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Development, physicochemical characterization and *in-vitro* evaluation of herbal sunscreen lotion

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Abstract: Ultraviolet radiations have shorter wavelengths and can reach earth's surface through penetrating clouds. UV-A rays leads to aging while UV-B rays causes burning of skin. Sunscreens protect the skin from harmful effects of sun including appearance of erythema, premature photo-ageing and facilitate to diminish the manifestation of facial red veins and blotchiness. In this investigation, herbal sunscreen was prepared using Shea butter, almond oil, raspberry oil, jojoba oil, zinc oxide and titanium dioxide as active ingredients. Fabricated lotions were evaluated for physicochemical parameters *i.e.* color, pH, viscosity and spreadability. Sun protection efficacy of lotion was determined in term of sun protection factor (SPF) by *in-vitro* spectrophotometric method. Total 10 formulations were made with different compositions F1-F10. The pH of formulations ranges from 6.10 (F6) to 8.34 (F5). The viscosity of formulations ranges from 1500 (F1) to 3586 (F10). The spreadability of formulations ranges from 10.56±0.8 (F1) to 30.65±0.7 (F10). The physicochemical parameters of formulation F6 and F10 were found to be in controlled range justifying its compatibility with skin and confirming good cosmetological property. Stability study of optimized lotion was performed after storage of formulation at 25°C and 60 % RH as well as 40°C and 75 % RH for three months. Stability of lotion was evaluated on the basis of changes in physicochemical parameters *i.e.* color, pH, viscosity and spreadability and SPF. F10 has SPF value of 15.71±0.07 (medium protection sunscreen). The optimized formulations might provide good moisturizer, emollient, anti-ageing and anti-wrinkle effect with good sun protection.

Keywords: Sun protection factor, Cosmetological, Spreadability, Stability study

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1. INTRODUCTION

About 80 percent of sun's UV rays can pass through visible mass of liquid droplets in atmosphere. Therefore, regardless of weather, the sun can cause damage to our skin. Here's another daunting fact that UV rays from sun can come in through windows. Therefore, it has been essential to apply sunscreen on everyday and by everybody nevertheless one has fair, dark or oily skin. All types of skin are susceptible to sun damage which can lead to dark spots, wrinkles and skin cancer (Sayre *et al.,* 1979).

There are several types of rays which are not visible *i.e.* ultraviolet radiation (UV-A, UV-B and UV-C) because they have shorter wavelengths than visible light. UV-C rays are absorbed by earth's ozone before reaching our skin, so we don't need to concern ourselves with these when conferring about sunscreen. UV-A rays leads to aging while UV-B rays causes burning of skin. UV-A rays are always able to reach the earth's surface through penetrating clouds and glass. Therefore, skin needs protection even on cloudy environment and days spent indoors. The environmental protection agency believes that 90 percent of skin changes associated with aging such as wrinkle is consequences of UV-A exposure. UV-B rays cause the reddening and burning of skin. They vary in intensity depending on time of day and season.

Since, ozone layer is depleting, therefore, body needs shielding from harmful rays. Nowadays, skin cancer rates are on the rise and sunscreen has been proven to decrease the development of skin cancer. Broad spectrum sunscreens provide protection against each of ultraviolet radiation. Therefore, it has been essential to lather up broad spectrum sunscreen for UVA/UVB protection to prevent skin diseases (https://www.solrx.com/blog/). Sunscreens protect the skin from harmful effects of sun, including appearance of erythema *i.e.* sunburn in short term, premature photo-ageing and skin cancers in long term. Sunscreen prevents facial brown spots and skin discolorations. It also facilitates to diminish the manifestation of facial red veins and blotchiness (http://www.huffingtonpost.com/2013/06/19/sunscreenbenefits) The efficacy and protective value of sunscreen is usually expressed by sun protection factor (SPF).

Various herbal sunscreen ingredients used in present investigation includes Shea butter, raspberry seed oil, almond oil and jojoba oil. Shea butter is the plant fat obtained from nuts of African Karite tree and contains allantoin, vitamin A & E. It has potent moisturizing, emollient and anti-ageing effect. It provides skin care protection against UV radiations (SPF 4-6) and has anti-inflammatory & soothing properties to heal minor wound & irritated skin. Almond oil is rich in β-sitosterol, squalene and vitamin E. It provides skin care protection against UV radiations (SPF 4). It is an excellent emollient which leaves skin soft, smooth and conditioned, good nourishing & revitalizing effect, moisturizer & lubricant, heals injured & chapped skin. Raspberry seed oil is excellent light & nourishing oil with valuable emollient for skin. It provides ultimate skin care protection against UV radiations (SPF 25-50). Jojoba oil is an excellent moisturizer & emollient which prevents transdermal water loss, anti-wrinkle-agent through providing smoothness & softness, good lubricant and protects partly from UV radiation (SPF 4). Zinc oxide provides physical barrier by reflecting or absorbing or blocking radiations from sun (SPF 4-6).

In this investigation, herbal sunscreen was prepared using Shea butter, almond oil, raspberry oil, jojoba oil, zinc oxide and titanium dioxide as active ingredients. Fabricated lotions were evaluated for physicochemical parameters *i.e.* color, pH, viscosity and spreadability. Sun protection efficacy of lotion was determined in term of sun protection factor (SPF) by *in-vitro* spectrophotometric method.

2. MATERIALS AND METHOD

2.1 Materials

Shea butter (CAS NO-91080-23-8), almond oil (CAS NO-8007-69-0), raspberry oil and jojoba oil (CAS NO-61789-91-1) were purchased from Making Cosmetics, USA. Zinc oxide (CAS NO-1314-13-2), stearic acid (CAS NO-57-11-4), glycerin, lactic acid, HPMC and glyceryl monostearate were purchased from Loba Chemicals Private Limited, Mumbai, India. All other chemicals used were of analytical grade

2.2 Methods

2.2.1 Preparation of sunscreen lotion

Ten formulations F1 to F10 were prepared as per Table 1. Accurate quantities of ingredients were weighed. Phase I [oil phase] ingredients (*i.e.* shea butter, almond oil, raspberry oil, jojoba oil, lavender oil, cetyl alcohol and tocopherol acetate) were heated to melt in a 100 ml beaker using hot plate. Dry powder (*i.e.* zinc oxide, titanium dioxide, calamine and hydroxy propyl methyl cellulose) were added to heated mixture followed by continuous heating till complete solubilization of powder in oil phase succeeded by addition of emulsifier's combination (*i.e.* glyceryl monostearate, stearic acid, sorbitan stearate, sorbitan monooleate, PEG-20 sorbitan monolaurate and tween 80) with required HLB. Phase II [aqueous phase] (glycerin, propylene glycol and small amount of rose water) was heated in separate 100 ml beaker to the same temperature as that of oil phase. Phase II was slowly poured into phase 1, a little at a time with constant stirring succeeded by addition of fragrance (*i.e.* mangosteen and mandarin berry) and color (*i.e.* carmoisine and erythrosine) in quantity sufficient amount. Stirring was continued in a glass mortar until a smooth and uniform paste was obtained. Rose water was added to make up the required volume.

2.2.2 Determination of physicochemical parameters

Determination of organoleptic acceptability

The color and odour of prepared lotions were visibly observed for their organoleptic acceptability.

2.2.3 Determination of viscosity

Viscosity is the degree of fluid friction which can be contemplated as the internal friction resulting when a layer of fluid is made to move in connection to another layer. Viscosity (in cps) of lotion was measured by Brookfield rotational digital viscometer model LVDV-II+P, USA using LV-spindle 64. The spindle was rotated at 6 rpm. Approximately 250 ml lotion was used for measurement which was maintained at temperature of 25°C during the measurements (Patel *et al.*, 2009). All measurements were taken in triplicate and represented as mean ± SD.

2.2.4 Determination of Spreadability

Spreadability is an important characteristic of lotions. It refers to the ease with which product can be spread without losing its firmness. Spreadability was determined by apparatus recommended by Mutimer *et al.*, which was suitably modified in the laboratory and employed for research (Multimer, 1956). It consists of a wooden block, which was provided by a pulley at one end. Spreadability was determined on the basis of 'Slip' and 'Drag' characteristics of lotion (Biradar *et al.,* 2011). A ground glass slide was fixed on this block. An excess of lotion (approximately 2 g) under investigation was positioned on fixed slide and sandwiched using another glass slides provided with hook. 1 Kg weight was placed on the top of two slides for 5 minutes to expel air and to impart uniform film of lotion between slides. Excess of lotion was scrapped off from edges. With the help of string attached to hook, top slide was subjected to pull of 80 g. The time (in seconds) required by top slide to cover a distance of 7.5 cm was noted. A shorter time interval indicated better Spreadability which was calculated using following formula (Chakole *et al.*, 2009):

$$S = M \times L/T \tag{1}$$

Table 1: Composition of various sunscreen formulations.

Ingredients (%w/v or %v/r)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**
Shea butter*	5	10	10	5	4	25	10	10	10	12.5
Almond oil*	10	10	10	5	2	4	2	3	3	3
Raspberry oil*	5	10	10	5	3	-	-	2	2	2
Jojoba oil*	-	10	10	3	1	3	1.5	2	2	2
Zinc oxide*	1.25	4	4	5	10	25	7.5	12.5	5	2.5
Titanium oxide*	1.25	4	1	1	1	1	0.5	-	-	-
Stearic Acid	-	-	-	-	4	4	-	3	3	3
Glyceryl monostearate	-	-	-	-	-	-	2.5	4	9	5
Lavender oil	-	-	-	2	2	1	-	1	1	1
Sorbitan stearate 60	-	-	-	-	-	-	-	5	5	5
Sorbitan monooleate 80	-	-	-	-	-	-	-	5	5	5
Propylene glycol	-	-	-	-	2.5	2.5	-	2	2	2
Tocopherol acetate	2.5	5	5	5	5	0.5	0.25	-	1.5	1.5
PEG-20 sorbitan monolaurate	0.1	0.1	0.2	-	-	5	0.25	5	-	5
Glycerine	-	-	1	1	10	5	2.5	2	3	-
Cetyl alcohol	-	-	2	2	10	15	2	-	2	-
Carbopol	-	-	-	-	-	1	-	-	-	-
Tween 80	-	-	-	5	5	5	-	-	-	-
Triethanolamine	-	-	-	-	0.6	0.6	-	-	-	-
Lactic acid	-	-	-	-	-	0.5	-	-	-	-
Methyl paraben	-	-	-	-	-	0.5	-	-	-	-
HPMC	-	-	-	-	-	-	5	-	-	-
Calamine	-	-	-	-	-	0.2	-	-	-	-
Mandarin berry	-	0.1	0.1	0.1	0.1	0.01	0.01	0.1	0.1	0.2
Mangosteen	0.1	-	-	-	-	-	-	-	-	-
Carmoisine	q.s.	-	-	-	q.s.	q.s.	-	-	-	-
Erythrosine	-	-	q.s.	q.s.	-	-	q.s.	q.s.	q.s.	q.s.
Rose water q.s. (in ml)	50	50	50	50	50	50	50	50	50	50

*Active ingredients

Where, S = Spreadability, M = Weight in pan (tied to upper slide), L = Length moved by glass slide and T = Time taken to separate the slide completely from each other. All measurements were taken in triplicate and represented as mean ± SD.

2.2.5 Determination of pH by pH meter and litmus paper

1gm of lotion was dissolved in 100 ml of distilled water and pH of formulations was measured using digital pH meter (361, Systronics, India) (Panda, 2011). All measurements were taken in triplicate and represented as mean ± SD. Lotion was placed at the end of glass rod and a drop of lotion was dropped on litmus paper. Note the colour change of litmus paper and compare with standard shades of pH strip.

2.2.6 Determination of sun protection factor (SPF)

SPF was determined by *in-vitro* method using double beam UV spectrophotometer (Systronics AU2701, India). SPF was calculated using the Eq. 2 and Normalized product function (Table 2) derived by Mansaur *et al.*, (Mansaur *et al.,* 1986; Sayre *et al.,* 1979; More *et al.,* 2013)

$$SPF_{spectrophotometric} = CF\sum_{290}^{320} EE(\lambda)\times I(\lambda)\times A(\lambda) \quad (2)$$

Where, correction factor, CF=10, EE (λ) = erythemogenic effect of radiation of wavelength, I (λ) = intensity of solar light of wavelength, A (λ) = spectrophotometric absorbance values at wavelength. All measurements were taken in triplicate and represented as mean ± SD.

Table 2: Normalized product function used in calculation of SPF

Wavelength (nm)	EE (erythemal factor) * I (Solar Intensity)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

2.2.7 Selection of optimized formulation

The optimized formulation was selected on the basis of physicochemical parameters such as color, pH, spreadability, viscosity and residual whiteness.

2.2.8 Stability study

Optimized formulation was stored at room temperature (25°C and 60 %±5% RH) and under accelerated conditions (40°±2ºC & 75 %±5% RH) for 6 months. Physicochemical parameters and SPF of lotion was investigated after storage for specified period. Stability of optimized formulation was also determined by centrifugation method (Butler, 2000). The centrifugation was performed at 8000 rpm for 10 minutes and observed for phase separation.

3. RESULTS AND DISCUSSION

3.1 Physicochemical studies

The results of physicochemical properties such as color, pH, spreadability and viscosity are summarized in Table 3. The pH of formulations ranges from 6.10 (F6) to 8.34 (F5). The lotion with pH around 6.5 was considered good because it complies with skin pH. The color of formulations was acceptable pink. The viscosity of formulations ranges from 1500 (F1) to 3586 (F10).

Table 3: Physicochemical evaluation parameters

Sunscreens	Color	pH	Viscosity (cps)	Spreadability (g.cm/sec)
F1	Dark pink	6.58 ± 0.03	1500 ± 12	10.56 ± 0.8
F2	Pink	6.63 ± 0.02	3467 ± 13	29.75 ± 0.9
F3	Pink	6.66 ± 0.04	3475 ± 15	29.65 ± 0.7
F4	Dark Pink	6.47 ± 0.03	1787 ± 16	12.76 ± 0.9
F5	Dark pink	8.34 ± 0.04	3397 ± 17	28.65 ± 0.8
F6	Dark pink	6.10 ± 0.05	2436 ± 15	19.47 ± 0.7
F7	Pink	6.49 ± 0.03	2654 ± 18	21.57 ± 0.6
F8	Pink	6.61 ± 0.04	2166 ± 16	16.75 ± 0.8
F9	Pink	6.56 ± 0.03	2677 ± 15	21.67 ± 0.9
F10	Pink	6.53 ± 0.02	3586 ± 14	30.65 ± 0.7

All values are represented as mean ± SD (n=3)

The spreadability of formulations ranges from 10.56 (F1) to 30.65 (F10). The important physicochemical parameters of formulation F6 and F10 were found to be in controlled range justifying its compatibility with skin and confirming good cosmetological property.

3.2 Sun protection factor

The absorbance values of formulations F1 to F10 and two marketed sunscreens (Lakme Sunexpert SPF 30 and Lakme Sunexpert SPF 24+) MS 1 and MS 2 were measured using UV spectrophotometer and SPF was calculated (Table 4 and Table 5). The results showed that F6 has highest SPF of 46.06±0.06 which may be attributed to the presence of higher concentration of zinc oxide but had residual slight whiteness left after application over skin, which could be adjusted with further formulation studies for optimization of ZnO amount F10 has an SPF value of 15.71±0.07 (medium protection sunscreen) which is sufficient for protection against sun burn for a period of about 3 h and shows better formulation characteristics. SPF determination of marketed sunscreens by *in-vitro* method and comparison with its claimed SPF indicated that the method is highly suitable and reliable.

3.3 Selection of optimized formulation

Formulation F1 was having less viscosity and it was interpreted that it needs addition of rheological modifier. F2 produced residual whiteness for more than 15 minutes and needs substantial reduction of concentration of zinc oxide. F3 was unstable indicated by foaming which may be due to addition of cetyl alcohol. F4 was sticky formulation and needs increased amount of emulsifier's addition. The pH of formulation F5 was basic (pH 8) which may be due to addition of triethanolamine (TEA). F6 was satisfactory in most aspects except optimization of ZnO and dark pink color. F7, F8, F9 and F10 were considered good formulation but due to higher viscosity and good pourability F10 was selected as optimized formulation than them.

3.4 Stability

The results of stability tests of optimized formulations F6 and F10 carried out by evaluation of physicochemical parameters and centrifugation method after storage period of 3 months under room temperature and accelerated conditions are given in Table 6 and 7. No significant changes in physicochemical parameters were observed which illustrated stability of formulation. Moreover, no phase

Table 4: Absorbance values of the formulated and marketed sunscreen

S.No.	Wavelength (nm)	290	295	300	305	310	315	320
F1	A	1.358±0.003	1.466±0.002	1.489±0.001	1.486±0.012	1.496±0.003	1.486±0.023	1.596±0.013
	EE (λ)*I(λ)*A	0.020	0.119	0.428	0.487	0.279	0.125	0.029
F2	A	1.685±0.014	1.785±0.002	1.894±0.004	1.883±0.013	1.874±0.012	1.863±0.003	1.968±0.002
	EE (λ)*I(λ)*A	0.025	0.146	0.544	0.617	0.349	0.156	0.035
F3	A	1.484±0.013	1.573±0.003	1.638±0.002	1.648±0.001	1.638±0.011	1.684±0.012	1.748±0.003
	EE (λ)*I(λ)*A	0.022	0.128	0.471	0.540	0.305	0.141	0.031
F4	A	1.604±0.021	1.726±0.005	1.842±0.015	1.875±0.002	1.846±0.014	1.804±0.003	1.945±0.002
	EE (λ)*I(λ)*A	0.024	0.141	0.529	0.615	0.344	0.151	0.035
F5	A	2.054±0.002	2.174±0.001	2.274±0.004	2.284±0.003	2.264±0.012	2.256±0.023	2.367±0.004
	EE (λ)*I(λ)*A	0.031	0.178	0.654	0.749	0.422	0.189	0.042
F6	A	4.176±0.003	4.496±0.002	4.547±0.001	4.632±0.011	4.685±0.022	4.677±0.001	4.748±0.002
	EE (λ)*I(λ)*A	0.063	0.367	1.307	1.519	0.873	0.392	0.085
F7	A	1.654±0.001	1.774±0.003	1.874±0.001	1.884±0.013	1.864±0.021	1.856±0.003	1.967±0.004
	EE (λ)*I(λ)*A	0.025	0.145	0.538	0.618	0.347	0.156	0.035
F8	A	2.338±0.001	2.498±0.002	2.52±0.011	2.566±0.001	2.59±0.003	2.55±0.002	2.62±0.023
	EE (λ)*I(λ)*A	0.035	0.204	0.724	0.841	0.482	0.214	0.047
F9	A	1.404±0.003	1.524±0.002	1.624±0.004	1.634±0.013	1.614±0.002	1.606±0.004	1.717±0.005
	EE (λ)*I(λ)*A	0.021	0.124	0.467	0.536	0.301	0.135	0.031
F10	A	1.369±0.002	1.449±0.033	1.460±0.023	1.483±0.043	1.495±0.035	1.475±0.023	1.510±0.043
	EE (λ)*I(λ)*A	0.020	0.118	0.419	0.486	0.278	0.123	0.027
MS1	A	3.01±0.043	3.01±0.053	3.02±0.024	3.03±0.053	3.04±0.053	3.05±0.061	3.05±0.053
	EE (λ)*I(λ)*A	0.045	0.245	0.867	0.993	0.566	0.255	0.054
MS2	A	2.40±0.062	2.01±0.013	2.51±0.043	2.62±0.041	2.72±0.027	2.73±0.051	2.84±0.037
	EE (λ)*I(λ)*A	0.036	0.164	0.663	0.760	0.441	0.203	0.047

MS- Marketed sunscreen, all values are represented as mean ± SD (n=3)

Table 5: SPF of the formulated and marketed sunscreen.

Sunscreens	SPF
F1	14.73±0.07
F2	18.72±0.06
F3	16.38±0.15
F4	18.39±0.07
F5	22.65±0.11
F6	46.06±0.06
F7	18.64±0.12
F8	25.47±0.07
F9	16.15±0.07
F10	15.71±0.04
MS1- Marketed sunscreen (Lakme Sunexpert SPF 30)	30.02±0.07
MS2- Marketed sunscreen (Lakme Sunexpert SPF 24+)	23.17±0.07

Table 6A: Stability evaluation by physicochemical parameters (F6)

Day	Color	pH	Viscosity (cps)	Spreadability (g.cm/sec)	Centrifugation at 8000 rpm
0	Dark Pink	6.10 ± 0.05	2436 ± 15	19.47 ± 0.7	Stable
3 Months (25^{o}C & 60 %RH)	Dark Pink	6.60 ± 0.02	2103 ± 12	17.67 ± 0.8	Stable
3 Months (40 ^{o}C& 75 %RH)	Dark Pink	6.61 ± 0.04	2095 ± 12	16.68 ± 0.8	Stable

Table 6B: Stability evaluation by physicochemical parameters (F10)

Day	Color	pH	Viscosity (cps)	Spreadability (g.cm/sec)	Centrifugation at 8000 rpm
0	Pink	6.58 ± 0.03	3586 ± 14	30.65 ± 0.7	Stable
3 Months (25^{o}C & 60 %RH)	Pink	6.60 ± 0.02	3203 ± 12	28.67 ± 0.8	Stable
3 Months (40 ^{o}C & 75 %RH)	Pink	6.61 ± 0.04	3105 ± 12	27.68 ± 0.8	Stable

Table 7A: Stability evaluation by SPF parameter (F6)

Day	Absorbance							SPF= (10 * ∑ EE(λ) * I(λ)*A)
	290	295	300	305	310	315	320	
0	4.176±0.003	4.496±0.002	4.547±0.001	4.632±0.011	4.685±0.022	4.677±0.001	4.748±0.002	42.73±0.07
3 Months (At 25^{o}C & 60 %RH)	4.176±0.003	4.496±0.002	4.547±0.001	4.632±0.011	4.685±0.022	4.677±0.001	4.748±0.002	39.66±0.06
3 Months (At 40 ^{o}C & 75 %RH)	4.176±0.003	4.496±0.002	4.547±0.001	4.632±0.011	4.685±0.022	4.677±0.001	4.748±0.002	36.18±0.09

Table 7B: Stability evaluation by SPF parameter (F10).

Day	Absorbance							SPF= (10*∑ EE(λ)*I(λ)*A)
	290	295	300	305	310	315	320	
0	1.369 ±0.002	1.449 ±0.033	1.460 ±0.023	1.483 ±0.043	1.495 ±0.035	1.475 ±0.023	1.510 ±0.043	15.73±0.07
3 Months (At 25°C& 60 %RH)	1.368 ±0.003	1.447 ±0.035	1.458 ±0.024	1.481 ±0.041	1.495 ±0.038	1.474 ±0.026	1.509 ±0.036	15.66±0.06
3 Months (At 40 °C& 75 %RH)	1.368 ±0.014	1.358 ±0.051	1.307 ±0.003	1.327 ±0.014	1.301 ±0.010	1.316 ±0.006	1.289 ±0.014	14.18±0.09

separation at 8000 rpm was observed indicating the stability of F6 and F10 at high stress conditions and revealed that it may bear different environmental changes during product transport.

CONCLUSIONS

The herbal sunscreens prepared using proposed formulae were found to have non-granular consistency with optimum viscosity and uniform spreadibility. They appear translucent with an acceptable pink color & acceptable aroma. They were found to have good moisturizing effect without leaving much residual whiteness. This composition showed acceptable adherence to primary packing surface, which may be glass as well as plastic bottle and further pourability. The pH of lotion was found 6.5 which comply with skin pH. Viscosity profile of lotion indicated good rheology during handling. No phase separation was observed after centrifugation which indicated stability of formulations. The optimized formulation might provide good moisturizer, emollient, anti-ageing and anti-wrinkle effect with SPF 15.73.

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CONFLICT OF INTEREST

The authors have reported no conflicts of interest in this work.

REFERENCES

[1] Biradar, S. Paradkari, A. and Mahadik, K. (2011) *In vitro* evaluation of topical gel prepared using silk fibroin at different concentration of gel accelerating agent-glycerol Int. J Pharma Bio Sci, **2**, 646-660.

[2] Butler, H. (2000) 'Poucher's perfumes, cosmetics and soap. Quality, stability and safety assurance'. Kluwer Academic Publishers, Dordrecht, 507–621. http://dx.doi.org/10.1007/978-94-017-2734-1

[3] Chakole, C.M, Shende, M.A, and Khadatkar, S.N. (2009) Formulation and development of novel combined halobetasol propionate and fusidic acid ointment Int. J Chem Tech Res, **1**, 103–116.

[4] Jain, B.D. Padsalg, A. Patel, K. and Mokale, V. (2007) Formulation, development and evaluation of Fluconazole gel in various polymer bases Asian J Pharma, **1**, 63 – 68.

[5] Mansur, J.S. Breder, M.N.R. Manusur, M.C.A. and Azulay, R.D. (1986) Determinacao do fato de potecao sola po espectrofotometrica. An. Bras. Dermatol, **61**, 121-124.

[6] More, B.H. Sakharwade, S.N. Tembhurne, S.V. and Sakarkar, D.M. (2013) Evaluation of Sunscreen activity of Cream containing Leaves Extract of Butea monosperma for Topical application'. Int. J Res Cosmetic Sci., **3**, 1-6.

[7] Multimer, M. (1956) Spreadability determination by an apparatus. J American Pharm Ass, **45**, 212–214.

[8] Panda, P. (2011) Formulation and evaluation of topical dosage form of Alangium salvifolium Linn. and their wound healing activity Asian J Pharm Sci and Res, **1**, 10-23.

[9] Patel, N. A. Patel, N.J. and Patel, R.P. (2009) Comparative development and evaluation of topical gel and cream formulations of psoralen. Drug Discovery and Therapeutics, **3**, 234-242.

[10] Sayre, R.M. Agin, P.P. Levee, G.J. and Marlowe, E. (1979) A Comparison of in-vivo and in-vitro testing of sun screening formulas. Photochem and Photobio, **29**, 559-566. https://www.solrx.com/blog/ http://dx.doi.org/10.1111/j.1751-1097.1979.tb07090.x