Assessment of Anti-oxidant Herbal Cream Formulation for Sun Protection Factor (SPF) Efficacy

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ABSTRACT

The primary aim of the study is to develop creams containing herbal extract and assess their effectiveness. The utilization of natural products is of great significance due to concerns regarding the toxicity and side effects associated with modern drugs. The objective of this research is to formulate sun protection creams that are cost-effective, non-toxic, and efficacious, and to ascertain if these formulations are as effective as those already on the market. The study on skin moisturization revealed high significance ($p<0.001$), while the In vitro occlusion study indicated that the formulation displayed a significant percentage of occlusion ($p<0.01$). The examination of the sun protection factor demonstrated that the formulation exhibited significant sun protection values ($p<0.01$) and was comparable to the marketed cream. Notably, the Formulation, incorporating gallic acid as a marker ingredient, displayed a considerable percentage occlusion and sun protection factor compared to the marketed formulation, confirming their ability to remain on the skin surface and safeguard against UV radiation. Stability assessments of the formulation by freeze-thaw testing showed no significant variation in the parameters. Additionally, the preservative efficacy study exhibited a significant reduction in bacterial colonies, with over 99.9% reduction within seven days, and over 90% reduction in fungal colonies within 28 days.

Key Words: *Herbal cream, Sun protection factor, In vitro occlusion, Preservative efficacy*

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INTRODUCTION

The demand for herbal products, including health items, medications, supplements, and cosmetics, is surging globally due to their safer profiles, fewer side effects, and economic appeal [1]. Cosmetics aim to improve appearance while minimizing flaws, but success depends on choosing suitable products for different skin and hair types and the skills of the user. Mastering cosmetic application requires both knowledge and artistic talent, which develop with practice [2]. Herbal cosmetics not only serve as body care items but also enhance skin health through their natural ingredients.

These botanical products contain various beneficial components like vitamins, antioxidants, oils, and bioactive compounds. Sunscreen agents play a crucial role in protecting against UV radiation [3], utilizing their ability to absorb, reflect, or scatter sunlight. The Sun Protection Factor (SPF) measures the effectiveness of sunscreen, with higher SPF values providing better protection against sunburn and assisting in the prevention of premature aging and skin cancer.

Recent research suggests that herbal cosmetics are better suited for sensitive skin due to their gentle nature and compatibility. Topical treatments, especially those incorporating herbal ingredients, are preferred by patients and frequently recommended by healthcare professionals for sunburn, given their mild side effects, ease of use, affordability, and accessibility. Effective herbal cosmetics for sun protection should include active sunscreen agents with antioxidant properties [4]. Hence, the detrimental effects of ultraviolet radiation can be alleviated by harnessing potent Phyto antioxidants, which exert their actions through various proposed mechanisms [5]. When

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combined with sunscreens, this approach holds promise for effectively reducing ultraviolet-induced reactive oxygen species-mediated photoaging and skin cancer in humans. Numerous herbs are renowned for their nutritional and therapeutic benefits. Therefore, a formulation rich in antioxidants can mitigate skin damage, thereby reducing the risk of basal cell and squamous cell carcinoma. Herbal cosmetics rich in antioxidant principles provide a timely solution to reduce the population's susceptibility to such afflictions. Therefore, it is crucial to create a sunscreen cosmetic that incorporates herbal antioxidants to protect the skin from sunburn and its related effects.

MATERIALS AND METHODS

Preparation of ethanolic extract

The measured and powdered fruits of Phyllanthus emblica underwent extraction through Cold Maceration using 70% ethanol at cold temperatures (15-25°C) for 48-96 hours [6]. Subsequently, the solution was filtered using Whatman filter paper no: 1, and the filtrate was concentrated under vacuum to obtain residues. These concentrated extracts were stored in desiccators for future use.

Anti-oxidant screening of the extract by DPPH radical scavenging effect

Phyto antioxidants react with DPPH, a persistent free radical, converting it into diphenyl-picryl hydrazine and resulting in a color shift from deep violet to pale yellow. The amount of DPPH scavenging activity in plant extracts may be determined by measuring the change spectrophotometrically at 518 nm. For the test, combine 25µl of plant extract with 0.48 ml of methanol and 0.5 ml of a methanolic solution containing DPPH [7, 8]. Allow the mixture to react at room temperature for 30 minutes. Methanol is utilized as a blank, whereas DPPH in methanol, excluding the plant extracts, works as a positive control, thereafter the reduction in purple color is measured at 518 nm.

Formulation of herbal extract creams

For making the cream, we dissolved all water-soluble ingredients in water, while oil-soluble ingredients were combined at a temperature of 75°C ± 50°C separately. The water phase was gradually combined with the oil phase while stirring continuously, and the resulting product was mixed for 30 minutes [9].

Measurement of viscosity

The cream's viscosity was measured using a Brookfield viscometer. Viscosity readings were taken with a Brookfield DV-II + viscometer equipped with an LV-4 spindle. The cream formulation was poured into the viscometer's adaptor, and the rotational speed was gradually increased from 0.5 to 20 rpm [10].

Skin moisturizing effect study

When skin undergoes photodamage, it can become pale, thin, and dry. Therefore, an important function of cosmetics is to replenish the skin's moisture balance, with skin hydration being a key parameter to measure. Skin hydration levels are assessed using a chronometer, which is attached to a multiprobe adapter. This device detects and evaluates capacitance changes, which vary according to the water content in the outermost layer of the skin, known as the stratum corneum [11, 12].

In vitro occlusion studies

The formulation occlusivity was assessed using the occlusion factor. In this in vitro model, 200 mg of the formulation is equally dispersed over an 18.8 cm2 surface on filter paper placed on top of water in a beaker. A reference control is established by putting filter paper in a separate beaker without any formulation. An occlusion factor of zero signifies no occlusion impact in comparison to the reference, whilst a maximum occlusion factor of 100 represents total occlusion. The occlusion factor is determined by a certain formula [13]:

$$F = 100(A - BA)\; F = 100(AA - B) \tag{1}$$

Where AA represents water loss without the sample (reference), and BB represents water loss with the sample.

In vitro sun protection factor determination by UV spectrophotometer

The effectiveness of a sunscreen cream is defined by its Sun Protection Factor (SPF), which is calculated as the ratio of the amount of UV energy needed to generate a minimum erythemal dose (MED) in protected skin to the UV energy necessary for a MED in unprotected skin. The least amount of UV light exposure needed to cause slight redness on unprotected skin is referred to as the minimal erythemal dose (MED). A higher SPF indicates better protection against sunburn.

The in vitro approach measures the decrease of UV irradiation by assessing the transmittance after passing through a layer of the product. One frequent method is to measure the spectral transmittance from 280 to 400 nm throughout UV wavelengths. A particular formula is used to calculate the absorbance values recorded at 5 nm intervals [14]:

$$SPF = CF \times \sum_{290}^{230} (EE(\lambda) \times Abs(\lambda)) \tag{2}$$

Where:

CF represents the correction factor (10),

EE(λ) represents the erythrogenic effect of radiation at wavelength λ,
Abs(λ) represents the spectrophotometric absorbance values at wavelength λ.

The values of EE(λ)×I are constant and determined by Sayre *et al*. The aliquots are scanned throughout the range of 290 to 320 nm, and the resulting absorbance readings are then multiplied by the corresponding EE(λ)×I(λ) values. The total of these numbers is thereafter multiplied by the adjustment factor of 10.

Preservative efficacy study of the formulation

The preservative efficacy of formulation was assessed following the guidelines outlined by the US Pharmacopeia (USP). Aspergillus niger ATCC 16404, Escherichia coli ATCC 8739, Candida albicans ATCC 10231, Pseudomonas aeruginosa ATCC 9027, and Staphylococcus aureus ATCC 6538 were utilized in this study. Freshly cultured bacteria and yeast were collected in sterile tryptone sodium chloride and adjusted to a 1x108 cfu/ml inoculum concentration (measured by spectrophotometer). The mold was harvested using sterile saline (with Tween80 added) and adjusted to a spore count of 1x108 cfu/ml [15].

Fifty grams of formulation were weighed in an aseptic environment and inoculated with 0.5 ml of each inoculum suspension. All inoculated samples were thoroughly mixed and then incubated at 25°C for 28 days. Aseptic samples of two grams each were withdrawn on days 0, 7, 14, and 28 and transferred to neutralizing medium. The number of viable microorganisms within the inoculum suspensions was determined via the plate count method. Dey-Engley Neutralizing Agar (Difco) and Sabouraud Dextrose Agar (SDA) were utilized for bacterial and yeast/mold counts, respectively [16]. Plates were then incubated at 37°C for 24-48 hours and at 25°C for 5 days. Following the incubation period, colony counts were recorded for each plate and the results were expressed as colony-forming units per plate (cfu/plate).

Stability of herbal cream formulation through three-cycle temperature testing

The chosen formulation underwent three to four freeze-thaw cycles, involving freezing at -10°C for 24 hours followed by thawing at 30°C for an additional 24 hours [17, 18]. Following the study period, the formulations were assessed for any changes in pH, color, consistency, fragrance, and viscosity.

RESULTS AND DISCUSSION

The ethanolic extract of Phyllanthus emblica (PE) was standardized by evaluating the presence of foreign matter, ash value, total ash, acid-insoluble ash, water-soluble ash, loss on drying and extractive values. The results were almost the same as that of the standard value. The DPPH radical scavenging activity of extracts used in the formulation is represented in **Table 1**. The Phyllanthus emblica exhibited good anti-oxidant activity with IC50=22.453 µg/ml. The technique of neutralizing the stable DPPH radical is a commonly employed approach for swiftly assessing antioxidant capabilities compared to other methods. In this research, all extracts demonstrated robust antioxidant properties, with P. emblica showing particularly high free radical inhibition. This heightened activity could be attributed to the potent phytochemicals present in the extract.

Table 1. *In vitro* anti-oxidant activity of *Phyllanthus emblica* extract by DPPH radical scavenging activity.

PE Extract		ASCORBIC ACID	
Conc (µg/ml)	**% Scavenging**	**Conc (µg/ml)**	**% Scavenging**
10	42.21 ±2.22	10	21.6 ±1.64
20	45.48 ±2.77	20	31.7 ±1.52
30	56.48 ±6.26	30	49.02 ±2.35
40	62.45 ±6.40	40	54.30 ±2.65
50	73.08 ±11.0	50	68.20 ±3.03 (IC_{50}=22.453 µg/ml)

[a]Data are presented as the mean ± SEM (n = 6)

The viscosity study of the formulation showed that the viscosity of the cream is inversely proportional to the rate of shear. The viscosity of cream at 0.5 rpm was found to be significant (p<0.01). The viscosity of the formulation was found to be good and it confirms the consistency of the prepared herbal cream (H.C). The result of the viscosity study is given in **Table 2**.

Table 2. Viscosity of the prepared formulation

RPM	VISCOSITY (cps)	
	H.C	**Blank**
20	1638±10	1658±10
10	1954±15	1986±15
5	16120±10	16180±10
1	32381±20	33431±5
0.5	16798±20**	16896±15

[a] Values are Mean±SEM, n=6, **Paired t-test P<0.01(Significant)

In the study on the Moisturizing Effect on Skin, moisture levels rise from the baseline after applying the formulation, peak at 30 minutes, then gradually decline until the fourth hour. There's a slight increase and subsequent decrease over the next 24 and 48 hours. Notably, there's a significant difference in moisture content between the formulation and the blank. This suggests that the moisturizing treatment

provided by the formulation involves repairing the skin barrier, retaining water, distributing and holding moisture, and preserving skin health and appearance. The result is summarized in **Table 3**.

Table 3. The study of the Skin moisturizing effect of prepared herbal cream

Formulation	OBSERVATION				
	Baseline	30 min	4 hr	24 hr	48 hr
H.C	47.3 ± 10.7	60.5 ± 10.3	58.6 ± 10	59.7 ± 10.2	58.2 ± 7.4
Blank	46.8 ± 9.1	49.4 ± 7.0	47.0 ± 9.8	47.0 ± 10.7	48.7 ± 8.0
Mean difference	0.50±1.6	11.10±3.3***	11.60±0.2***	12.70±0.5***	9.50±0.6***

[a] Values are Mean±SEM, n=6, *** Paired t-test, highly Significant (p-value <0.001).

In the *In vitro* occlusion study the occlusive effect of the cream is based on the occlusive factor which is based on the water loss due to the application. In this study the formulation, displays significant occlusive effect and it is given in **Table 4**.

Table 4. *In vitro* occlusion study

Formulation	Water loss (ml)	% Occlusion factor (F)
Control	0.9 ±0.020	-----
H.C	0.35 ± 0.050**	61.106
Marketed Cream	0.33± 0.042**	62.444

[a] Values are Mean±SEM, n=6, ** t-test Significant (p-value < 0.01)

The Sun Protection Factor (SPF) study helps determine how long someone can be in the sun before getting sunburned compared to not using sunscreen. A higher SPF indicates better protection. The herbal cream (H.C) demonstrated a notable SPF value compared to the commercial product, likely because of the potent plant compounds in the extract. Increasing the extract concentration in the cream could further enhance its SPF value. The result of the SPF study is summarized in **Table 5**.

Table 5. The Sun Protection Factor Activity

Formulations	SPF value
Blank	2.67 ± 0.039
H.C	20.96±0.14**
Marketed cream	19.94 ± 0.054

[a] Values are Mean±SEM, n=6, ** t-test Significant (p-value < 0.01)

The preservative is an essential ingredient in the formulation of cream to prevent the growth of contaminating microorganisms present in the atmosphere. In this study, the efficacy of the preservative is tested against bacteria (S.aureus, P. aeruginosa and E.coli) and fungi (C.albicans and A.niger). The results of the study reveal the antimicrobial efficacy of the preservative which is evident from the reduction in bacterial population (expressed in terms of number of microorganisms/plate) at 28 days of incubation when compared to the control plates and the results are given in **Table 6**.

Table 6. The preservative efficacy study of the prepared cream

Microbial Strains	Products	Days (cfu/plate)			
		0	07	14	28
Staphylococcus aureus	**Control**	6.5×10^5	6.7×10^5	5.5×10^5	4.5×10^4
	H.C	5.7×10^5	4.0×10^2	10^1	<10
Pseudomonas aeruginosa	**Control**	3.3×10^5	3.5×10^5	3.0×10^5	2.0×10^4
	H.C	3.1×10^5	2.0×10^5	<10	<10
Escherichia coli	**Control**	3.5×10^5	3.7×10^5	3.1×10^5	2.3×10^4
	H.C	3.3×10^5	2.3×10^2	<10	<10
Candida albicans	**Control**	2.0×10^5	1.7×10^5	1.3×10^4	1.1×10^3
	H.C	1.1×10^5	6.0×10^3	2.0×10^3	1.0×10^2
Aspergillus niger	**Control**	2.5×10^5	1.9×10^5	1.5×10^4	1.1×10^3
	H.C	2.0×10^5	9.0×10^4	5.1×10^3	1.2×10^2

The stability study by three cycles temperature testing showed excellent stability for the prepared herbal cream (H.C) when the formulation was exposed to three cycles of freezing and thawing and maintained the physical properties as that of the unexposed formulation and the results are given in **Table 7**.

Table 7. The stability study of Prepared formulation by three cycles of temperature testing

Formulation	Parameters studied	Initial	After 3 cycles of storage at -10°C and 30°C.
H.C	PH	5.9	5.7
	Color	Creamish white	Creamish white
	Consistency	Smooth and nongreasy	Smooth and nongreasy
	Fragrance	Pleasant	Pleasant
	Viscosity	16798	16738

CONCLUSION

The study demonstrated the promising antioxidant activity of *Phyllanthus emblica* with an IC_{50} value of 22.453 µg/ml, utilizing the DPPH scavenging model as a rapid assessment method. The viscosity of the herbal cream was notably significant at 0.5 rpm, confirming its consistency, while also exhibiting a substantial change in moisture content compared to the blank formulation. Additionally, the herbal cream displayed a significant SPF value, suggesting its potential as a skin protectant, further enhanced by the inclusion of higher concentrations of the plant extract. Importantly, the formulation maintained its physical properties even after exposure to multiple cycles of freezing and thawing. The preservative efficacy study demonstrated a remarkable reduction in bacterial colonies, with a decrease of over 99.9% observed within seven days, while fungal colonies exhibited a reduction of over 90% within 28 days. These findings collectively indicate the feasibility of developing antioxidant-rich herbal creams as effective skin barrier solutions, offering potential benefits for skin protection and health.

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