



Formulation, Characterization and *In-vitro* Sun Protection Factor of a Lemongrass Sunscreen Lotion

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Abstract: Sunburn, pigmentation disorders, photocarcinogenesis, immunosuppression and other skin related damages are caused by the ultraviolet radiation present in the sunlight. The aim of this study was to determine the sun protection factor (SPF) value of the lemongrass extract, also the formation of its stable sunscreen lotion and to determine its SPF value. For the determination of SPF value of the developed formulation, spectrophotometric method was used along with Mansur equation. The SPF value of lemongrass sunscreen lotion was 22 with the antioxidant potential of 94.20%. The hydrogen ion concentration (pH) of the lotion was 5.5, which complied with skin pH. Viscosity profile of lotion indicated good rheology which is an important aspect of a formulation during application. The formulation was stable as there was no phase separation observed after centrifugation, freeze-thaw and thermal stress tests. Harmful effects of chemicals are evident and therefore, the natural source could become a good, economical, easily accessible and safe alternative formulation ingredient in sunscreen products due to its beneficial effects and safety.

Keywords: Emulsions, formulation/stability, lemongrass, spectroscopy, sun protection, sunscreen

1. INTRODUCTION

Skin, the outermost layer of the body that acts as a sensory organ is a part of the integumentary system that defends the body from surrounding environment. Epidermis, dermis and hypodermis are the three main layers of the skin [1]. Melanin naturally present in the skin is responsible for protection of skin from the harmful effects of sun [2]. Skin aging is not hazardous for a person but it can have negative effects on psychology of a person. The interaction of the skin with environment directly or indirectly is the main cause of premature ageing [3]. Photo carcinogenesis, inflammation, erythema, immunosuppression, pigmentation, photo aging, hyperplasia and vitamin D synthesis are certain skin responses induced by ultraviolet radiations (UV-R). [4]. The radiations from sun comprises of 50% visible light lying in the range of 400-800 nm, 40% infrared radiation lying in the

range of 1300-1700 nm and 10% UV-R lying in the range of 100-400 nm [5-6].

The substances that absorb or block UV rays of sunlight are called sunscreens or sun blocks. All compounds used as sunscreen filter are by their nature and chemicals that can absorb UV-A and / or UV-B light [7]. The ideal sunscreen product should provide good protection throughout the whole range of UV spectrum, even after sunlight exposure. An ideal sunblock should be non-irritating, non-toxic and not produce any type of allergy [4]. Due to antioxidant power and UV-R absorption, natural ingredients extracted from plants have been recently considered as potential sunscreen resources [8].

The measurement of the effectiveness of sunblock in protecting the human skin from UV-R is called Sun protection factor (SPF). **Table 1** shows various ratings of SPF. To prevent any possible

Table 1. Sun Protection Factor Ratings [9]

SPF Range	Effective UV- R transmission (%)	SPF Rating	Protection Category
15-24	6.7-4.2	15, 20	Good
25-39	4.1-2.6	25, 30, 35	Very Good
40-50, 50+	Less than 2.5	40, 45, 50, 50+	Excellent

damage to skin by UV rays, the sunscreen products should have adequate SPF values and a wide range of absorbance between 290 to 400 nm to absorb and reflect enough amount of UV ray photons [10].

Nanotechnology reveals its great potential in the field of research and development by increasing the efficacy of the product. . To overcome certain disadvantages associated with the traditional products, application of nanotechnology is rising in the area of cosmeceuticals [11]. Because natural compounds are capable of attenuating some of the UV-induced aging effects in the skin, increased attention has been generated in the area of cosmetic sciences [12].

The substances which delay or prevent the oxidation of an oxidizable substrate are called the “Antioxidants” They may be synthetic or natural antioxidants. Biological systems produce the natural antioxidants. Natural antioxidants are present in many plants including lemongrass, garlic, turmeric, onions, celery seed, basil, rosemary, ginseng, and coriander [13]. In previous studies, it was concluded that lemon grass has high antioxidant capacity than many botanicals [14] [15].

Principal compounds were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) and identified as neral, nerolic acid, geraniol, geranic acid and geranial in lemongrass oil [16]. Along with other biological activities reported in the literature, potent tyrosinase inhibitory activity has been found in lemongrass. Geranic acid was discovered as one of the active substances through assay-guided purification [17] and percentage values of geranic acid have been found to be 0.3%, 1.1%, 0.4% in various studies[18] [19]. As lemongrass has antioxidant potential, the objective of this study was to determine the SPF value of lemongrass extract as well as to make the sunscreen lotion of lemongrass and to check its SPF value.

2. MATERIALS AND METHODS

2.1 Materials

All chemicals used were of analytical grade. Materials used in the study were Lemongrass (Local market, Lahore, Pakistan), Ethanol (Riedel-de Haen, Germany), Salicylic acid (Local market, Lahore, Pakistan), DPPH(2,2-Diphenyl-2picrylhydrazyl) (Sigma), GMS (glyceryl mono stearate) (Local market, Lahore, Pakistan), Cetyl alcohol (BDH, U.K), Silicone oil (Local market, Lahore, Pakistan), Glycerin (Riedel-de Haen, Germany), Bees wax (Local market, Lahore, Pakistan), Ascorbic acid (Sigma, Germany), Potassium hydroxide (Omicron science ltd, U.K), Tween 80 (Merck-Schuchardt, America), Palmitic acid (Sigma-Aldrich, Germany), Stearic acid (Daejung, Korea), Amaranth red (U.S.A) and Distilled water (Research lab University of Central Punjab, Lahore, Pakistan).

2.2 Plant collection and identification

Mature, healthy and disease-free lemongrasses (*Cymbopogon citratus*) plant grown in a nursery of Lahore were collected and shade-dried leaves were used in the present study. For the identification of the lemongrass, the plant was provided to a botanist for confirmation of its taxonomical classification. Voucher specimens (voucher no. GC. Herb. Bot.3319) were deposited in the herbarium maintained by Government College University Lahore.

2.3 Preparation of lemon grass extract

100 gm of lemon grass was weighed precisely on the weighing balance (Shimadzu, Japan) and then grinded into blender into coarse powder, which was then transferred to an amber, colored bottle having a capacity of 2.5 L. For the extraction purpose, ethanol and water were used as menstruum in a ratio

of 70:30. For the menstruum preparation, 1400 ml ethanol was transferred to an amber colored bottle after being measured in a measuring cylinder and then 600 ml of distilled water was transferred to the same bottle using a measuring cylinder. Finally, the lemon grass was macerated in 2 L of menstruum with shaking of the bottle on alternate days for a period of 15 days.

2.4 Filtration and evaporation of extract

For the filtration of the lemongrass extract, Whatman (grade 54) filter paper was used. The volume obtained after filtration was then concentrated in a rotary evaporator (Heidolph- VAP, Germany). The crude extract was kept in air-tight amber bottle and stored at cold temperature until it was further used [20].

2.5 Dilution of extracts for SPF determination

For determination of SPF value, dilution was done, so that the final concentration obtained should be 10,000 μ g/ml.

2.6 Determination of SPF value of lemongrass extract

For the determination of SPF value, absorption spectra of the lemongrass extract was obtained in the range of 290nm to 320nm using the quartz cell. Ethanol was used as blank.

Absorption values for the lemongrass extract were recorded at the wavelength ranging from 290nm to 320nm with an interval of 5nm. Triplicate readings were taken for each wavelength and then their average was calculated which was then used for the determination of SPF by using the Mansur equation [10].

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

Where EE - erythral effect spectrum; I-solar intensity spectrum; Abs-Absorbance of sunscreen product; CF-correction factor (=10). The value of EE x I are constant and preset as shown in **Table 1**.

2.7 DPPH radical scavenging capacity assay

2, 2-diphenyl-1-picryl-hydrazyl (DPPH), a stable free radical was used to determine the antioxidant

activity as described previously with slight modification [21]. The concentration of DPPH 100 μ M in methanol was used. Total assay volume was 100 μ l, containing 10 μ l of the test solution and 90 μ l of DPPH solution in a 96 well plate. The contents were mixed and incubated at 37°C for 30 minutes. Synergy HT BioTek® USA microplate reader was used to determine the diminution in absorbance at 517nm. Standard antioxidant used was ascorbic acid (100 – 1000 μ g / mL). All experiments were carried out in triplicate.

The percent inhibition was determined by the following formula.

$$\text{Inhibition (\%)} = (\text{Abs. of control} - \text{Abs. of test solution}) / \text{Abs. of control} \times 100$$

Where,

Absorbance of Control = Total radical activity without inhibitor.

Absorbance of Extract = Total radical activity in the presence of test/standard compound [21].

2.8 Preparation of lemongrass lotion

13 trials were done to get the desired formulation as shown in **Table 2**. Different concentrations of the oil phase and aqueous phase were prepared to get the right consistency of the formulation. A selection criterion was designed to select the optimal dosage form. Method adopted for the formation of the desired formulation is described below. Oil phase i.e. glyceryl mono- stearate (GMS) and silicone oil were transferred to a beaker which was then placed in water-bath (Jiangsu Zhengji instruments CO. LTD, China) having a temperature of 75°C for the melting of GMS and for homogeneous mixing of the oil phase.

An aqueous phase i.e. distilled water and Tween 80 were mixed in a beaker and were heated to the same temperature as that of the oil phase i.e. 75 °C. Finally, oil phase was added to the aqueous phase dropwise using a mixer SILENT CRUSHER M (Heidolph, Germany) for 10 minutes at 10,000 rpm and for further 5 minutes at 5,000 rpm until mixture gets cooled down. At 30 °C, the lemon grass extract was added in the formulation and it was further homogenized by using the SILENT CRUSHER M (Heidolph, Germany) for 5 minutes to obtain

Table 2. Composition of different trialed formulations

	Composition (% w/w)										
	GMS	P.A	B.W	TW.80	S.O	C.A	Gly	KOH	S.A	L.G.E	Water (Q.S)
T ₁	1	1	-	1	-	-	-	-	-	5	100
T ₂	2	1	-	1	-	-	-	-	-	5	100
T ₃	2	2	-	1	-	-	-	-	-	5	100
T ₄	1	2	-	-	-	-	-	-	-	5	100
T ₅	2	2	-	-	-	-	-	-	-	5	100
T ₆	5	2	1	-	-	-	-	-	-	5	100
T ₇	5	2	-	-	-	-	-	-	-	5	100
T ₈	-	-	-	-	-	2	2	0.8	8	5	100
T ₉	-	-	0.5	-	-	-	2	0.8	8	5	100
T ₁₀	-	-	-	-	-	2	2	0.8	6	5	100
T ₁₁	-	-	-	-	-	2	2	0.8	4	5	100
T ₁₂	2	-	-	2	5	-	-	-	-	5	100
T ₁₃	5	-	-	2	10	-	-	-	-	5	100

GMS= Glyceryl mono stearate.

P. A= Palmitic acid.

TW.80= Tween 80.

C.A= Cetyl Alcohol.

KOH= Potassium hydroxide.

L.G.E= Lemon Grass Extract

Q.S = Quantum satis

B. W= Bees wax.

S. O= Silicone oil.

Gly= Glycerine.

S. A= Stearic acid.

a stable formulation of the desired consistency. Similar procedure was followed for different trial formulations.

2.9 Emulsion type confirmation

For the confirmation of O/W emulsion 1 drop of emulsion was mixed with 1 drop of distilled water on a slide and mixing was done using a tooth pick then a small quantity of amaranth was added to the slide and a smear was made and then slide was observed under microscope (Libomed, USA). As amaranth is miscible in water so the continuous phase would gain the color of amaranth i.e. red and dispersed phase i.e. oil droplets were seen against red background. This confirms the emulsion type i.e. O/W type.

2.10 Short term stability (Centrifugation tests)

Centrifugal tests were employed for emulsions right after their preparation. Those tests were repeated after 1 day and 7 days of storage at the

temperatures of 25° C and 40° C. They were subjected to centrifugation at 5,000 rpm and 25° C for 10 min by placing 10 g of sample in centrifugal tubes of centrifuge machine (Model: 800) [22].

Relative Amount (%) = $V_o / V \times 100$

V_o = Volume Instability Phenomenon

V = Total Volume

2.11 Thermal stress tests

Stability tests were done at different settings for emulsions to check the effect of these conditions on the stability of emulsions. These tests were performed on samples kept in refrigerator (Dawlence model # 9122M, Pakistan) at 4° C ± 2° C, room temperature at 25° C ± 2° C and in thermal electric thermostatic drying oven (DHG-9202, SANFA, China) at 40° C ± 2° C. Phase separation and liquefaction of emulsions were observed at various time intervals during 28 days. pH values of freshly prepared emulsions and emulsions kept at different conditions were determined by a digital pH-Meter . The pH tests were repeated after 24 hours, 3 days, 7 days, 14 days, 21 days and 28 days

of preparation [22]. Creaming property for 5 ml of lotion was determined by keeping it at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$, $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for a period of 28 days and separation of solid and liquid phase was also observed.

2.12 Rheological studies

Since 1960's rheological measurement has been becoming progressively essential to illustrate the "consistency" of semisolid gels, ointments and creams, ("so-called complex or structural fluids"), in a meaningful fashion [23]. Rheological profiles are now mandatory in numerous pharmaceutical and cosmetics industries. DV-III-Ultra Rheometer (Brookfield Engineering Lab) was used to check the rheological profile of the lemongrass formulation i.e. sunscreen lotion.

2.13 Freeze thaw test

Stability testing was done by using freeze thaw cycling method. The temperature was altered every 24 hours between 25°C and -5°C for ten cycles and samples were observed for physical stability. To check the physical stability of emulsion, its color, pH, liquefaction, phase separation and creamy

texture were observed at the end of each cycle for 10 cycles.

3. RESULTS AND DISCUSSION

3.1 2, 2-Diphenyl-2picrylhydrazyl radical scavenging capacity assay

DPPH radical scavenging capacity assay was done to check the antioxidant potential of lemongrass extract. Ascorbic acid (vitamin C) was taken as standard. Assay indicates the antioxidant capacity of lemongrass extract to be 94.20% and that of vitamin C was 92% as shown in **Table 3**. The results indicate that lemongrass extract possesses slightly more antioxidant potential as compared to the standard i.e. vitamin C.

3.2 Selection of the desired formulation:

For the selection of desired formulation, selection criteria were developed, according to which the most stable and suitable sunscreen formulation was selected for further study as shown in **Table 4**. Thirteen (13) trials were performed and for the selection of desired one the following parameters were set; phase separation, sedimentation/

Table 3. Antioxidant activity of lemongrass extract in comparison with vitamin C

Compositions	Antioxidant activity (%)
Lemongrass Extract	94.2
Ascorbic Acid (Vitamin C)	92

Table 4. Selection criteria of desired formulation

	Phase separation	Sedimentation /creaming	Liquefaction	Consistency	Microbial Growth	Phase Inversion
T ₁	✓	✓	✓	Watery	×	×
T ₂	✓	✓	✓	Watery	×	×
T ₃	✓	×	✓	Watery	×	×
T ₄	×	×	×	Watery	×	×
T ₅	✓	✓	✓	Cream	×	×
T ₆	×	×	×	Cream	✓	×
T ₇	×	×	×	Cream	✓	×
T ₈	×	×	×	Thick cream	×	×
T ₉	×	×	✓	Cream	×	×
T ₁₀	×	×	×	Cream	×	×
T ₁₁	×	×	×	Cream	✓	×
T ₁₂	×	×	×	Watery	×	✓
T ₁₃	×	×	×	Lotion	×	×

creaming, liquefaction, consistency, microbial growth and phase inversion. T₁ (trial 1) and T₂ (trial 2) were rejected due to its watery consistency, phase separation, sedimentation and liquefaction. In T₃ (trial3), phase separation and liquefaction was observed and its consistency was watery. There was no physical instability observed in T₄ (trial 4), T₈ (trial 8) and T₁₀ (trial 10) but these were rejected due to their poor consistency - T₄ was watery; T₈ and T₁₀ were of creamy consistency. T₅ (trial 5) was rejected due to phase separation, sedimentation, liquefaction and creamy consistency. In T₆ (trial 6) and T₇ (trial 7), there was visible microbial growth along with creamy consistency, so these were also rejected. In T₉ (trial 9), liquefaction was observed along with creamy texture so it was also excluded. The rejection of T₁₁ (trial 11) was due to microbial growth and creamy consistency while that of T₁₂ (trial 12) was of watery consistency along with phase inversion. T₁₃ (trial 13) was selected because of its desired characteristics, there was no physical instability observed in this trial also it had the desired consistency of a lotion.

Creaming/sedimentation leads to phase separation and often occurs due to density differences amongst the two phases under the effect of gravity [24]. From the very beginning, the

temperature and time processes begin to contribute to emulsion separation, leading to a decrease in viscosity, and as a consequence liquefaction occurs [25].

3.3 Characterization tests

The pH of human skin usually varies from 4.5 to 6.0. So, it is essential for a formulation that its pH value should lie within this range [26]. Determining the pH value is critical for assessing the emulsions' stability. In fact, pH fluctuations show the incidence of chemical reactions that can indicate the quality of the final formulation [27]. That is why pH is included in all the stability studies such as centrifugation, freeze thaw test and thermal stress test.

3.4 Short-term stability (Centrifugation tests)

Apparently, there was no effect of centrifugation on the emulsion. No phase separation, liquefaction, sedimentation was observed as shown in **Table 5**. This was probably due to the appropriate homogenization speed during emulsion formulation which might have prevented the breakage of the formulations during testing [28].

Table 5. Short-term stability of lotion (centrifugation test)

	Stability of lotion at different time intervals			
	At day 1		At day 28	
	25° C	40° C	25° C	40° C
Liquefaction	×	×	×	×
Phase separation	×	×	×	×
Creaming	×	×	×	×

×=no change.

Table 6. Thermal stability of lotion

	Stability of lotion at time intervals														
	1 st day			7 th day			14 th day			21 st day			28 th day		
	4°C	25°	40°	4°	25°	40°	4°	25°	40°	4°	25°	40°	4°C	25°	40°C
Co	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
pH	5.5	5.5	5.5	5.5	5.5	5.4	5.5	5.5	5.3	5.5	5.5	5.3	5.5	5.5	5.24
L	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
P.S	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Cr	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×

C = Cream, **Co** = Color, **Cr**=Creaming, **L**=Liquefaction, **P. S**= Phase separation, ×= no change

3.5 Thermal stress tests

There were no visible signs of lotion instability after thermal stress test as shown in **Table 6**. No changes were observed in any of the parameter except pH and the possible reason was explained earlier that it could be due to destabilization of emulsion caused by hydrolysis process at high temperature [27].

3.6 Rheological studies

Rheological behavior is adjusted as per need and purpose of usage of a particular cosmetic product. For example, body lotions need a certain yield stress (or high viscosity) at rest to stay in the hands of the user while being taken out of the bottle. However, a consequent shear thinning manner (or low viscosity at high shear) is essential for easiness of spreading and applying the lotion onto the skin. The low viscosity at high shear is also important for lotion to form a uniform thin layer that will more easily penetrate the skin and help the skin absorb the active ingredients without feeling oleaginous or gluey [29].

The rheological results obtained by using rheometer (DV-111-ultra Rheometer, Brookfield Engineering Lab) indicate the non-Newtonian behavior of the formulation; it has pseudo-plastic and shear thinning behavior as shown in **Table 7** which indicates that it can be handled without difficulty while its application on skin.

3.7 Freeze thaw Test

There was no significant change in the formulation stability as shown by the results of this test in the **Table 8**. No changes were observed in the color, liquefaction, phase separation and creaming. However, slight difference was observed in the pH that may be due to the changing temperature but overall the pH change was negligible to have any influence on the stability of the formulation because the pH at the end of the freeze thaw test was 5.18 which lies in the range of skin pH i.e. 4.5 to 6.0 [26] and is not a skin irritating pH [30].

3.8 SPF determination

SPF of the extract was determined using UV-visible spectrophotometer (HALO DB-20) and by applying Mansur equation. The absorbance was determined via spectrophotometer at a wavelength range of 290nm to 320nm and then by applying the Mansur equation the SPF was determined. The calculated value of the extract is given in the **Table 9**. For the determination of SPF of the formulation, three different dilutions i.e. 2000 μ g/ml, 400 μ g/ml and 80 μ g/ml were made and SPF values were determined as done for the lemongrass extract. Maximum SPF value was obtained at 2000 μ g/ml i.e. 22 and at decreased concentrations, SPF values also showed a decreasing trend as shown in **Table 9**.

Table 7. Rheology of Lotion

NO.	Viscosity (η) (cP)	Speed (RPM)	% Torque (%)	Shear Stress ($\dot{\sigma}$) (D/cm ²)	Shear Rate ($\dot{\gamma}$) (1/sec)
1	3.75	5.00	0.1	0.25	10.00
2	3.68	10.00	0.3	0.74	20.00
3	3.27	15.00	0.4	0.98	30.00
4	3.07	20.00	0.5	1.23	40.00
5	2.75	25.00	0.7	1.72	50.00
6	2.46	30.00	0.6	1.47	60.00
7	2.46	35.00	0.7	1.72	70.00
8	2.46	40.00	0.8	1.96	80.00
9	2.46	45.00	0.9	2.21	90.00
10	2.46	50.00	1.0	2.46	100.00

Rheocalc V2.6 Brookfield Engineering Labs

Math Model: Power Law

Consistency Index: 3.95 cP

Flow Index: 0.91

Confidence of Fit: 87.3 %

Spindle: CP41

Table 8. Freeze thaw of sunscreen lotion

Time interval	Temperatures									
	-5°C					25°C				
	Color	pH	Liquefaction	Phase separation	Creaming	Color	pH	Liquefaction	Phase separation	Creaming
Day1	-	-	-	-	-	Cream	5.51	×	×	×
Day2	Cream	5.5	×	×	×	-	-	-	-	-
Day3	-	-	-	-	-	Cream	5.47	×	×	×
Day4	Cream	5.47	×	×	×	-	-	-	-	-
Day5	-	-	-	-	-	Cream	5.45	×	×	×
Day6	Cream	5.42	×	×	×	-	-	-	-	-
Day7	-	-	-	-	-	Cream	5.42	×	×	×
Day8	Cream	5.41	×	×	×	-	-	-	-	-
Day9	-	-	-	-	-	Cream	5.40	×	×	×
Day10	Cream	5.39	×	×	×	-	-	-	-	-
Day11	-	-	-	-	-	Cream	5.35	×	×	×
Day12	Cream	5.33	×	×	×	-	-	-	-	-
Day13	-	-	-	-	-	Cream	5.31	×	×	×
Day14	Cream	5.28	×	×	×	-	-	-	-	-
Day15	-	-	-	-	-	Cream	5.25	×	×	×
Day16	Cream	5.23	×	×	×	-	-	-	-	-
Day17	-	-	-	-	-	Cream	5.21	×	×	×
Day18	Cream	5.20	×	×	×	-	-	-	-	-
Day19	-	-	-	-	-	Cream	5.19	×	×	×
Day20	Cream	5.18	×	×	×	-	-	-	-	-

Table 9. SPF value determination of lemongrass extract and sunscreen lotion

S. No.	Wavelength λ (nm)	EE×I Normalized	LEMONGRASS EXTRACT		LEMONGRASS SUNSCREEN		
			10,000 μ g/ml	2000 μ g/ml	400 μ g/ml	80 μ g/ml	
1	290	0.0150	0.015	0.033	0.013	0.004	
2	295	0.0817	0.076	0.180	0.072	0.023	
3	300	0.2874	0.253	0.638	0.252	0.080	
4	305	0.3278	0.276	0.721	0.284	0.090	
5	310	0.1864	0.154	0.417	0.161	0.051	
6	315	0.0839	0.080	0.188	0.072	0.023	
7	320	0.0180	0.014	0.040	0.015	0.005	
SPF			8.5	22	8.7	2.8	

SPF of the extract was 8.5 at a dilution of 10,000 μ g/ml, while SPF values of formulation were 22, 8.7, and 2.8 at dilutions of 2000 μ g/ml, 400 μ g/ml, and 80 μ g/ml, respectively. The decreasing trend of SPF values observed with decreasing concentration is evident in **Table 9**. Results clearly indicate a dose dependent increase in SPF value and at 2 mg/ml concentration; lemongrass shows good photoprotective ability in *in-vitro* studies. These results are consistent with previous and recent studies in which plant extracts have been explored for photoprotection.

In a study, Calendula oil cream showed good photoprotection ability (SPF = 14.84 \pm 0.16) at 5 % concentration of extract [31]. Sri Lankan researchers focused on evaluation of photoprotective activity of some aqueous herbal extracts at a fixed concentration of 1 mg/ml and among the extracts, *Atalantia ceylanica*, *Hibiscus furcatus*, *Leucas zeylanica*, *Mollugo cerviana*, *Olox zeylanica* and *Ophiorrhiza mungos* have shown SPF value \geq 25, which were even higher than two commercial photoprotective creams used as reference compounds. Moreover, high antioxidant

activity of extracts was also observed in DPPH assay [32].

A group of researchers evaluated the photoprotective effect of cosmetic formulations containing hydroalcoholic extract of *N. variegata* (Nv-HA). Initially, the phenolic and flavonoid total content of Nv-HA were determined. The photoprotective activity of Nv-HA showed SPF values of 5.43 ± 0.07 and 11.73 ± 0.04 at the concentrations of 0.5% and 1.0% (v/v), respectively. It was also verified that Nv-HA potentiated the photoprotective effect of formulations in a dose dependent manner and formulations remained stable at the end of the study [33].

4. CONCLUSION

The *in-vitro* spectrophotometry method was used in this study which is a simple and an easy method for determination of SPF value of sunscreen emulsions. For the determination of antioxidant potential, DPPH radical scavenging capacity assay was performed. The results showed the antioxidant potential of lemongrass extract to be 94.20% and the lemongrass sunscreen lotion to have SPF value of 22. The pH of lotion was found 5.5 which comply with the skin pH. Viscosity profile of lotion indicated good rheology during handling. No phase separation was observed after centrifugation, freeze thaw and thermal stress tests which indicated stability of formulation.

Along with their many beneficial effects and safety, the natural product could become a good, cheap and easily available formulation ingredient in sunscreen products.

5. ACKNOWLEDGEMENTS

Authors are thankful to the University of Central Punjab for funding this project.

6. REFERENCES

1. Kishore, V.S., N. Balaji., K. Srikanth & J. Hemanth. Design and Development of Aloe vera Lotions and Determination of Their Sun Protection Factor. *Research Journal of Topical and Cosmetic Sciences*. 2(2): 65-9 (2011).
2. Lu, H., C. Edwards., S. Gaskell., A. Pearse & R. Marks. Melanin content and distribution in the surface corneocyte with skin phototypes. *British Journal of Dermatology*. 135(2):263-267 (1996).
3. Mbang, L., M. Mulenga., P. Mpiana., K. Bokolo., M. Mumbwa & K. Mvingu. Determination of Sun Protection Factor (SPF) of some body creams and lotions marketed in Kinshasa by Ultraviolet Spectrophotometry. *International Journal of Advanced Research in Chemical Science*. 1(8):7-13 (2014).
4. Suva, M.A. Evaluation of sun protection factor of Zingiber officinale roscoe extract by ultraviolet spectroscopy method. *Journal of PharmaSciTech*. 3(2):95-7 (2014).
5. Mensah, A., J. Sampson., P. Houghton., P. Hylands., J. Westbrook., Dunn, M., M.A. Hughes & G.W. Cherry. Effects of Buddleja globosa leaf and its constituents relevant to wound healing. *Journal of Ethnopharmacology*. 77(2):219-226 (2001).
6. Mukhtar, H & C.A. Elmets. Photocarcinogenesis: Mechanisms, Models and Human Health Implications: Introduction. *Photochemistry and Photobiology*. 63(4):356-7 (1996).
7. Westerhof, W., O. Estevez-Uscanga., J. Meens., A. Kammeyer., M. Durocq & I. Cario. The relation between constitutional skin color and photosensitivity estimated from UV-induced erythema and pigmentation dose-response curves. *Journal of Investigative Dermatology*. 94(6):812-6 (1990).
8. Maske, P.P., S.G. Lokapure., D. Nimbalkar., S. Malavi & J.I. D'souza. *In vitro* determination of sun protection factor and chemical stability of Rosa kordesii extract gel. *Journal of Pharmacy Research*. 7(6):520-4 (2013).
9. Das, B.R. UV radiation protective clothing. *Open Textile Journal*. 3:14-21 (2010).
10. Mansur, J.S., Breder, M.N.R., Mansur, M.C.A & R.D. Azulay. Determination of sun protection factor by spectrophotometry. *Anais Brasileiros De Dermatologia*. 61:121-124 (1986).
11. Shreya, K., N. Gulati., D. Verma., S. Mukherjee & U. Nagaich. Role of Nanotechnology in Cosmeceuticals: A Review of Recent Advances. *Journal of Pharmaceutics (Cairo)*. 2018: 1-19 (2018).
12. Spencer D., R. Odin., L. Zhou., L. Ji., Y. Zhang & A.L. Kadekaro. Natural Antioxidants: Multiple Mechanisms to Protect Skin From Solar Radiation. *Frontiers in Pharmacology*. 2018; 9: 392.
13. Craig, W.J. Health-promoting properties of common

- herbs. *American Journal of Clinical Nutrition*. 70(3):491s-9s (1999).
14. Rao, B., R. Shanbhoge., B. Rao., S. Adiga., D. Upadhy., B. Aithal & M.R. Kumar. Preventive efficacy of hydroalcoholic extract of *Cymbopogon citratus* against radiation-induced DNA damage on V79 cells and free radical scavenging ability against radicals generated in vitro. *Human & Experimental Toxicology*. 28(4):195-202 (2009).
 15. Nambiar, V.S. & H. Matela. Potential Functions of Lemon Grass *Cymbopogon citratus* in Health and Disease. *International Journal of Pharmaceutical & Biological Archive*. 3(5): 1-9 (2012).
 16. Sargenti, S. & F. Lancas. Supercritical fluid extraction of *Cymbopogon citratus* (DC.) Stapf. *Chromatographia*. 46(5-6):285-290 (1997).
 17. Masuda, T., Y. Odaka., N. Ogawa., K. Nakamoto & H. Kuninaga. Identification of geranic acid, a tyrosinase inhibitor in lemongrass (*Cymbopogon citratus*). *Journal of Agricultural and Food Chemistry*. 56(2):597-601 (2007).
 18. Vyshali, P., K.T. Saraswathi & G. Mallavarapu. Chemical Composition of the Essential Oils of *Cymbopogon citratus* (DC.) Stapf Grown in Three Locations in South India. *Journal of Essential Oil Bearing Plants*. 18(1):230-235 (2015).
 19. Gupta, A.K. & D.A. Ganjewala. Study on developmental changes in essential oil content and composition in *Cymbopogon flexuosus* cultivar Suvarna. *Acta Biologica Szegediensis*. 59(2):119-125 (2015).
 20. Arif, N.J., A. Yahya., M.A Hamid., H. Yaakob & R.M. Zulkifli. Development of Lightening Cream from Mangosteen Pericarp Extract with Olivoil Emulsifier. *International Proceedings of Economics Development and Research*. 81:58 (2014).
 21. Ratshilivha, N., M. Awouafack., E. du Toit, & J. Eloff. The variation in antimicrobial and antioxidant activities of acetone leaf extracts of 12 *Moringa oleifera* (Moringaceae) trees enables the selection of trees with additional uses. *South African Journal of Botany*. 92:59-64 (2014).
 22. Akhtar, N., M. Ahmad., Gulfishan, M.I. Masood & M. Aleem. Formulation and in vitro evaluation of a cosmetic emulsion from almond oil. *Pakistan Journal of Pharmaceutical Sciences*. 21(4):430-7 (2008).
 23. Davis, S.S. Viscoelastic properties of pharmaceutical semisolids III: Nondestructive oscillatory testing. *Journal of Pharmaceutical Sciences*. 60(9):1351-1356 (1971).
 24. Rousseau, D. Fat crystals and emulsion stability—a review. *Food Research International*. 33(1):3-14 (2000).
 25. Herbert, A., m. Martin & S. Gilbert. Pharmaceutical Emulsions, Pharmaceutical Dosage Forms: Disperse Systems. Vol. 1. *Marcel Dekkar, New York and Basel*. 199-240 (1988).
 26. Matousek, J.L., K.L. Campbell, I. Kakoma., P.F. Solter & D.J. Schaeffer. Evaluation of the effect of pH on in vitro growth of *Malassezia pachydermatis*. *Canadian Journal of Veterinary Research*. 67(1):56 (2003).
 27. Some, I.T., P. Bogaerts., R. Hanus., M. Hanocq & J. Dubois. Improved kinetic parameter estimation in pH-profile data treatment. *International Journal of Pharmaceutics*. 198(1):39-49 (2000).
 28. Nour, A.H. & R.M. Yunus. Stability investigation of Water-in-Crude oil emulsion. *Journal of Applied Sciences*. 6:2895-2900 (2006).
 29. Yao, M.L. & J.C. Patel. Rheological characterization of body lotions. *Applied Rheology*. 11(2):83 (2001).
 30. Smaoui, S., H.B. Hlima., I.B. Chobba, & A. Kadri. Development and stability studies of sunscreen cream formulations containing three photoprotective filters. *Arabian Journal of Chemistry*. 10: 1216-1222 (2013).
 31. Mishra A.K., A. Mishra, & P. Chattopadhyay. Assessment of In vitro Sun Protection Factor of *Calendula Officinalis* L. (Asteraceae) Essential Oil Formulation. *Journal of Young Pharmacists*. 4(1): 17–21 (2012).
 32. Mayuri T. N., B.M.A.S. Malkanthi., S.A.K. Abayawardana., M.M. Qader & L. Jayasinghe. Photoprotective potential in some medicinal plants used to treat skin diseases in Sri Lanka. *BMC Complementary and Alternative Medicine*. 16:479 (2016).
 33. Oliveira-Júnior R.G., G.R. Souza., C.A.A. Ferraz., A.P. de Oliveira., C.S. Araújo., S.R.G. Lima-Saraiva., S.A.G.B. Reis., T.M. Gonçalves., L.A. Rolim., P.J. Rolim-Neto., F.C.S. César & J.R.G.S. Almeida. Development and Evaluation of Photoprotective O/W Emulsions Containing Hydroalcoholic Extract of *Neoglaziovia variegata* (Bromeliaceae). *Scientific World Journal*. 2017: 1-8 (2017).