



FORMULATION AND EVALUATION OF HERBAL SUNSCREENS: AN ASSESSMENT TOWARDS SKIN PROTECTION FROM ULTRAVIOLET RADIATION

Ruchi Tiwari¹, Indu Singh¹, Monisha Gupta¹, Laliteshwar Pratap Singh², Gaurav Tiwari^{1*}

1. *Pranveer Singh Institute of Technology (Pharmacy), Kanpur, Uttar Pradesh, India.*
2. *Narayan Institute of Pharmacy, Gopal Narayan Singh University, Jamuhar, Rohtas Bihar, India.*

ARTICLE INFO

Received:

05 April 2022

Received in revised form:

07 Jun 2022

Accepted:

09 Jun 2022

Available online:

28 Jun 2022

Keywords: Herbal sunscreens with carbopol 934 base, Herbal sunscreens with a coconut oil base, Antioxidant activity, Mutagenic activity, SPF, stability activity

ABSTRACT

Sunscreen lotion is a sort of product that protects against the sun's harmful rays by containing ultraviolet radiation (UV rays), which is divided into two types: ultraviolet radiation A (UVA) and ultraviolet radiation B (UVB). The incorporation of herbal materials into sunscreen is one of the most effective and natural ways to protect against the sun, as measured by the sun protection factor (SPF), as well as the detrimental side effects of toxic chemicals. The present study aimed to develop herbal sunscreens containing turmeric (strong antiseptic property which protects skin from bacteria caused by excess sweat), coconut oil (used as a sun-block agent and helps to protect skin from sun damage), aloe vera (give a cooling effect to the skin and work as skin barrier), lemon (used to protect skin from sunburn) which will be effective for skin and protect skin against harmful sun rays, sunburn, and skin cancer. Prepared herbal sunscreens were evaluated for physicochemical characteristics, SPF, thermal stability, antioxidant activity, *in vitro* mutagenicity, and stability. Results showed that the F5 and F6 herbal sunscreens were of good consistency and viscosity with excellent antioxidant, non-mutagenic, nonirritant, stability activity and possessed 33.50 SPF for normal skin. In comparison to F1 through F4, formulations with a coconut oil base and carrot seed extract (F5 and F6) were shown to be stable and effective, with a high SPF.

This is an **open-access** article distributed under the terms of the [Creative Commons Attribution-Non Commercial-Share Alike 4.0 License](#), which allows others to remix, and build upon the work non commercially.

To Cite This Article: Tiwari R, Singh I, Gupta M, Singh LP, Tiwari G. Formulation and Evaluation of Herbal Sunscreens: An Assessment Towards Skin Protection from Ultraviolet Radiation. *Pharmacophore*. 2022;13(3):41-9. <https://doi.org/10.51847/svzLRFMP5F>

Introduction

It is extremely difficult to create sunscreen solutions that are effective, adhere to current regulatory standards, are marketable, and satisfy consumer demands. The four fundamental criteria of effectiveness, safety, registration, and patent freedom serve as checkboxes to verify that a sunscreen product has a chance of being successful following development [1]. Sun-block formulae must be created for repair, reduction of sunburn, sun tanning, skin melanoma, and early fine lines and wrinkles, as well as increasing the degree of sun protection factor (SPF) [2]. Sunscreens are frequently applied to the skin to protect it from the sun's harmful rays and to reduce the risk of skin disorders caused by the sun's rays. Broad-spectrum sunscreens are now being researched to reduce the long-term effects of high UV radiation [3]. Polyphenols and phenolic compounds, particularly natural oil, are essential ingredients for UV protection because of their high UV-A absorption efficiency and antioxidant activity [4]. UV rays are absorbed by certain bio-active substances in the environment, which protects the skin from their harmful effects. Because of their safety, absence of unpleasant responses, lack of dangerous chemical components, and environmental integrity, biologically active compounds have become more popular in cosmetics formulations in recent years [5]. Because synthetic photo-protective chemicals are more likely to be dangerous and carcinogenic, phytoconstituents are gaining favor as major cosmetics ingredients due to their natural anti-cancerous, anti-mutagenic, and non-toxic properties. Genuine herbal elements in sun-screen are the least irritating to the skin, especially for sensitive skin [6], include natural components, can regenerate the skin, and give enough protection against pollution and climate changes in the atmosphere. The most often used herbs in natural sunscreen include aloe vera, vitamin E, turmeric, and cucumber [7-9].

Corresponding Author: Gaurav Tiwari; Pranveer Singh Institute of Technology (Pharmacy), Kanpur, Uttar Pradesh, India. E-mail: dr_gauravtiwari81@gmail.com

Aloe vera is a well-known and ancient Liliaceae medicinal plant. This plant is a shrubby or arborescent perennial xerophytic succulent with a pea-green color. Aloe-vera leaf extracts include a lot of polyphenol components and chemicals in them. Aloe vera's 75 potentially active elements include vitamin supplements, minerals, carbohydrates, enzymes, lignin, saponins, salicylic acids, and amino acids [10-12]. It has successfully treated sunburns, including both first and second-degree burns [13]. Aloe vera gel has been found to protect human skin from all harmful effects of rays. Due to its anti-inflammatory, antibacterial, and wound-healing characteristics, aloe vera has long been used to treat digestive issues as well as skin injuries (burns, wounds, insect bites, and eczemas). The goal of research on this medicinal plant has been to confirm its historical applications, understand its mode of action, and pinpoint the chemicals that are responsible for these effects. The active ingredients that have received the greatest research are acemannan, aloe-emodin, aloin, aloesin, and emodin [14, 15]. Aloe vera contains both antimicrobial and antibacterial properties. It decreases the creation and secretion of immunosuppressive cytokines such as interleukin-10, which are secreted by epidermis keratinocytes, and prevents a protracted type of hypersensitivity when exposed to UV radiation (IL-10) [16].

Turmeric is produced by *Curcuma Longa*, a Zingiberaceae rhizomatous perennial herbaceous plant. Essential oil, tannin, and curcumin are all present in this plant. The extract of curcuma longa has anti-flatulent, anti-inflammatory, anti-fungal, anti-parasitic, anti-inflammatory, and anti-cancer properties. Additionally, Curcuma longa has the advantage of inhibiting apoptosis, according to a 2009 survey conducted at the University of Texas [17]. Curcumin has antioxidant and anti-inflammatory properties. Curcumin contains over a hundred different constituents, all of which have been identified. Turmeric is primarily made up of essential oil called turmerone, as well as curcuminoids, which are coloring compounds. Curcuminoids are antioxidants found in the environment, including curcumin de-methoxycurcumin, di-hydrocurcumin, and 5'-methoxycurcumin [18, 19].

Vitamin E is a fat-soluble antioxidant with photoprotective capabilities that is vital for human health. Vitamin E from food (RRR—tocopherol) is different from vitamin E from pills (all-rac-tocopherol). Because photosynthetic processes can create vitamin E, it should only be received in small amounts from outside sources [20].

Coconut oil is derived mostly from the dried seeds of edible coconut trees, commonly known as copra [21]. Lauric acid makes up a major portion of coconut oil. Coconut oil has long been used as a body lotion and for the prevention and healing of dry skin due to its thick, silky texture. Sunburn compositions including photoprotective coconut oil can lower inorganic UV radiation concentrations, reducing manufacturing challenges and meeting customer desire for more natural goods, however, humans have also been discovered to have considerable SPF values [22, 23].

Vitamin C and polyphenols, which are powerful antioxidants, are abundant in lemons. Lemon is a Rutaceae family medicinal plant that grows in tropical and subtropical Southeast Asia [24]. Even though this Citrus fruit has excellent nutritional characteristics, its significant biological effect in current phytotherapy and cosmetics is still unappreciated [25]. Vitamin C is required for the formation of elastin, the organic anatomical structure of the skin, as well as for sunburn protection.

In the present research, herbal sunscreens were prepared using Carbopol 934 base and coconut base with different herbal ingredients. Color, pH, viscosity, spreadability, thermal stability, in vitro antioxidant activity, in vitro mutagenicity activity, in vitro occlusion, and stability of fabricated herbal sunscreen lotions were all examined. By using an in-vitro spectrophotometric approach, the sun protection efficiency of the lotion was assessed in terms of SPF.

Materials and Methods

All the herbal ingredients such as Lemon (*Citrus Limon*), Turmeric (*Curcuma Longa*), Aloe vera (*Aloe Barbadensis*), and carrot seeds were collected from the botanical garden of Pranveer Singh Institute of Technology, Kanpur. Vitamin E was collected from Procter and Gamble Ltd.; Isca Guard PEG was collected from BRM Chemicals; Carbopol 934 was purchased from SD Fine Chemical Limited. All solvents were of analytical grade.

Methods

Prepared Sunscreen with Carbopol 984 Base

Herbal sunscreens were created by combining several herbs with carbopol 934 as a foundation (**Table 1**). One gm of carbopol 934 was soaked in distilled water, and additional components such as aloe vera, turmeric, vitamin E, and lemon were added and mixed continuously for one hour before adding the preservative. Evaluation studies began after one hour.

Table 1. Composition of herbal sunscreen formulations

Ingredients	F1	F2	F3	F4	F5	F6
Carbopol 934	1 g	1.5 g	5g	7g	-	-
Coconut oil	0.2 ml	0.4 ml	1 ml	0.5 ml	25 ml	15 ml
Aloe vera gel	20 ml	30 ml	40 ml	50 ml	20 ml	10 ml
Lemon juice	0.3 ml	1 ml	5 ml	3 ml	-	-
Turmeric	375 mg	1125 mg	375 mg	375 mg	375 mg	150 mg

Vitamin E	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg
Carrot seed	-	-	-	-	2 g	2 g
Green tea	-	-	-	-	2.836 g	2.836 g
Beeswax	-	--	-	-	500 mg	1.5 g
Preservative	0.5 %	0.5 %	0.5 %	0.4 %	0.5 %	0.5 %
Distilled water	100 ml	100 ml	100 ml	100 ml	-	-

Prepared Sunscreen with Coconut Oil Base

In a water bath, the required amount of coconut oil, green tea, bee wax, and carrot seeds was heated for 1 hour. When the oil phase was at room temperature, other ingredients such as aloe vera, turmeric, and vitamin E were gradually added while stirring constantly until a smooth and homogeneous paste was created. Following that, a preservative was added to the combination (**Table 1**). The manufactured herbal sunscreen was then stored in a cool location for further research or evaluation.

Evaluation of Herbal Sunscreen Formulations

Physical Parameters

Appearance, color, and homogeneity were determined.

Determination of Viscosity

The Brookfield viscometer (RVDV-II+PRO) was used to test viscosity, with the proper number of spindles selected. A 50 ml beaker was used to hold 50 g of preparation until the spindle groove was dipped and the rpm was set. Herbal sunscreen viscosity was measured at 5, 10, 20, 50, and 100 rpm. The viscosity was computed using the factor obtained from the reading.

Determination of pH

The pH of herbal sunscreens was determined using a digital pH meter. pH was measured after 1 g of the formulation was dissolved in 100 ml of newly prepared distilled water for 2 hours. The purpose of this study was to guarantee that the pH of the produced herbal sunscreens is similar to the pH of the skin after 24 hours of use. The results were triple-checked, and S.D. was recorded.

Extrudability Study

The extrudability of herbal sunscreens was determined in this study by calculating the percentage of formulation extruded from the collapsible tube based on the weight in grams necessary to extrude at least 0.5 cm of gel ribbon in 10 seconds [22]. After that, the extrudability was estimated using the formula:

$$\text{Extrudability} = \frac{\text{Applied weight to extrude gel from tube (gm)}}{\text{Area (cm}^2\text{)}} \quad (1)$$

Spreadability

The spreadability of herbal sunscreens determined their therapeutic efficiency. The appropriate amount of herbal sunscreen was applied between two slides, and under specified load directions, and the two sides took the time in seconds to slide off. Spreadability was defined as the amount of time it took to separate two slides in less time [22]. The formula for calculating it is:

$$S = M \times L / t \quad (2)$$

Where M = weight tied to upper slide

L = length of glass slide

T = time taken to separate the slides

Thermal Stability

The oil separation from herbal sunscreens was evaluated in a humidity chamber at 60-70 % RH and 37±1°C. A 20 mm wide and 5 mm thick stripe of herbal sunscreens was applied to the internal wall of a 100 ml capacity chamber in its whole heights. The beaker was stored in a humidity chamber for 8 hours at 60-70 % relative humidity and 37°C. There should be no oil separation in the herbal sunscreen to pass the test [23].

Skin Irritation Study

Three healthy rat groups (1273/PO/Re/S/09/CPCSEA), each with six rats of either sex, were used in the skin irritation investigation. The animals were fed conventional animal feed and had unlimited access to water. Hair was shaved from the

backs of the rats on one of the study days, and 5 cm² of the area was marked on both sides, with one side serving as a control and the other being tested. No reaction, slight patchy erythema, slight but confluent or moderate but patchy erythema, and severe erythema with or without edema were graded as 0, 1, 2, 3 for no reaction, slight patchy erythema, slight but confluent or moderate but patchy erythema, and severe erythema with or without edema, respectively [26].

Determination of SPF

A UV Visible spectrophotometer was used to examine the in-vitro efficacy of herbal sunscreens. A 0.10 percent solution (w/v) of herbal sunscreen lotions in ethanol was made by dissolving 0.050 g of herbal sunscreen lotions in 50.0 ml of ethanol. Between 290 and 320 nm, aliquots of each herbal sunscreen were scanned at 5 nm intervals. SPF was calculated using the equation below. Three times each sample was analyzed [27].

$$SPF = CF \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times A(\lambda) \quad (3)$$

Whereas, CF= Correction factor; EE= Erythemogenic effect; I= Intensity of solar light of wavelength; A= Absorbance

DPPH Method for in-vitro Antioxidant Activity Determination

In different vials, 1 ml of varying concentrations of herbal sunscreens and ascorbic acid as standard were taken. 5 mL of DPPH methanolic solution was added to this, shaken thoroughly, and incubated at 37°C for 20 minutes. At 516 nm, the absorbance was measured against methanol as a blank. The DPPH absorbance was used as a control [24]. The following formula was used to compute the percentage of antiradical activity:

$$\% \text{ Anti - radical activity} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100 \quad (4)$$

In-vitro Occlusion Studies

Skin occlusion is indicated by complete coverage of the skin's surface. The following equation can be used to determine the occlusivity of herbal sunscreens:

$$\text{Occlusion factor (F)} = \frac{(A - B)}{A} \times 100 \quad (5)$$

where A= water loss without sample and B = water loss with the sample.

This approach employs a filter paper-covered water-filled beaker. The occlusion factor ranges from zero to one hundred, with zero indicating no occlusion effect and one hundred indicating complete surface covered by topical application. 50 to 200 mg of each herbal sunscreen were evenly placed on the filter paper surface to form a solid film with an 8.5 mg/cm² concentration. A beaker covered with filter paper was used as a reference control, with no samples applied. For 48 hours, keep the samples at 32°C and 50–55% RH. Meanwhile, water flow or evaporation through the filter paper was measured by weighing the samples after 6, 24, and 48 hours. Each experiment was duplicated three times [25].

Stability Testing

Centrifugation and the freeze-thaw method were used to test the stability of each herbal sunscreen. For 10 minutes, the centrifugation was carried out at 10000 rpm with 500 rpm intervals, and phase separation was observed. All herbal sunscreens were stored at 20°C and 40°C in freeze-thaw research, and phase separation was noted. All of the tests were done three times [28].

Safety Evaluation by Mutagenicity Assay

Salmonella typhimurium strain TA 100 without the S9 mix was employed in the investigation. Sodium azide (CAS Number: 26628-22-8) was used as a positive control for TA 100: 5 g/plate. As a negative control, sterile distilled water was used. Before the start of each experiment, fresh solutions of the reference mutagen were created. The samples were dissolved in dimethyl sulfoxide (DMSO) and preincubated in phosphate buffer with the test strain for 20 minutes at 37°C. The plates were incubated at 37°C for 48 hours after the test samples (herbal sunscreens) were added. The mutagenic reactions of the sunscreen compounds were assessed using a triplicate assay for each sample [26, 28-30].

Results and Discussion

By graphing viscosity against sheer speed, the viscosity of herbal sunscreens was determined (**Figure 1**). With an increase in sheer speed (rpm), the viscosity of herbal sunscreens was observed to decrease, indicating pseudoplastic behavior, which is a desirable property for cosmetic formulations since it enables maximum area coverage when applied. When greater force is

given to a pseudoplastic fluid, its viscosity drops. "Shear thinning" is the term for this effect. The bulk of cosmetic products is pseudoplastic because this behavior allows a lotion to flow through a pump and disseminate on the skin. pH values of herbal sunscreens (F1-F4) were found between 6.7 to 6.8, while for F5 and F6 pH was found to be 5.6 and 5.7 respectively (**Table 2**). For two reasons, an acidic pH is essential for barrier homeostasis: first, two ceramide-synthesizing enzymes have low pH optima; and second, a low pH promotes the 13-nm long periodicity phase required for barrier construction. An acidic pH, in other words, generates a more effective barrier [26, 29, 31]. A lower skin surface pH is associated with greater resistance to SLS-induced irritating dermatitis, whereas patients with skin pH 5 have less scaling and higher moisture levels than those with skin pH > 5 [28, 30]. The improved defense against invading microorganisms in an acidic rather than a neutral or alkaline environment is one of the topics that comes constantly when analyzing the scientific literature on skin surface pH. Propionibacteria, for example, thrives effectively at pH 6.0 to 6.5 but slows down around pH 5.5. Staphylococcus aureus thrives best at pH 7.5, while at pH 5.0 to 6.0, it grows poorly. The acidic pH of the stratum corneum discourages pathogenic flora colonization and promotes the survival of healthy microbial flora. The skin's surface is acidic, but as you go deeper, the pH of the SC rises until it reaches neutral levels in the viable epidermis. The identification of acidic membrane compartments (also known as microdomains) throughout the SC has revealed the complexity of the pH gradient [32]. The pH of a topical formulation should ideally be slightly acidic in the range of 5 to 5.5, especially in the case of topical formulations that are used often. In actuality, topical preparations are appropriate in the pH range of 5-7 [27, 33]. Extrudability of herbal sunscreens was assessed because high consistency formulations may not extrude from tubes, whilst low viscous formulations may flow fast, necessitating the use of an appropriate consistency to extrude gel from tubes. Herbal sunscreens had a range of extrudability from good to exceptional [25]. Herbal sunscreens have great homogeneity (**Table 2**). When compared to other formulations, the spreadability of F5 and F6 was considered high because of the low time spread. Gels' medicinal efficacy is determined by their distribution. Because gel spreadability aids in uniform administration of the gel to the skin, produced gels must have good spreadability and meet the optimum quality in the transdermal application (**Table 2**). Furthermore, this is seen as a critical element in patient treatment adherence.

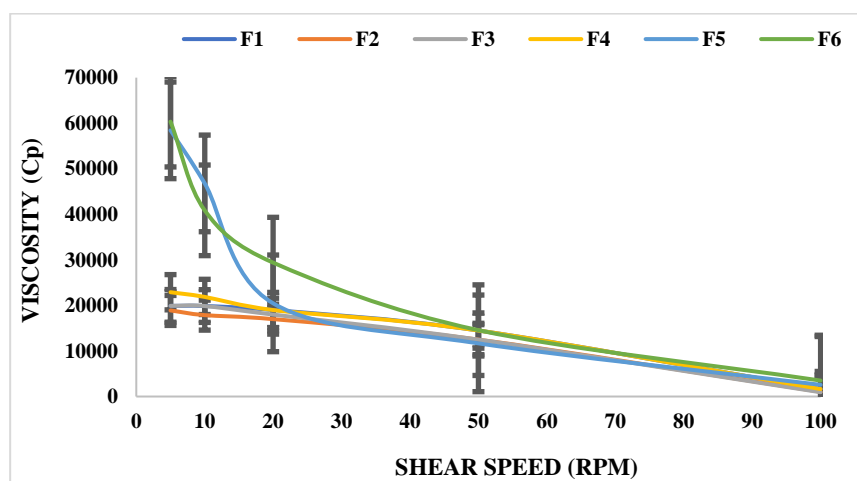


Figure 1. Comparative viscosities of herbal sunscreen formulations. n= 3

Table 2. Physical characteristics and SPF of herbal sunscreen lotions

	F1	F2	F3	F4	F5	F6
Appearance	Cream like	Cream like	Cream like	Cream like	Cream like	Cream like
Color	Greenish-yellow	Greenish-yellow	Greenish-yellow	Greenish-yellow	Greenish-yellow	Greenish-yellow
pH	6.7±0.923	6.5±1.23	6.8±2.03	6.9±0.923	5.6	5.8
Extrudability	++++	++++	++++	++++	+++	+++
Homogeneity	Uniform and homogenous	Uniform and homogenous	Uniform and homogenous	Uniform and homogenous	Uniform and homogenous	Uniform and homogenous
Spreadability (gm.cm/sec)	11.98±1.91	12.01±2.09	12.45±2.73	11.23±3.85	18.91±1.09	19.23±1.42
Thermal stability	unstable	unstable	unstable	unstable	Stable	Stable
Skin irritation	No	No	No	No	No	No
SPF	1.06	7.31	1.21	0.30	33.43	33.47
Occlusion factor	72±1.02	75±2.42	79±1.95	81±2.87	94±1.36	97±1.20
Stability						
(i) Centrifugation	Phase separation	Phase separation	Phase separation	Phase separation	No phase separation	No phase separation
(ii) Freeze-thaw	unstable	unstable	unstable	unstable	stable	stable

++++= Excellent, +++= Good, ++= Fair

Thermal Stability

The phase separation was evaluated in a humidity room at 37°C and 60-70 % relative humidity. F1 to F4 formulations showed phase separation, however, F5 and F6 formulations were determined to be stable with no phase separation at 37°C. The evaporation of water content at high temperatures may be the cause of instability in F1 to F4 formulations, whereas F5 and F6 herbal sunscreens were oil-based lotion (**Table 2**) [28].

Skin Irritation Study

After seven days of using herbal sunscreens, a skin irritation study found no irritation, sensitivity, or minor or patchy erythema (**Table 2**) [31].

In-vitro Determination of SPF by UV-spectrophotometer

Despite being a source of life and energy, sunlight causes serious health problems such as sunburn, pigmentation, wrinkles, dermatitis, urticaria, aging, immunological suppression, and a variety of skin malignancies. SPF is a measure of how effective sunscreens are at preventing sunburn SPF. The absolute protection efficacy of sun care products against erythema-effective UV light was determined using *in vitro* transmittance measurements and weighted with the erythema action spectrum and the "standard" output spectrum of a UV solar simulator used for SPF testing [23, 34]. The SPF of herbal sunscreens was estimated using the equation. The aliquots were scanned between 290 and 320 nm, and the absorbance values obtained were multiplied by the corresponding EE (λ) and I (λ) values. Then, their summation was taken and multiplied with the correction factor i.e., 10. All herbal sunscreens revealed significant SPF (**Table 3**). When comparing herbal sunscreens, the F5 and F6 formulations showed promising results, with higher SPF ratings than other formulations. This could be because of the presence of carrot seed extract, which has an SPF of roughly 20, or because of the synergistic activity of all herbal compounds utilized in herbal sunscreen formulations, such as coconut oil, turmeric, and aloe vera [26, 28-30, 32]. Efficacy of photoprotection found in following order F6 > F5 > F2 > F3 > F1 > F4. These results reveal that the prepared F5 and F6 herbal sunscreens have good SPF and good sun protection activity.

Table 3. SPF determination of herbal sunscreens

Wavelength (nm)	EE (λ)*I (λ)	F1		F2		F3		F4		F5		F6	
		Abs (λ)	Abs (λ)*EE (λ)*I (λ)	Abs (λ)	Abs (λ)*EE (λ)*I (λ)	Abs (λ)	Abs (λ)*EE (λ)*I (λ)	Abs (λ)	Abs (λ)*EE (λ)*I (λ)	Abs (λ)	Abs (λ)*EE (λ)*I (λ)	Abs (λ)	Abs (λ)*EE (λ)*I (λ)
290	0.01	0.28	0.004	0.95	0.014	0.06	0.0009	0.00	0.0001	3.17	0.0476	3.17	0.047
295	0.08	0.14	0.011	0.79	0.065	0.08	0.0065	0.01	0.0010	3.02	0.2468	2.97	0.243
300	0.28	0.10	0.030	0.74	0.214	0.10	0.0298	0.02	0.0068	3.11	0.8941	3.13	0.900
305	0.32	0.10	0.033	0.72	0.236	0.12	0.0419	0.03	0.0108	3.50	1.1468	3.56	1.168
310	0.18	0.09	0.017	0.70	0.131	0.14	0.0272	0.03	0.0072	3.42	0.637	3.61	0.673
315	0.08	0.09	0.007	0.69	0.058	0.14	0.0123	0.03	0.0032	3.60	0.3014	3.09	0.258
320	0.01	0.08	0.001	0.68	0.012	0.14	0.0025	0.03	0.0006	3.83	0.0689	3.08	0.055
Total			0.1061		0.7315		0.1213		0.0300		3.3435		3.3475
SPF			1.06		7.32		1.21		0.30		33.44		33.50

In-vitro Antioxidant

Exogenous causes such as UV radiation and pollution, as well as endogenous generation of radicals from cellular metabolism, can harm the skin at the cellular and tissue levels. Although the body has a sophisticated defense system in place to avoid radical damage, this system can be overburdened, resulting in oxidative stress, immunological suppression, and even cancer. Antioxidant supplements applied topically can help to neutralize reactive oxygen species produced by both endogenous and exogenous sources. 1, 1-diphenyl-2-picrylhydrazyl (diphenyl—picrylhydrazyl; DPPH) is a stable free radical that can delocalize to produce a violet color shift in ethanol. However, after reacting with antioxidant molecules, there is a reduction in violet color intensity, which may be detected at 517 nm. 2 ml of 0.5 millimolar DPPH solution was combined with 0.2 ml of methanolic extract. After 30 minutes, the absorbance of the samples and standards were measured at 517 nm. The anti-radical activity of equally diluted materials was determined using the DPPH method, and activity was found to be concentration-dependent. Ascorbic acid's antioxidant activity in F1, F2, F3, F4, F5, and F6 was determined to be 96.45, 68.87, 71.23, 76.78, 77.89, 87.54, and 89.56 %, respectively (**Figure 2**). However, as compared to ordinary ascorbic acid, F5 and F6 herbal sunscreens had the best antioxidant activity [33], while formulations with a higher dose of carbopol 934 had the lowest. Because the improved herbal sunscreens F5 and F6 have high antioxidant activity, they can be used as photo protectors.

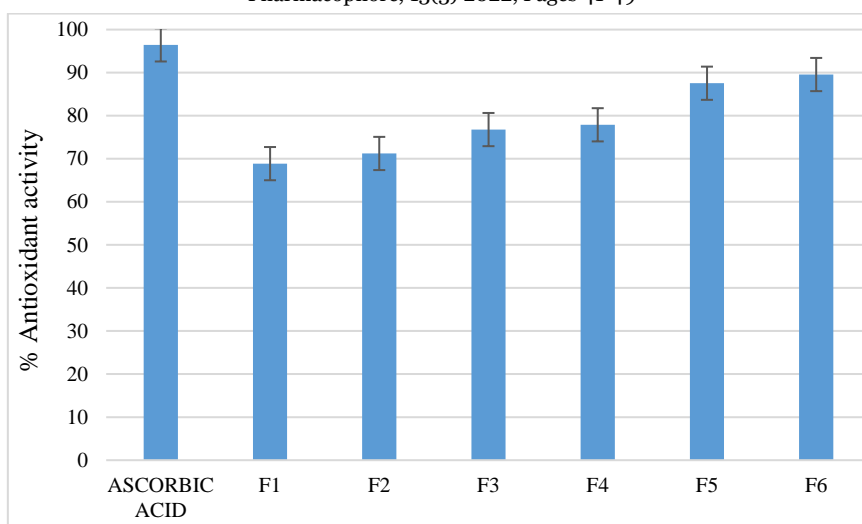


Figure 2. Percentage antioxidant activity of herbal sunscreens compared to standard (ascorbic acid). n =3

In vitro Occlusion Studies

The in-vitro filter paper-coated water-filled beaker method was used to measure the occlusion factor of herbal sunscreens. Herbal sunscreens were found to have an occlusive factor ranging from 72 ± 1.02 to 97 ± 1.20 (**Table 2**). Occlusion is important for moisturizing and improving skin partitioning, which is directly proportional to the thickness of the applied sample layer [30].

Stability Testing

Phase separation was found for F1 to F4 at 10000 rpm, showing that these formulations are unstable under high stress. Water was removed from these formulations during the freeze-thaw research, indicating that they may not be able to withstand various environmental changes during product shipping. In comparison to F5 and F6 formulations, which were proven to be stable under stress conditions, herbal sunscreens created with a carbopol base require more attention and protection from temperature variations and environmental pressures. Furthermore, the viscosity of herbal sunscreens was discovered to have a direct correlation with centrifugal results/phase separation. Herbal sunscreen with a higher viscosity was shown to be more stable against centrifugation. As a result of their greater viscosity compared to other formulations, F5 and F6 herbal sunscreens demonstrated no phase separation [26, 28-30, 32].

Mutagenicity Study

All herbal sunscreens were found to be non-mutagenic in mutagenicity experiments. As a result, the created formulations were thought to be safe. According to the psychometric examination, the formulation performed well in terms of handling, firmness, and skin radiance. Due to the elimination of dead cells, the skin glow effect of F5 and F6 herbal sunscreens was found to be long-lasting [26].

Conclusion

The current study aimed to create a stable herbal sunscreen with a suitable SPF. Coconut oil-based sunscreens (F5 and F6) were found to be stable, have good antioxidant activity, and have high SPF values of 33.43 and 33.50, respectively. These herbal sunscreens have also shown to be non-mutagenic. It can be stated that the current study will hopefully lead to improvements in the treatment of sunburns produced by UV radiation exposure. The study also demonstrates that UV Spectroscopy is the most efficient, acceptable, and repeatable approach for determining the performance of herbal sunscreens. As a result, the findings of this study can help regulatory agencies, scientific organizations, and manufacturers set standardized standards for herbal sunscreens.

Acknowledgments: Authors would like to acknowledge Pranveer Singh Institute of Technology for providing laboratory and chemical facilities.

Conflict of interest: None

Financial support: None

Ethics statement: None

References

1. Hanay C, Osterwalder U. Challenges in Formulating Sunscreen Products. *Curr Probl Dermatol*. 2021;55:93-111. doi:10.1159/000517655
2. Skotarczak K, Osmola-Mańkowska A, Lodyga M, Polańska A, Mazur M, Adamski Z. Photoprotection: facts and controversies. *Eur Rev Med Pharmacol Sci*. 2015;19(1):98-112.
3. Supriya S. Design and Evaluation of Polyherbal Formulation for Treatment of Malaria. *Int J Pharm Phytopharmacol Res*. 2021;11(3):1-5. doi:10.51847/R4Y4UUoZG8x
4. Chawla A, Kaur R, Sharma AK. *Ficus carica* Linn.: A Review on its Pharmacognostic, Phytochemical and Pharmacological Aspects. *Int J Pharm Phytopharmacol Res*. 2012;1(4):215-32.
5. Mishra A, Mishra A, Chattopadhyay P. Herbal Cosmeceuticals for Photoprotection from Ultraviolet B Radiation: A Review. *Trop J Pharm Res*. 2011;10(3):351.
6. Cefali LC, Ataide JA, Moriel P, Foglio MA, Mazzola PG. Plant-based active photoprotectants for sunscreens. *Int J Cosmet Sci*. 2016;38(4):346-53.
7. Shanbhag S, Nayak A, Narayan R, Nayak UY. Anti-aging and Sunscreens: Paradigm Shift in Cosmetics. *Adv Pharm Bull*. 2019;9(3):348-59. doi:10.15171/apb.2019.042
8. Dutta D, Goyal N, Kumar Sharma D. Formulation and development of herbal microsphere sunscreen gel. *J Cosmet Dermatol*. 2022;21(4):1675-87.
9. Sajjad A, Subhani Sajjad S. Aloe vera: An Ancient Herb for Modern Dentistry—A Literature Review. *J Dent Surg*. 2014;2014:1-6.
10. Singh B, Mohan R, Maurya A, Mishra G. Phytoconstituents and biological consequences of Aloe vera: A focused review. *Asian J Pharm Pharmacol*. 2018;4(1):17-22.
11. Hashim DM, Sheta NM, Elwazzan VS, Sakran W. Enhancing the sunscreen efficacy of bemotrizinol micropigment by using o/w nanoemulsion topical preparations. *Int J Pharm Pharm Sci*. 2019;11(7):47-56.
12. Radha MH, Laxmipriya NP. Evaluation of biological properties and clinical effectiveness of Aloe vera: A systematic review. *J Tradit Complement Med*. 2014;5(1):21-6.
13. Patel DK, Patel K, Dhanabal S. Phytochemical standardization of Aloe vera extract by HPTLC techniques. *J Acute Dis*. 2012;1(1):47-50.
14. Kryczyk-Poprawa A, Kwiecień A, Opoka W. Photostability of Topical Agents Applied to the Skin: A Review. *Pharmaceutics*. 2019;12(1):10. doi:10.3390/pharmaceutics12010010
15. Sánchez M, González-Burgos E, Iglesias I, Gómez-Serranillos MP. Pharmacological Update Properties of Aloe Vera and its Major Active Constituents. *Molecules*. 2020;25(6):1324. doi:10.3390/molecules25061324
16. Munir A, Malik SI, Aslam S, Mehmood A, Amjad S, Malik KA, et al. Medicinal plants are effective inhibitors of type i and ii diabetes. *Pharmacophore*. 2018;9(5):1-7.
17. Shahrzad K, Seyyed MJM, Meisam A, Esmaeil K, Nasrin MA, Hossein M. The effect of Curcuma longa Rhizome Extract on Blood Cells of Mice: An Animal Trial. *Pharmacophore*. 2017;8(3):19-23.
18. Ruby AJ, Kuttan G, Babu KD, Rajasekharan KN, Kuttan R. Anti-tumour and antioxidant activity of natural curcuminoids. *Cancer Lett*. 1995;94(1):79-83.
19. Selvam R, Subramanian L, Gayathri R, Angayarkanni N. The anti-oxidant activity of turmeric (*Curcuma longa*). *J Ethnopharmacol*. 1995;47(2):59-67.
20. Colombo ML. An update on vitamin E, tocopherol, and tocotrienol perspectives. *Molecules*. 2010;15(4):2103-13.
21. Nevin KG, Rajamohan T. Beneficial effects of virgin coconut oil on lipid parameters and in vitro LDL oxidation. *Clin Biochem*. 2004;37(9):830-5.
22. Saifee M, Atre M, Toshniwal R. Formulation and In-vitro Evaluation of Ethosomal Gel of Repaglinide for Transdermal Delivery. *Int J Pharm Phytopharmacol Res*. 2021;11(4):11-7. doi:10.51847/IQKgwU11
23. Herzog B, Sohn M. The Formula for Best Sunscreen Performance: Beer-Lambert's Law Under the Microscope. *Curr Probl Dermatol*. 2021;55:133-43. doi:10.1159/000517663
24. Klimek-Szczykutowicz M, Szopa A, Ekiert H. Citrus limon (Lemon) Phenomenon-A Review of the Chemistry, Pharmacological Properties, Applications in the Modern Pharmaceutical, Food, and Cosmetics Industries, and Biotechnological Studies. *Plants (Basel)*. 2020;9(1):119.
25. Goetz P. Citrus limon (L.) Burm. f. (Rutaceae) citronnier. *Phytothérapie*. 2014;12(2):116-21.
26. Shakib Z, Shahraki N, Razavi BM, Hosseinzadeh H. Aloe vera as an herbal medicine in the treatment of metabolic syndrome: A review. *Phytother Res*. 2019;33(10):2649-60. doi:10.1002/ptr.6465
27. Ahmady A, Amini MH, Zhakfar AM, Babak G, Sediqi MN. Sun Protective Potential and Physical Stability of Herbal Sunscreen Developed from Afghan Medicinal Plants. *Turk J Pharm Sci*. 2020;17(3):285-92. doi:10.4274/tjps.galenos.2019.15428
28. Bouwstra JA, Gooris GS, Dubbelaar FE, Ponc M. Cholesterol sulfate and calcium affect stratum corneum lipid organization over a wide temperature range. *J Lipid Res*. 1999;40(12):2303-12.
29. Hachem JP, Crumrine D, Fluhr J, Brown BE, Feingold KR, Elias PM. pH directly regulates epidermal permeability barrier homeostasis and stratum corneum integrity/cohesion. *J Invest Dermatol*. 2003;121(2):345-53.

30. Behne MJ, Meyer JW, Hanson KM, Barry NP, Murata S, Crumrine D, et al. NHE1 regulates the stratum corneum permeability barrier homeostasis. Microenvironment acidification assessed with fluorescence lifetime imaging. *J Biol Chem.* 2002;277(49):47399-406.
31. Lin YY, Lu SH, Gao R, Kuo CH, Chung WH, Lien WC, et al. A Novel Biocompatible Herbal Extract-Loaded Hydrogel for Acne Treatment and Repair. *Oxid Med Cell Longev.* 2021;2021:5598291. doi:10.1155/2021/5598291
32. Lukić M, Pantelić I, Savić SD. Towards Optimal pH of the Skin and Topical Formulations: From the Current State of the Art to Tailored Products. *Cosmetics.* 2021;8(3):1-18. doi:10.3390/cosmetics8030069
33. Noor A, Islam M, Anisuzzaman S, Roy J, Begum AA. Evaluation of Antioxidant Activity of Ethanolic and Chloroformic Extracts of *Nymphaea nouchali* Leaves. *Int J Pharm Phytopharmacol Res.* 2013;2(6):417-20.
34. Balkrishna A, Singh S, Srivastava D, Mishra S, Sharma S, Mishra R, et al. A systematic review on traditional, ayurvedic, and herbal approaches to treat solar erythema. *Int J Dermatol.* 2022. doi:10.1111/ijd.16231