RESEARCH ARTICLE

Physical Characterization and *In vitro* Sun Protection Factor Determination of Anti-Ageing Poly Herbal Cream

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ABSTRACT

Aging may be due to simple chronological passing of years or maybe due to photoaging induced by prolonged exposure to the sun. Herbal cosmetics are the preparations used to enhance human appearance by playing a major role in impeding and reversing the ageing of the skin. Thus, herbal creams are safe to use and can be used as the provision of a barrier to protect skin. In the present study, four creams (AA1, AA2, AA3, and AA4) were formulated by varying the concentrations of herbal oils like tamanu oil, lavender oil, etc., for physical characterization and also to determine the *in vitro* sun protection factor of anti-aging polyherbal cream. The evaluations were done for all the formulations on the parameters like pH, spreadability, extrudability, stability, etc. The Ultraviolet-B protection of a sun care product is given by the sun protection factor (SPF). *In vitro* SPF was determined using Mansur equation by UV-spectrophotometer at the range of 200 to 320 nm. All formulations showed good spreadability, good consistency, homogeneity with good appearance, pH, no evidence of phase separation, and ease of removal. But a little change in color was observed for creams AA2 and AA4. The formulations AA1 and AA3 showed no redness, edema, inflammation, and irritation to the skin during irritancy studies on rabbits. *In vitro* SPF of AA1 and AA3 were found to be 2.16 ± 0.57 and 2.21 ± 0.6, respectively. Stability studies were also performed over a period of 3 months, while maintaining the products at 4, 25, and 40°C, as per ICH guidelines for the parameters like pH, spreadability, extrudability, and viscosity. Statistical analysis was also performed using SPSS software 17.0 version. Ultimately, AA1 and AA3 were found no change and maintained their stability during the period of 3 months and by statistical analysis.

Keywords: Anti-ageing cream, Herbal oils, *In vitro,* Sun protection factor.

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INTRODUCTION

Skin is the outermost, largest organ which shows first line of defense for the body. It is most sensitive to photodamage as it is directly exposed to solar radiation and other environmental factors.[1] Solar light consists of different ultraviolet (UV) radiations: UV-A in the 320 to 400 nm, UV-B 290 to 320 nm, and UV-C 100 to 290 nm range, respectively.[2] Skin aging is a natural phenomena, complex and dynamic process induced by constant exposure to UV radiations which leads to changes in the physiology of the skin.[3] Aging is of two types: chronological or intrinsic aging and photoaging. While, intrinsic aging is natural and mainly due to the passage of time (influence of genetic factors, oxidative stress, cellular senescence, etc.) and its consequences. Photoaging is mainly linked with the detrimental effects of solar exposure on the skin, although pollution, diet, and smoking are also contributing factors.[4] Photoaging is also called as premature skin aging.[5] Cosmetic products are created for the purpose of cleansing, beautifying or altering appearance, and enhancing attractive features of the human body.[6] To minimize the deleterious effect of UV-rays, photoprotection is important.

Various herbal formulations and chemicals are being available to block UV rays and to prevent all types of skin damages.[7] Oily vehicles are more effective for producing a uniform and long-lasting film of sunscreen on the skin, and their emollient properties protect the skin against the drying effects of exposure to wind and sun. Volatile oils are used as perfumes in cosmetics. The use of skincare products supplemented with several effective agents working through different pathways in conjunction with the use of sunscreens may be an effective approach for reducing UV-B generated ROS-mediated photoaging.[8] Sunscreen preparations are used to protect the skin from harmful aspects of ultraviolet radiation particularly UV-B radiation. Sunscreen products are considered as any preparation which intended to be applied on skin to protect it from UV radiation through absorbing,

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scattering, or reflecting radiation.[9] Formulations such as creams, ointments, oils, gels, and sprays are most widely used. Most of the sunscreens are applied topically on skin surface, which leads to penetration of the ingredients into deeper layers of skin, which may cause toxic effects.[10] The ideal sunscreen product should provide good protection against the entire range of UV spectrum, even after sunlight exposure. It should be non-toxic, non-irritating, and should not produce any type of allergy.

The present study aims at developing and evaluating anti-aging creams made of naturally available herbal oils having sun protection activity, which may cause no irritation and also gives nourishment to the skin.

METHODOLOGY

Materials

Herbal oils used in the study include are tamanu oil, grapeseed oil, peppermint oil, sandalwood oil, clove leaf oil, and lavender oil (Dr. Jain's Forest Herbals Pvt. Ltd.). Stearic acid and cetyl alcohol (Himedia Labs Pvt. Ltd., Mumbai). Triethanolamine, glycerin, and propylene glycol are from Merck Company, Mumbai. Parabens were purchased from Sd. Fine. Chem. Ltd., Mumbai. All other analytical grade solvents were used. *Aloe vera* juice and tomato juice were freshly prepared and used.[11,12]

Methods

Preparation of Anti-Aging Cream

All the oil-soluble ingredients were taken in one beaker, and all aqueous soluble substances were added to another beaker. Both the phases were heated to 70 to 74°C. Then, aqueous phase was added to oily phase with continuous stirring till a homogeneous mixture was formed (Tables 1 and 1A).

EVALUATION

Physical appearance of the formulations

Organoleptic Test

The prepared formulations were observed for the color and odor.

Homogeneity

The prepared creams were tested for homogeneity by visual appearance and by touch.

After Feel

Emolliency, slipperiness, and amount of residue left after the application of fixed amount of cream was checked.

Removal

The ease of removal of the cream applied was examined by washing the applied part with tap water.[13]

pH Measurement

A 0.5 grams of the weighed formulation was taken in a beaker and dispersed in 50 mL of distilled water.[14] The pH was measured using digital pH meter in triplicates, n = 3.

Viscosity Measurement

Viscosity measurement of the formulations were done using a rotational viscometer[15] (Brookfield DVIII + Rheometer/ model: LV, with spindle no. 52 having a speed of 50 rpm, 25 ± 1°C). Measurements were taken in triplicates, n = 3.

Spreadability

Excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1,000 grams weight for 5 minutes. Weight was added to the pan. The time

Table 1: Composition of optimized base cream

Oil phase	
Ingredients	*Concentration (% w/w)*
Stearic acid	10
Cetyl alcohol	2
Almond oil	0.75
Olive oil	0.75
Rose oil	0.75
Carrot seed oil	1.5
Tulsi oil	1.0
Propylparaben	0.05
Aqueous phase	
Triethanolamine	3
Glycerine	5
Propylene glycol	3
Methylparaben	0.05
Zinc oxide	0.5
Purified water	Up to 100 mL

Table 1A: Composition of herbal oils and juices used in various anti-aging creams

Herbal oils	*Cream's codes*			
AA1 (mL)	*AA1 (mL)*	*AA2 (mL)*	*AA3 (mL)*	*AA4 (mL)*
Tamanu oil	0.25	0.5	0.75	1
Grape seed oil	0.5	0.5	0.5	0.5
Peppermint oil	0.25	0.5	0.75	1
Sandalwood oil	0.25	0.25	0.25	0.25
Clove leaf oil	0.5	0.5	0.5	0.5
Lavendor oil	0.25	0.5	0.75	1
Juices (freshly prepared)				
A. vera juice	5	10	20	30
Tomato juice	2.5	5	7.5	10

required to separate the two slides, i.e., the time in which the glass slide moves over the lower plate was taken as measure of spreadability[16] (g.cm/sec). The method was repeated in triplicates (n = 3).

$$S = m \times l/t$$

Where,

m = weight tide to upper slide, l = length moved on glass slide, and t = time taken

Extrudability

The apparatus consists of a wooden block inclined at an angle of 45ºC fitted with a thin, long metal strip (tin) at one end, while the other end was free. The aluminium tube containing 20 grams of cream was positioned on inclined surface of wooden block. One kilogram weight was placed on free end of the aluminium strip and was touched for 30 seconds. The quantity of cream extruded from each tube was noted.[17] The test results were taken as n = 3 and expressed as percentage extrudability.

Performance and Drying Test

It was performed for about 48 hours after the preparation of formulations. Samples are taken on a glass slide using brush, forming a uniform thick layer of about 1 mm. Then the slide was placed in an oven at 36.5 ± 2.0ºC for 1-hour simulating the body temperature. The formulations were monitored up to 10 minutes until the drying process was completed and allowed the complete removal of the film from the glass slide.[18]

Centrifugation Test

All the formulations immediately after preparation were subjected to centrifugation test at 24 hours, 7, 14, and 30 days at 25ºC at 5,000 rpm for 10 minutes by placing the 5 grams sample in stopper centrifugal tubes.[19]

Primary Skin Irritation Studies

Primary skin irritation studies of the formulations were performed using albino rabbits. Rabbits were kept in cages, and supplied with fresh food and water during the test period, 24 hours prior to test. Hair was removed from neck and thigh region and cleaned with surgical spirit to expose sufficient test area.[20] The creams were applied to the test area and observed for erythema and edema for 24, 48, and 72 hours. KU Animal Ethical Committee approval number: IAEC/21/UCPSc/KU/2016.

Table 2: Relationship between erythemogenic effect and radiation intensity

Wavelength (λ_{nm})	*EE × I (normalized)*
290	0.015
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.018
Total	1

***In vitro* SPF using Mansur Equation**

A 1-gram of sample was weighed and taken in 100 mL volumetric flask and dilute with ethanol. Filtered through Whatman filter paper. First, 10 mL was rejected, a 5 mL aliquot was taken and transferred into 50 mL volumetric flask. Once again diluted with ethanol. Then, 5 mL aliquot was transferred into 25 mL volumetric flask, diluted with ethanol. From these absorbance spectra of each aliquot prepared was determined from 290 to 320 nm using UV spectrophotometer (SL-159, Syntonics, India), taking ethanol as blank at every 5 nm, performed in triplicate (n = 3), followed by the application of Mansur equation.[21] EE(λ) × I(λ) values are mentioned (Table 2).

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

Where, CF is correction factor (= 10); EE(λ) = erythemal effect of radiation with wavelength λ; I(λ) = solar intensity spectrum; Abs (λ) = absorbance of sunscreen product. The values of EE(λ) × I(λ) are constants as given determined by Sayre *et. al.* (1979). The obtained absorbance values Abs(λ) were multiplied with the respective EE(λ) × I(λ) values, and then summation was taken and multiplied with the correction factor 10.

Stability Study

All the prepared creams were subjected to stability studies at different temperature conditions, i.e., 25ºC (room temperature), 4ºC (refrigerator), and 40ºC (stability chambers) with 75% relative humidity (RH) and the parameters like pH, spreadability, viscosity, and extrudability were measured for a period of 3 months as per ICH guidelines.[22]

Statistical Analysis

The evaluation of data was done using SPSS software (version 17.0). One way ANOVA followed by Dunnet's test was used for data expressed as mean ± standard deviations. For all comparisons, a value of $p < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Physical Appearance of the Formulations

Organoleptic parameters like color—there is a little change for the formulations AA2 and AA4. No phase separation was observed for all creams. All the data was given (Table 3).

Table 3: Physical appearance of the formulations

Formulation code	*Color*	*Odor*	*Centrifugation (phase separation)*	*After feel*	*Removal*
AA1	Creamy white	Pleasant	No	Good	Easily removed
AA2	Little change	Pleasant	No	Good	Easily removed
AA3	Creamy white	Pleasant	No	Good	Easily removed
AA4	Little change	Pleasant	No	Good	Easily removed

Homogeneity

All formulations showed uniform distribution of ingredients in cream. By visual appearance and touch, it was confirmed.

pH Measurement

The pH of the formulations was found to be between 6.01 to 7.00 in 3 months' stability studies and was nearer to skin pH. The results were expressed as mean ± standard deviation and were shown (Table 4).

Viscosity

All the prepared formulations were tested for viscosity for 3 months to observe for their stability by comparing with standard. The data was presented (Table 5) and expressed in mpas (SI system). The formulations AA1 and AA3 maintained their viscosity in 3 months period than other two formulations and were found to be stable.

Spreadability

When formulations were subjected to spreadability studies for 3 months, it was found that the AA1 and AA3 creams took less time to spread than AA2 and AA4, when compared with standard. The results were expressed in g.cm/sec and shown in Table 6.

Extrudability

All the prepared formulations were subjected to extrudability and compared with the standard. The data of extrudability was shown (Table 7). Among all the formulations, AA1 and AA3 showed percentage extrudability between 65 to 75%, respectively.

Table 4: pH measurement

Formulation code	*Temperature (°C)*	*0 day*	*1st-month*	*2nd-month*	*3rd-month*
AA1 (avg ± SD)	25	6.44 ± 0.07	6.04 ± 0.35	6.9 ± 0.1	6.29 ± 0.51
	4	6.22 ± 0.1	6.06 ± 0.05	6.87 ± 0.02	6.01 ± 0.42
	40	6.05 ± 0.05	6.40 ± 0.14	6.56 ± 0.49	6.34 ± 0.26
AA2 (avg ± SD)	25	6.48 ± 0.03	6.56 ± 0.27	6.9 ± 0.1	6.46 ± 0.25
	4	6.11 ± 0.1	6.10 ± 0.4	6.79 ± 0.02	6.05 ± 0.81
	40	6.14 ± 0.1	6.37 ± 0.29	6.97 ± 0.02	6.14 ± 0.57
AA3 (avg ± SD)	25	6.65 ± 0.02	6.22 ± 1.05	6.56 ± 0.49	6.07 ± 0.05
	4	6.36 ± 0.04	6.29 ± 0.23	6.33 ± 0.49	6.29 ± 0.32
	40	6.60 ± 0.08	6.55 ± 0.17	6.2 ± 0.17	5.74 ± 0.55
AA4 (avg ± SD)	25	6.25 ± 0.05	6.4 ± 0.26	6.89 ± 0.02	6.33 ± 0.16
	4	6.09 ± 0.02	6.54 ± 0.06	6.61 ± 0.49	6.44 ± 0.05
	40	6.75 ± 0.12	6.38 ± 0.35	6.39 ± 0.50	6.21 ± 0.2
Dabur anti-aging cream (standard) (avg ± SD)	25	6.57 ± 0.01	6.48 ± 0.11	6.44 ± 0.01	6.43 ± 0.01
	4	6.42 ± 0.1	6.43 ± 0.21	6.54 ± 0.23	6.34 ± 0.32
	40	6.41 ± 0.01	6.40 ± 0.01	6.12 ± 0.01	6.43 ± 0.41

Table 5: Viscosity measurement

Formulation code	*Temperature (°C)*	*0 day*	*1st-month*	*2nd-month*	*3rd-month*
AA1 (avg ± SD) (mpas)	25	23.99 ± 1.02	24.25 ± 3.7	18.58 ± 1.6	18.81 ± 1.19
	4	20.91 ± 7.1	22.4 ± 3.33	17.45 ± 1.67	17.91 ± 1.48
	40	19.39 ± 1.8	21.07 ± 3.66	19.18 ± 0.53	18.62 ± 2.45
AA2 (avg ± SD) (mpas)	25	18.34 ± 0.89	21.74 ± 1.4	13.66 ± 0.65	18.57 ± 2.56
	4	18.8 ± 4.44	18.28 ± 1.43	18.18 ± 0.9	15.04 ± 2.95
	40	15.81 ± 1.67	16.3 ± 2.93	19.37 ± 2.94	18.37 ± 0.36
AA3 (avg ± SD) (mpas)	25	20.83 ± 3.28	23.09 ± 1.07	23.98 ± 3.45	23.53 ± 10.9
	4	21.9 ± 5.68	20.33 ± 3.54	23.02 ± 11.32	21.63 ± 2.32
	40	20.7 ± 2.28	19.79 ± 1.61	19.61 ± 0.06	20.36 ± 1.57
AA4 (avg ± SD) (mpas)	25	19.19 ± 5.74	19.84 ± 1.17	16.74 ± 4.11	19.64 ± 4.63
	4	16.31 ± 5.34	16.98 ± 3.12	18.31 ± 1.53	15.83 ± 0.38
	40	14.5 ± 3.7	15.96 ± 0.68	18.42 ± 1.9	16.30 ± 1.59
Dabur anti-aging cream (standard) (avg ± SD) (mpas)	25	19.87 ± 1.22	25.85 ± 1.5	22.23 ± 8.88	22.87 ± 4.27
	4	20.8 ± 1.88	21.75 ± 5.18	20.03 ± 1.01	19.72 ± 6.1
	40	21.12 ± 2.42	18.97 ± 2.65	18.92 ± 2.32	21.37 ± 2.18

Table 6: Spreadability

Formulation code	*Temperature (°C)*	*0 day*	*1st-month*	*2nd-month*	*3rd-month*
AA1 (avg ± SD) (g.cm/sec)	25	9.06 ± 0.95	8.44 ± 2.27	10.9 ± 1.05	10.07 ± 0.55
	4	5.09 ± 0.2	6.44 ± 2.58	8.44 ± 2.58	7.8 ± 1.42
	40	6.53 ± 0.81	8.66 ± 1.2	11.22 ± 2.01	11.7 ± 1.11
AA2 (avg ± SD) (g.cm/sec)	25	13.41 ± 1.55	9.07 ± 3.08	8.54 ± 1.83	10.97 ± 2.92
	4	7.98 ± 1	7.36 ± 0.85	12.88 ± 2.91	7.86 ± 1.75
	40	7.9 ± 1.37	9.62 ± 2.93	10.77 ± 2.79	11.96 ± 1.15
AA3 (avg ± SD) (g.cm/sec)	25	8.22 ± 0.28	8.23 ± 1.96	7.55 ± 3	9.27 ± 2.74
	4	6.43 ± 1.12	6.22 ± 0.28	8.99 ± 0.57	7.07 ± 0.78
	40	7.90 ± 1.37	7.99 ± 1.51	7.22 ± 1.01	8.23 ± 2.02
AA4 (avg ± SD) (g.cm/sec)	25	11.77 ± 1.67	9.60 ± 2.96	8.99 ± 4.41	11.84 ± 2.09
	4	13.18 ± 3.55	7.32 ± 1.01	12.74 ± 1.08	8.48 ± 0.90
	40	10.13 ± 1.99	10.04 ± 2.5	12.26 ± 2.15	10.79 ± 3.13
Dabur anti-aging cream (standard) (avg ± SD) (g.cm/sec)	25	8.98 ± 1.4	8.26 ± 0.97	10.16 ± 1.74	7.34 ± 3.12
	4	7.16 ± 2.34	6.12 ± 0.34	9.10 ± 1.23	6.57 ± 0.12
	40	10.16 ± 1.74	7.36 ± 0.23	10.16 ± 3.41	9.12 ± 0.23

Table 7: Percentage extrudability

Formulation code	*Temperature (°C)*	*0 day*	*1st-month*	*2nd-month*	*3rd-month*
AA1 (%) (avg ± SD)	25	77.73 ± 23.24	73.97 ± 8.54	73.51 ± 0.15	76.19 ± 1.82
	4	72.32 ± 3.54	70.96 ± 7.03	89.88 ± 0.05	73.13 ± 3.54
	40	74.58 ± 7.42	65.96 ± 3.37	79.8 ± 0.09	72.96 ± 2.64
AA2 (%) (avg ± SD)	25	73.23 ± 3.73	72.28 ± 1.08	69.66 ± 0.09	70.04 ± 5.41
	4	59.71 ± 6.1	61.7 ± 6.63	70.55 ± 0.05	70.33 ± 6.03
	40	60.2 ± 4.4	65 ± 3.19	52.66 ± 0.05	69.61 ± 3.31
AA3 (%) (avg ± SD)	25	74.14 ± 7.53	72.69 ± .84	77.72 ± 0.09	71.77 ± 2.19
	4	71.56 ± 7.04	67.8 ± 2.42	93.86 ± 0.05	73.59 ± 4.61
	40	73.47 ± 2.39	70.58 ± 2.81	74.72 ± 0.55	71.16 ± 1.25
AA4 (%) (avg ± SD)	25	70.28 ± 5.74	71.18 ± 5.74	72.85 ± 0.05	67.38 ± 1.32
	4	68.27 ± 5.76	65.09 ± 3.5	81.92 ± 0.05	69.82 ± 0.09
	40	63.38 ± 10.59	59.85 ± 5.54	63.23 ± 0.05	71.98 ± 0.89
Dabur anti-aging cream (standard) (%) (avg ± SD)	25	73.63 ± 2.02	72.49 ± 1.17	71.14 ± 1.03	72.58 ± 8.18
	4	71.64 ± 7.38	73.77 ± 7.38	65.97 ± 3.32	74.02 ± 4.1
	40	74.82 ± 6.41	65.51 ± 10.12	65.04 ± 3.1	71.2 ± 4.01

Performance and Drying Test

All the formulations showed good performance on *in vitro* drying time test, which means that they formed a resistant film on the glass slides at maximum of 20 minutes, which were easily removed and among them, AA1 and AA3 took lesser time of 17.8 minutes for drying.

Primary Skin Irritation Studies

None of the prepared creams showed any erythema or edema, indicating that the prepared formulations were non-irritant on the skin of animals (Figures 1A to D).

***In vitro* SPF Determination**

In vitro SPF of all formulations was checked by UV-visible spectroscopy using Mansur equation method and were shown in Table 8.

Stability Studies

The result of stability studies showed that the formulations were stable during 3 months. The pH, viscosity, spreadability, extrudability formulations AA1 and AA3 did not change during 3 months.

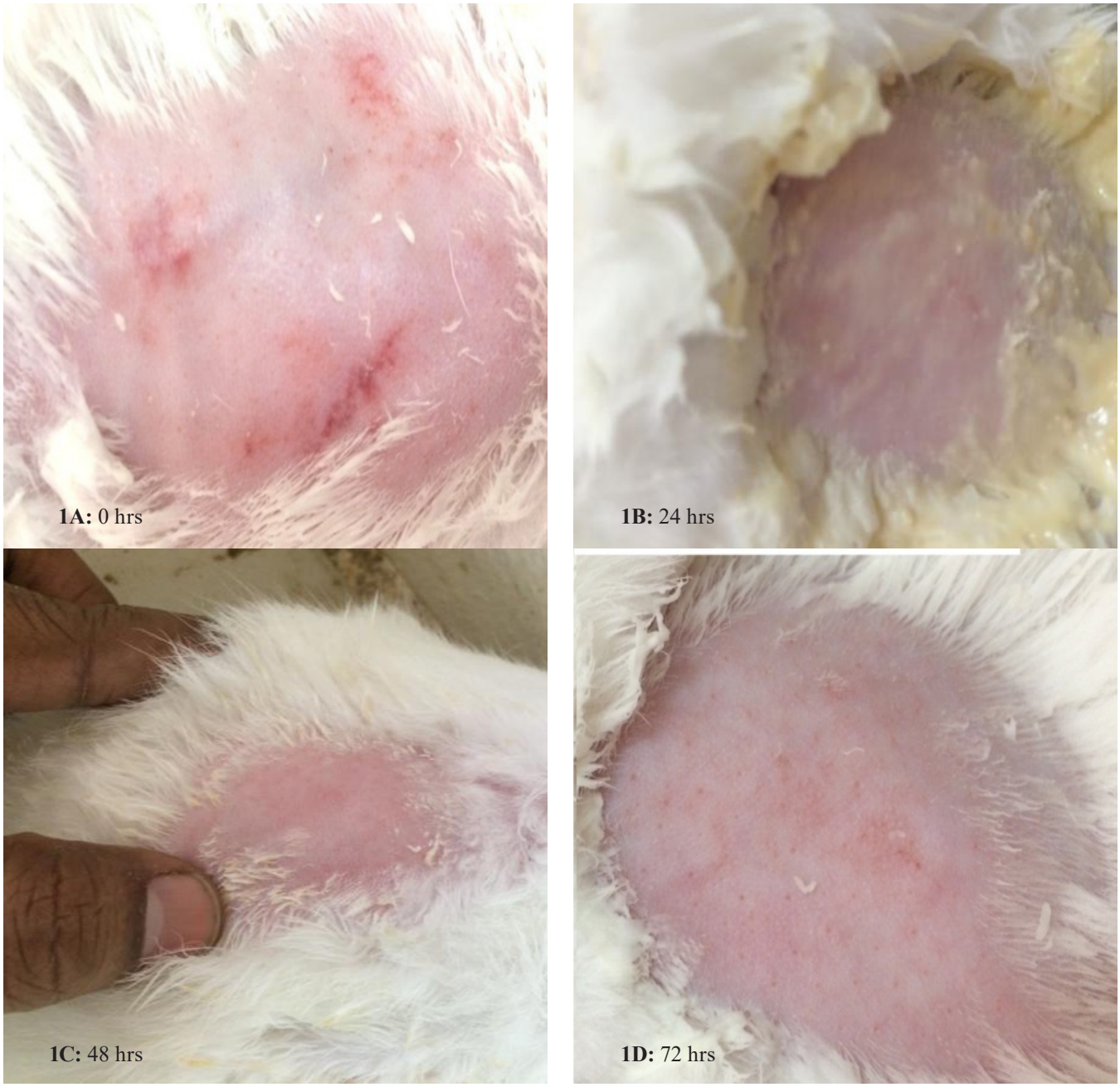
Selection of Formulation

Measurement of SPF is ultimate way to determine the effectiveness of the formulations. Sunscreens protect the body's natural defense mechanisms from exposure to harmful UV radiations of the sun, by the ability to absorb, reflect, or scatter the sunrays. An effective sunscreen should have a wide range of absorbance between 290 to 320 nm. Due to the cosmetic unacceptability of using inorganic and organic sunscreens, the researchers are now showing interest in using

Table 8: Results of *in vitro* SPF of anti-aging creams

S. No.	*Wavelength (λ_{nm})*	*EE × I (Normalized)*	*AA1*	*AA2*	*AA3*	*AA4*	*Standard*
1	290	0.015	0.00854	0.00172	0.010833	0.00338	0.000325
2	295	0.0817	0.006563	0.026207	0.03309	0.008507	0.001498
3	300	0.2874	0.053648	0.015613	0.110073	0.023437	0.005077
4	305	0.3278	0.134398	0.015203	0.033107	0.026463	0.005354
5	310	0.1864	0.007705	0.008263	0.02007	0.01563	0.00292
6	315	0.0839	0.003524	0.004317	0.0106	0.007547	0.000783
7	320	0.018	0.00297	0.000793	0.002793	0.00151	0.000252
SPF			2.16 ± 0.57*	0.72 ± 0.32	2.21 ± 0.6*	0.86 ± 0.54	0.16 ± 0.03

*Dunnet t test was performed for multiple comparisons and the mean difference is significant at the 0.05 level



Figures 1A to D: Photographs showing no erythema or edema

herbal sunscreen agents, which are effective with less or no side effect.

In the present study, out of all four developed formulations, AA1 and AA3 were selected for further evaluations based upon the physical appearance of the creams, studies conducted like pH, spreadability, percentage extrudability, and viscosity of the creams which shown statistically significant difference with the standard. The *in vitro* SPF determinations are particularly useful for screening the formulations during product development, thus, AA1 and AA3 were finally chosen for further evaluations.

CONCLUSION

Herbal formulation share growing demand in the world market. From the above results, it can be concluded that on combining different herbal oils in different concentrations showed good sunscreen protective effect. Thus, on increasing the concentrations of herbal oils the sun protection factor may increase proportionally and sun-protective effect may also increase. The method used in this work is simple, fast, economical, and also easy-to-use for the evaluation of creams to observe the sun-protective effect on the skin. This study can be helpful for upcoming researchers to select herbal oils for the formulation and evaluation of other cosmetic applications, which can be claimed for their efficacy with scientific data.

REFERENCES

1. IMAM S, AZHAR I, MAHMOOD ZA. In-vitro evaluation of sun protection factor of a cream formulation prepared from extracts of Musa Accuminata (L.), Psidium Gujava (L.) and Pyrus Communis (L.). IN-VITRO. 2015;8(3): 234-237.
2. Kale S, Bhandare S, Gaikwad M, Urunkar V, Rajmane A. Formulation and in vitro evaluation for sun protection factor of Lutein ester extracted from Tagetes erecta Linn flower (Family-Asteraceae) sunscreen creams. Research journal of pharmaceutical, biological and chemical sciences. 2011;2(3):947-955.
3. Rasul A, Akhtar N. Formulation and in vivo evaluation for anti-aging effects of an emulsion containing basil extract using non-invasive biophysical techniques. DARU: Journal of Faculty of Pharmacy, Tehran University of Medical Sciences. 2011;19(5):344–350.
4. JUNCAN A, LUNG C. FORMULATION AND OPTIMIZING OF A ANTI-AGING COSMETIC CREAM. Studia Universitatis Babes-Bolyai, Physica. 2016 Dec 1;61(2):101-110.
5. Duraivel S, Shaheda A, Basha R, Pasha EJ. Formulation and evaluation of anti-wrinkle activity of cream and nanoemulsion of moringaoleifera seed oil. IOSR-JPBS. 2014;9:58-73.
6. Arora N, Agarwal S, Murthy RS. Latest technology advances in cosmaceuticals. International Journal of Pharmaceutical Sciences and Drug Research. 2012;4(3):168-182.
7. Saraf AA, Totkekar DD, Saraf AY. An *In vitro* Evaluation of Flowers of Tagetus Spp.(L) as A Natural Anti-Solar Agent. Nat. Sci. 2010;8:274-276.
8. Kaur CD, Saraf S. *In vitro* sun protection factor determination of herbal oils used in cosmetics. Pharmacognosy research. 2010 Jan;2(1):22–25.
9. Suva MA. Evaluation of sun protection factor of Zingiber officinale Roscoe extract by ultraviolet spectroscopy method. J. Pharm. Sci. Tech. 2014;3:95-97.
10. Raghavamma. STV, *et al.* Assessment of Efficacy and Safety of Newly Formulated Natural Sun Protective Cream. IOSR. JPBS 2016;11:119-124.
11. Surjushe A, Vasani R, Saple DG. Aloe vera: a short review. Indian journal of dermatology. 2008;53(4):163-166.
12. Goswami PK, Samant M, Srivastava R. Natural sunscreen agents: A review. Sch Acad J Pharm. 2013;2(6):458-463.
13. Arshad. AA and Bipul. N. Formulation and *In vitro* Evaluation of Poly-Herbal Anti-Aging Face Cream of Coriandrum Sativum and Rose Hip Oil. Int. J. Curr. Pharm. Res. 2011;l9:75-78.
14. Chris. AA, *et al*. Evaluation of the Anti bacterial activity of Herbal ointments formulated with Methanolic extract of Cassia alata. Asian. J. Biomed. Pharmaceut. Sci. 2012;2:15-19 .
15. Evren. A.Y., Özge. Ý. and Sedef. EM Design and *In-Vivo* Evaluation of Emulgel formulations including Green tea extract and Rose oil, Acta. Pharm. 2013;63:531-44 .
16. Pratibha. N., *et al*. *In vitro* and In-Vivo assessment of Polyherbal topical gel formulation for the treatment of Acne Vulgaris. Int. J. Drug. Deliv. 2012;4:434-442 .
17. Suvidha. SP, *et al.* Novel Cosmeceutical Herbal Emulgel for Skincare. WJPPS. 2014;3:801-811.
18. Rafael. PV, *et al.* Physical and Physicochemical stability evaluation of cosmetic formulations containing Soybean extract fermented by Bifidobacterium animals. Braz. J. Pharm. Sci. 2009;45:515-525.
19. Sonika. P., *et al.* Development and Evaluation of antimicrobial herbal cosmetic preparation. Afr. J. Pharm. Pharmacol. 2014;8:514-528.
20. Pople. PV and Singh. KK Development and Evaluation of Topical Formulation Containing Solid Lipid Nanoparticles of Vitamin A. AAPS. PharmSciTech. 2006;E1-E7.
21. More. BH, *et al.* Evaluation of Sunscreen activity of Cream containing Leaves Extract of Butea monosperma for Topical application. Int. J. Res. Cosmetic. Science. 2013; 3: 1-6.
22. Surya. P.M., *et al.* Formulation and Evaluation of Anti Aging Poly Herbal Cream. Int. J. Pharm. Sci. Rev. Res. 2014;24:133-136.