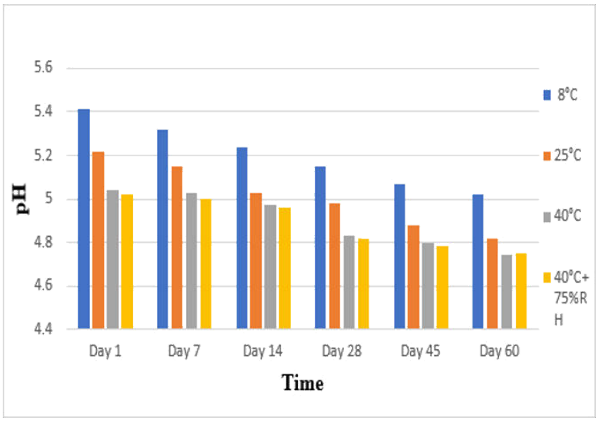

FIGURE 5: pH Analysis of F4 (PSO).

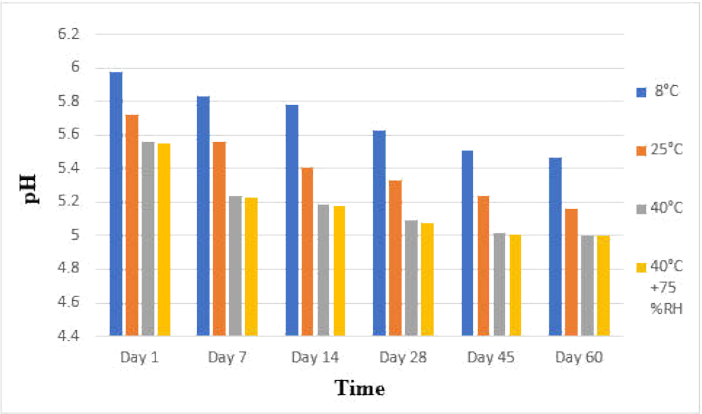

FIGURE 6: pH Analysis of F5 (Combination of all oil).

Conductivity Analysis

Conductivity tests were carried out to identify the emulsion type and were then extended for performing the stability tests at 8°C, 25°C, 40°C, and 40°C ± 75% RH.

Parameter	Time	Temperature			
		8°C	25°C	40°C	40°C ± 75% Rh
Conductivity analysis of F1 (SSO Formulation)	Day 1	41.7	44.3	48.5	49.2
	Day 7	41.5	43.1	47.4	48.5
	Day 14	39.2	41.8	45.2	47.5
	Day 28	37.6	40.7	44.8	46.4
	Day 45	36.1	38.2	43.1	N/A
	Day 60	35.8	37.9	N/A	N/A
Conductivity analysis of F2 (BCSO Formulation)	Day 1	102.5	107.3	111.4	114.2
	Day 7	99.1	106.2	110.1	112.3
	Day 14	97.8	105.6	109.8	105.8
	Day 28	96.7	103.5	107.4	N/A
	Day 45	95.9	101.4	104.2	N/A
	Day 60	94.6	98.7	N/A	N/A
Conductivity analysis of F3 (FSO Formulation)	Day 1	43.4	46.9	49.8	50.1
	Day 7	42.2	45.1	48.2	49.2
	Day 14	40.8	43.8	47.3	48.3
	Day 28	39.5	42.2	46.7	47.9
	Day 45	38.7	40.1	45.4	45.8
	Day 60	36.0	39.9	43.9	44.7
Conductivity analysis of F4 (PSO Formulation)	Day 1	58.6	63.1	66.7	68.2
	Day 7	57.1	62.9	64.8	67.9
	Day 14	56.5	61.2	63.4	66.0
	Day 28	55.3	60.3	62.2	64.7
	Day 45	53.4	58.7	63.0	63.6
	Day 60	51.6	57.4	60.9	62.1
Conductivity analysis of F5 (Combination Formulation)	Day 1	62.4	65.6	69.1	69.8
	Day 7	61.8	64.2	68.2	68.7
	Day 14	60.3	62.9	67.8	67.9
	Day 28	60.0	66.5	66.5	66.2
	Day 45	58.1	64.6	64.6	65.9
	Day 60	56.4	63.4	63.4	64.1

Centrifugation Analysis

Separation of the phase of all formulations was examined using a centrifugation analysis maintained at 8°C, 25°C, 40°C, and 40°C ± 75% Rh.

Parameter	Time	Temperature			
		8°C	25°C	40°C	40°C ± 75% Rh
Centrifugation analysis of F1 (SSO Formulation)	Day 1	S	S	S	S
	Day 7	S	S	S	S
	Day 14	S	S	S	S
	Day 28	S	S	S	S
	Day 45	S	S	SS	SS
	Day 60	S	S	SS	SS
Centrifugation analysis of F2 (BCSO Formulation)	Day 1	S	S	S	S
	Day 7	S	S	S	S
	Day 14	S	S	S	S
	Day 28	S	S	S	SS
	Day 45	S	S	S	SS
	Day 60	S	S	SS	SS
Centrifugation analysis F3 (FSO Formulation)	Day 1	S	S	S	S
	Day 7	S	S	S	S
	Day 14	S	S	S	S
	Day 28	S	S	S	S
	Day 45	S	S	S	S
	Day 60	S	S	S	S
Centrifugation analysis of F4 (PSO Formulation)	Day 1	S	S	S	S
	Day 7	S	S	S	S
	Day 14	S	S	S	S
	Day 28	S	S	S	S
	Day 45	S	S	S	S
	Day 60	S	S	S	S
Centrifugation analysis of F5 (Combination Formulation)	Day 1	S	S	S	S
	Day 7	S	S	S	S
	Day 14	S	S	S	S
	Day 28	S	S	S	S
	Day 45	S	S	S	S
	Day 60	S	S	S	S

S= Stable. SS = slight sedimentation.

Spreadability Analysis

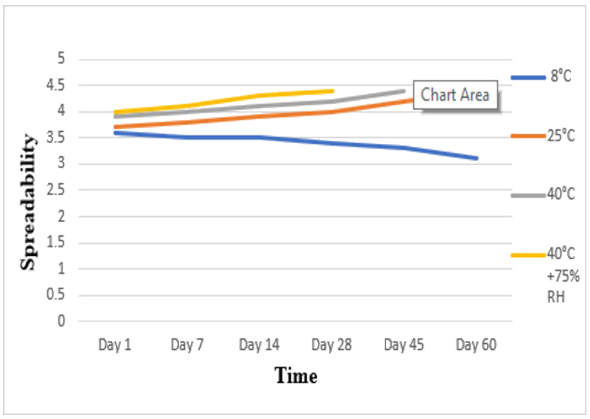

FIGURE 7: Spreadability Analysis of F1 (SSO).

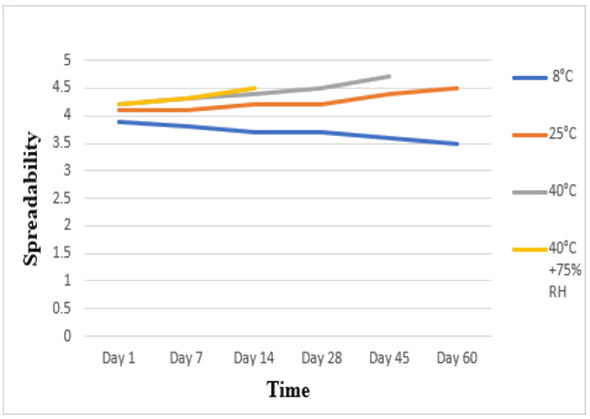

FIGURE 8: Spreadability Analysis of F2 (BCSO).

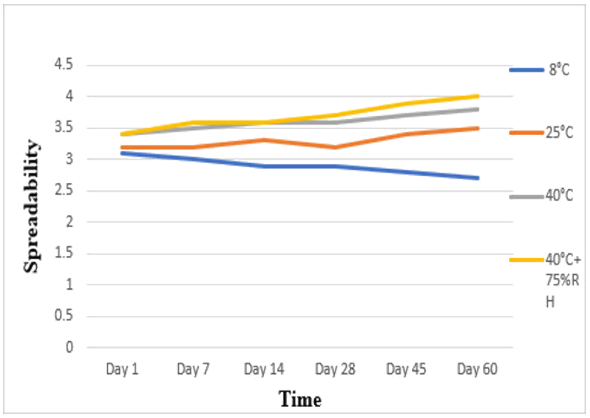

FIGURE 9: Spreadability Analysis of F3 (FSO).

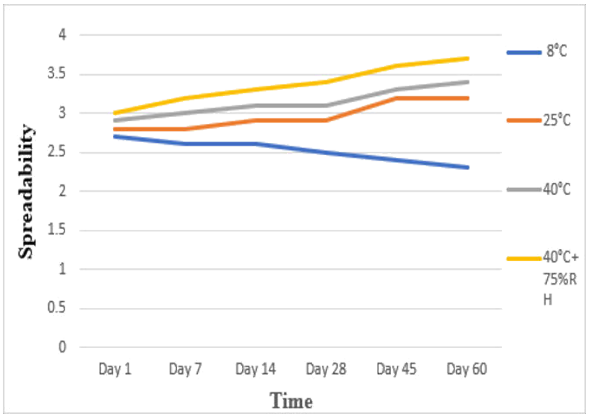

FIGURE 10: Spreadability Analysis of F4 (PSO).

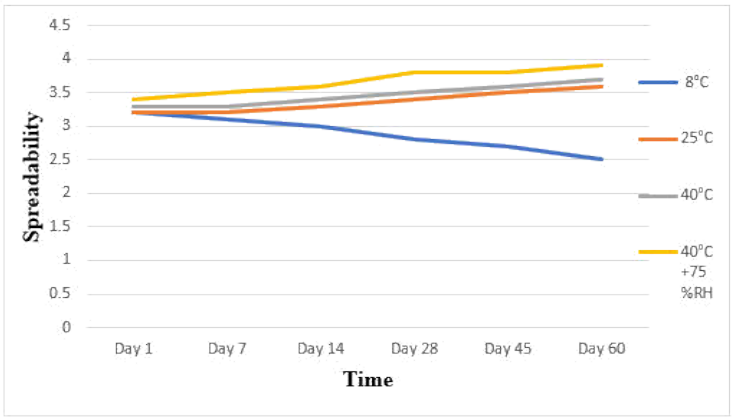

FIGURE 11: Spreadability Analysis of F5 (Combination of all seed oils).

DISCUSSION

Anti-oxidant assay

The DPPH assay is indeed a quick, simple, popular, as well as affordable way to test the antioxidant, H-donor, or free-radical scavenger activity of natural substances. 63% DPPH value of SSO showed that it has strong antioxidant activity such reading matches the findings of other investigations [13].59% DPPH value of BCSO is mainly due to high levels of unsaturation, natural phenolic compounds, as well as tocopherols. All these factors contributed to BCSO's relative oxidative stability improvement. Such findings were approximately similar to another study having 61% DPPH analysis value [14].

The 72% DPPH values of FSO is mainly due to omega-3 fatty acids, which is present in flax seed, inhibit the formation of interleukin-1, tumor necrosis factor, leukotriene B4, as well as oxygen free radicals from monocytes (LTB4) and neutrophils which was justified by other investigator findings which showed that 70% DPPH values of FSO [15].Poppy seeds contain vitamin E in its naturally existing form, which is potent antioxidant results in 47% DPPH value approximately similar to another research which was 49% [16].

SPF analysis

The SPF assesses the extent of UV radiation damage that reaches the skin's surface. SPF indirectly suggests how long can be stayed in the sun without producing damage to the skin as well as without protection [17].Freshly made F2 formulation with BCSO showed SPF values such as 6.28, 6.29, 6.27 and 6.26 at day 60 when kept at 8˚C, 25˚C, 40˚C, and 40˚C +75% RH. The BCSO has a comparatively high protection factor of UVA (PFA) and shielding power (SPF) rating. It offers defense against UV-A as well as UV-B rays, which are sources of oxidative damage to skin [18]. Freshly made F3 formulation with FSO showed relatively higher SPF values such as 8.17, 8.16, 8.12 and 8.11 at day 60 when kept at 8˚C, 25˚C, 40˚C, and 40˚C ± 75% RH which is mainly due to Omega-3 fatty acids[15].

Freshly made F4 formulation with PSO showed relatively lower SPF values such as 6.15, 6.12, 6.09 and 6.09 at day 60 when kept at 8˚C, 25˚C, 40˚C, and 40˚C +75% RH, but have sun protection properties. Tocopherols, a type of radical scavenger, are abundant in PSO [19]. Tocopherols as well as tocotrienols play a vital role in providing the body with good anti-oxidative stress-protective and UV-ray protective actions, along with other antioxidants such phenolics justified by others study findings [20].

Freshly made F5 formulation with combination of all oils showed relatively higher SPF values such as 12.09, 12.08, 12.02 and 12.01 at day 60 when kept at 8˚C, 25˚C, 40˚C, and 40˚C +75% RH. As result of the synergistic interactions between the active components of all four distinct seed oils which were employed to formulate F5, better photo-protective outcomes where observed& it demonstrated the potential to offer significant protection against the harmful effects of sunlight.

Colour

No deterioration was seen during the 60-day study period, with the exception of the F1 and F2 held at 40 C + 75 % RH. Due to high temperatures and the moisture content, some formulation shows change in colour[21].

Odour

No offensive odour was noticed from any of the tested formulations while stored for a 60 days analysis period at 8 °C, 25 °C, 40 °C, as well as 40°C ± 75% RH.

Phase Separation

Three phenomena, aggregation, flocculation, as well as coalescence, when coupled, can lead to phase separation along with destabilizations of emulsion. At greater temperatures, phase separation hastendency to proceed more quickly. Phase separation can be avoided with adequate homogeneity of the formulation [22]. Except for F1 formulation, none of the five formulations tested throughout the 60-day testing period showed evidence of phase separation while stored at 8 °C, 25 °C, 40 °C, and 40°C ± 75% RH.

pH Analysis

The freshly prepared formulations F1, F2, F3, F4 and F5 were found to have pH values of 4.75 to 5.02 while stored at temperatures such as 8°C, 25°C, 40°C, and 40°C ± 75% RH. The pH of all five formulations tends to drop during the evaluation period of two months [23].

Conductivity Analysis

Regardless of the amount of the mS/cm, a conductivity test can assist distinguish between the kind of emulsion generated, either O/W or W/O [24]. Results revealed that preparation contain the oil as internal phase and water as external phase.

Centrifugation Analysis

A typical technique for accelerating phase separation as well as destabilizing an emulsion composition is centrifugation[25]. Experiment revealed that F3, F4, & F5 formulations remained stable at a higher temperature for an extended period of time.

Spreadability

Results revealed that spreadability was increased when the formulation was stored at a higher temperature for longer period of time [26].

CONCLUSION

The study results provided credible evidence that combining natural oils produces effective outcomes without affecting the system's stability. Combined formulation, with all five natural plant oils, has high antioxidant and UV protection potential which help in deceleration of the aging process. Furthermore, this formulation is quite stable & performed well when stored at different temperatures.

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