Development and Evaluation of Herbal Sunscreen

Mukund Manikrao Donglikar[1] and Sharada Laxman Deore[2*]

ABSTRACT
Thus present research work deals with the development and evaluation of topical photo protective formulation, containing antioxidant, wound healing, anti-inflammatory and rather photo protective poly phenols like curcumin, quercetin, resveratrol and safranal. The present research work provides stable natural photo protective formulation with antioxidant potential, high SPF and more important uniform UVA/UVB protection.
Key words: Sunscreen, Resveratrol, Quercetin, Curcumin, Safranal, SPF.

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INTRODUCTION

From the dawn of mankind, Sun is source of life and energy. But recent studies accepts sun as main culprit of deleterious effects including acute effects (e.g., sunburn and drug-induced photo toxicity) and chronic risks of frequent sun ray exposure like sunburn, crack, melanoma and pigmentation, cancer and immune suppresion.[1] Sun rays are most harmful environmental factor which affects skin, cause sun burn, skin cancers and photo ageing. Due to these harmful effects of UV radiations there is need to develop sunscreen formulation to heal, prevent sun burn, suntan, skin cancer and premature skin ageing and to increase level of Sun Protection Factor.[2]

The goal of sunscreen formulation is to block UV-rays and increase the level of protection from the UV-rays. The key components of UV protection are flavonoids, phenolic compounds or herbal oils due to their UV rays absorption capacity in UV-A region and their antioxidant activity.[3] Cell mutation, DNA damage, hormone alteration and eczema like allergic reaction are some adverse effects of the synthetic sunscreen agents. Sunscreen formulations available in market don not have properties like wound healing, anti-inflammatory, cooling and anti-ageing. Again free radical mediated skin damages cannot be cured until and unless free radical scavengers are not available in photo protective products.[4]

During the market survey, it is found that there are many sunscreen formulations available in markets which are used in protection of skin from UV rays. Various formulations have different sun protection activity on basis of their efficacy of UV rays absorption but maximum formulations are of high cost and incorporated synthetic molecules are with potential toxicity and even carcinogenesis.[4] Hence there is need to develop and evaluate effective and safe sunscreen product which can give solution to sunburn, wounds, cracks, wrinkles, premature ageing and antioxidant ingredients to help in protection of long term damaging effects of sunrays mediated free radicals.

Curcumin, quercetin, resveratrol and safranal belong to class of poly phenolics and are potent antioxidants as well as photo protective. But additionally curcumin is wound healing, antiseptic; quercetin is anticancer, resveratrol is antiaging and safranal is emollient.[5-10] So sunscreen product incorporated with these ingredients can give desired all-in-one product.

Curcumin (diferuloylmethane) is a yellow odorless pigment isolated from the rhizome of turmeric (*Curcuma longa*). Curcumin possesses anti-inflammatory, antitumoral, and antioxidant properties. It has been found that topical application of curcumin in epidermis of CD-1 mice significantly inhibited UVA-induced ornithine decarboxylase ornithine decarboxylase (ODC) activity. The inhibitory effects of curcumin were attributed to its ability to scavenge reactive oxygen species reactive oxygen species (ROS). Curcumin can prevent UV irradiation-induced apoptotic changes in human epidermoid carcinoma A431 cells.[5]

Quercetin is polyphenolic compound present in *citrus* species shows strong immune modulatory, antioxidant, anti-inflammatory effects and act as a. Quercetin and rutin were tested as potential topical sunscreen factors in human beings and found to provide protection in the UVA and UVB range.[6]

Resveratrol is chemically fat soluble stilbenes belong to polyphenolic class. It is of trans and a cis configuration. It acts as a potent antioxidant and as well anticancer and anti-inflammatory.[7-8]

Safranal, an organic aromatic compound present in stigmas of crocus flowers (*Crocus sativus*). It exhibits high antioxidant and free radical scavenging efficacy. It is also found to be anticancer.[9-10]

MATERIALS AND METHODS

Pre-formulation studies

Quercetin, curcumin and resveratrol were purchased from THS, Mumbai. Safranal was purchased from KuberImpex Limited, Indore. Drugs were identified by various physical parameters (Table 2), spectroscopic studies[11] (Figure 1 and 2) and chromatographic studies (Figure 3 and 4).[12] Various confirmatory chemical tests performed to identify purchased chemicals. And thus selected phy to chemicals further processed to know SPF values and antioxidant activity.

In vitro SPF determination of active phyto chemicals

Initial stock solution was prepared by taking 1% w/v pure drug (curcumin, quercetin, resveratrol and safranal) in ethanol: distilled water (40: 60). Then from this stock Solution, 0.1% stock solution was prepared. Thereafter, absorbance values of each aliquot prepared were determined from 290-320 nm, at 5 nm intervals, taking ethanol: distilled water (40: 60) solution as blank, using Shimadzu UV-Spectrophotometer. Determinations were made in triplicate at each point. SPF of active drugs for were calculated by the application of equation:[13]

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

The aliquot prepared were scanned between 290-320 nm and the obtained absorbance values were multiplied with the respective EE (λ) and I (λ) values. Then, their summation was taken and multiplied with the correction factor (10)

In-vitro antioxidant activity determination by DPPH Method

1 ml different concentration of active drug and standard were taken in different vials. To this 5 ml of methanolic solution of DPPH was added, shaken well and mixture was incubated at 37°C for 20 min. The absorbance was measured against methanol as blank at 516 nm. Absorbance of DPPH was taken as control.[14-15] Percentage antiradical activity calculated by using following formula;

$$\% \text{ Anti} - \text{radical activity} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

Development of Formulation

About 20 cream bases were formulated in the preliminary study. The components used were in a generally recognized as safe (GRAS) status. The cream bases were prepared via emulsification process. Briefly, an oil phase containing lipophilic substances and an aqueous phase containing hydrophilic substances were separately heated in a water bath to 80°C. Afterwards, the aqueous phase was gradually added into the oil phase with constantly stirring until the mixture was congealed at the room temperature.[16] The resulted cream bases were optically observed for appearance, texture and spread ability. It was found that three cream bases had desirable properties; however, only two bases provided good characteristics after incorporated with pure phyto chemicals. Cream formulations of varying phyto chemicals composition were developed. All studied concentrations were in the legislated range. Compositions are shown in Table 1.

Evaluation of Formulation
Physical Parameters

Appearance, color and homogeneity are determined. Results are shown in the Table 12 and Figure 5.

Subjective Properties

Consistency, feel on application and irritation parameters are determined and results are shown in the Table 12.

Spread ability

Two glass slides of standard dimensions (20 × 5 cm) were selected. The formulation was over one of the slide. The other slide placed on the top of the cream such a that the formulation sandwiched between the two slides in an area occupied by a distance of 7.5 cm, alongside 100 gm weight was placed uniformly to form a thin layer. The weight was removed and the excess of cream adhering to the slides was scrapped off. The two slides in a position were fixed to stand (45° angle) without slightest disturbance and in such a way that only the lower slide held firmly by the opposite fangs of the clamps allowing the upper slide to slip off freely by the force of weight tied to it. 60 gm of weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 5 cm and separate away from the lower slide under the direction of weight was noted. The experiment repeated for 3 times and the mean taken for three such dimensions was calculated.[17] The results were recorded. The Spread ability is calculated by using formula: S = M*L/T Where, S= Spread ability, L= Length of glass slide, M= Weight tied to the upper slide and T= Time. In present experiment M= 60 gm and L= 7.5 cm. The data showing the Spread ability of different formulation are in the Table 12.

Extrudability

The cream formulation was filled in the standard capped collapsible aluminum tubes and sealed by crimping the ends. The weight of the tubes was recorded. The tube was placed between two glass slides and was clamped. A 500 gm cream was placed over the glass slides and then the cap was removed. The amount of cream extruded was collected and weighed.[16] The percent of cream was calculated and graded as fallow:

90%	Extrudability = + + + + Excellent		
80%	Extrudability =	+ + + Good	
70%	Extrudability =	+ +	Fair
50%	Extrudability =	+	Poor

The Extrudability of all the formulation is tabulated in Table 12.

Thermal stability

In this test the oil separation from the cream were tested at 60-70% RH and 37±1°C in humidity chamber. In this a 20 mm broad and 5 mm stripe of cream were spread on the internal wall of the chamber of 100 ml capacity, in its total heights. The beaker kept for 8 hrs in humidity chamber at 60-70% RH and temperature 37 ± 1°C. To pass the test there should not be any oil separation in the cream.[17] the results are shown in the Table 12.

pH Determination

Cream might have variety of pH mostly ranging from 5 to 9. The cream in general has a pH 6 to 9. Hazelton reported that there is little correlation between pH and irritancy. The electrode must be washed and free from any residue of acid and alkali to ensure the accurate reading. *Procedure:* All the formulations were oil in water semisolid emulsions. As pH of the cream not to be directly measured, here 10% dilutions were made with distilled water and the resulting pH of mixture was determined with a pH meter.[18] The results are shown in the Table 12.

Rancidity

This test is performed by using the Phloroglucinol solution. The rancidity is due to the oxidation of the fats and oils; during oxidation free fatty acids are liberated. These free fatty acids react with the Phloroglucinol solution and gives pink colour. This indicates the rancidity of the product.

Table 1: Formulae for development of trial batches of Photo protective cream formulations

Ingredients	F -I	F -II	F- III	F-IV	F-V
Curcumin	4	-	-	-	4
Quercetin	-	3	-	-	3
Resveratrol	-	-	4	-	4
Safranal	-	-	-	3	3
Cetostearyl alcohol	5	5	5	5	5
Stearic acid	2	2	2	2	2
PEG-200	2	2	2	2	2
Cetyl alcohol	1	1	1	1	1
Methyl paraben	0.3	0.3	0.3	0.3	0.3
Propyl paraben	0.06	0.06	0.06	0.06	0.06
Carbopol 940	0.5	0.5	0.5	0.5	0.5
Disodium EDTA	q.s	q.s	q.s	q.s	q.s
Triethanolamine	0.5	0.5	0.5	0.5	0.5
Distilled Water q. s. to 100 gm					

10 ml of melted cream was taken then added 10 ml of concentrated hydrochloric acid and 10 ml of Phloroglucinol solution and shaken for one minute. The material shall be taken to have passed the test if no pink colour developed. The results are shown in the Table 12.

Viscosity

Viscosity of creams was measured by the Brookfield viscometer. The correct spindle was selected (spindle no. 4) for the given product then the operating condition was setup. Then the viscosity was measured directly at 6 rpm speed by keeping the torque constant.[17] The mean was obtained. The results are shown in the Table 12. The viscosity is determined by following formula:

Viscosity = Dial Reading × Factor. For LV-4 at 6 RPM Factor is 1M (1000)

Photo stability determination

2 mg/cm2 of each sunscreen cream was weighed and spread evenly between two plates of polished fused quartz silica (thickness 5 mm and diameter 25 mm). The amount applied was. To avoid absorption distortion, thinner layer was applied. The AUC for UVA, UVA1 (340–400 nm), UVA2 (320–340 nm) and UVB was measured for each spectrum before (AUC before) and after (AUC after) UV art (980 kJ/m2 UVA and 12 kJ/m2 of UV radiation (UVB included) and before and after UV nat. If the AUCI (AUCI = AUC after/AUC before) was >0.80, the sunscreen was considered photostable.[18] The AUC was calculated with the following equation:

$$\sum_{\lambda\,\min}^{\lambda\,\max} A(\lambda)\,\Delta\lambda$$

where A is absorption and λ is wavelength. It was measured in steps of 1 nm.

For UVA λ_{max} = 400 nm and λ_{min} = 320 nm.

The same measurement was done for every UV range respectively, before and after UVart and before and after UV nat.

In-vitro occlusion studies

Complete coverage of the surface of the skin indicates occlusion of skin. The occlusivity of cream can be measured by occlusion factor F = 100* A-B/A where A= water loss without sample and B = water loss with sample. Filter paper covered water-filled beaker method is used here. The minimum occlusion factor is 0 which indicates no occlusion effect and maximum

occlusion factor is 100 which indicate complete surface coverage by topical formulation.[19]

50 to 200-mg of each sunscreen cream was applied evenly on the filter paper surface to create solid film which was found about amount of 8.5 mg/cm². Reference control was actually a beaker covered with filter paper without sample application. Store the samples at 32ºC and 50-55% RH for 48 hours. Meanwhile the samples were weighed after 6, 24, and 48 hours to determine water flux or evaporation through the filter paper.[19] Every experiment was performed in triplicate.

In-vitro skin permeation studies

In-vitro skin permeation measurements are done by a piece of the dorsal full thickness skin of Wistar rats devoid of hair and fat. 0.5 g of cream was applied to skin piece and mounted in Franz Diffusion cell. PBS serves as a receptor fluid. After 24 h, the amount of drug in the receptor compartment, the drug remaining on the skin, and the drug in the skin was determined by UV-vis spectrophotometer followed by extracting skin piece in alcohol.[19]

Total poly phenolic content determination

The Folin-Ciocalteu reagent (FCR) or Folin's phenol reagent or Folin-Denis reagent or Gallic Acid Equivalence method (GAE) uses a mixture of phosphomolyb date and phosphotungstate for the colorimetric assay of phenolic and polyphenolic antioxidants. It works by measuring the amount of the substance needed to inhibit the oxidation of the reagent. However, this reagent does not only measure total phenols but will react with any reducing substance. The reagent, therefore, measures the total reducing capacity of a sample, not just the level of phenolic compounds. This reagent forms part of the Lowry protein assay and will also react with some nitrogen-containing compounds such as hydroxylamine and guanidine.

Reagents

- Dilute Folin-Ciocalteu reagent with equal volume of distilled water,
- 20% sodium carbonate in water, and
- Gallic acid.

Prepare calibration curve of standard Gallic acid (10-100 µg/ml in water). Prepare 1 milligram/ml of extract solutions. Mix 1 ml of each sample with 0.25 ml of FolinCiocalteu reagent and 1.25 ml of 20% sodium

carbonate solution. Allow the mixture to react for 40 min. at room temperature. After the reaction period, mix the contents and measure the blue color at 725 nm in comparison with standards. Calculate the amount of total phenols from calibration curve as a Gallic acid equivalent by the following formula.[20]

$$T = \frac{C \times V}{M}$$

Where,

T is total content of phenolic compounds, (milligram per gram of plant extract),

C: the concentration of gallic acid established from the calibration curve(milligram per milliliter),

V: the volume of extract (milliliter) and M is the gram weight of plant extract.

In-vitro Antioxidant Activity

1 ml different concentration of active drug and standard were taken in different vials. To this 5 ml of methanolic solution of DPPH was added, shaken well and mixture was incubated at 37ºC for 20 min. The absorbance was measured against methanol as blank at 516 nm. Absorbance of DPPH was taken as control. Percentage antiradical activity calculated by using following formula.[14,15] the results are shown in the Table 3.

$$\% \, Anti-radical \, activity = \frac{Control \, Absorbance - Sample \, Absorbance}{Control \, Absorbance} \times 100$$

Determination Sun Protection Factor (SPF)

In-vitro Determination Sun Protection Factor (SPF) by UV-spectrophotometer

Creams: 1 gm quantity of formulated cream was weighed, transferred to 100 ml volumetric flask and diluted to volume with ethanol. Further, it was kept for ultra-sonication for 5 min and filtered through cotton filter, discarded the initial 10 ml. Afterwards 5 ml aliquot was transferred to 25 ml volumetric flask and the volume was adjusted with ethanol. The absorption spectra of samples in solution were obtained in the range of 290-450 nm using 1 cm quartz cell and ethanol as blank. The absorption data obtained in the range of 290-320 nm every 5 nm interval and 3 determinations were made at each point.[13] The absorbance values and results of formulation I, II and III are shown in Table 9.

Marketed Sunscreen Products: Two marketed formulations were selected. The results of marketed formulation I and formulation II are shown in the Table 9.

Synthetic Sunscreen Agents: The *in-vitro* determination SPF of synthetic sunscreen agents (Oxybenzone and Avobenzone) was done by UV-Spectrophotometer. These sunscreen agents are widely used in the sunscreen formulation. The results of oxybenzone and avobenzone are shown in the Table 9.

Sun Protection Factor Determination: SPF of formulated creams, marketed sunscreen products and synthetic sunscreen agents were calculated by the application of equation:

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

The aliquot prepared were scanned between 290-320 nm and the obtained absorbance values were multiplied with the respective EE (λ) and I (λ) values. Then, their summation was taken and multiplied with the correction factor (10)

In-vitro Determination of SPF by UV 2000S Ultraviolet Transmittance Analyzer (Labsphere)

Procedure: Approximately 110 mg of the prepared investigational sample was applied and spread on the 56 cm² area to obtained a sample film thickness of 2 µl/ cm² on Transpore Tape to get an even film as suggested in the operation manual of the UV-2000S Ultraviolet Transmittance Analyzer for the sample preparation and application technique. The sample thus prepared was exposed to Xenon flash Lamp for determining the Sun Protection Factor. The calculation and results are shown in the Table 9. Critical Wavelength (λc) associates with level of protection- 340 ≤ λc 370 nm- Some (UVA/UVB), λc 370 nm- More (Broad Spectrum). The topical natural formulation evaluated for all cream parameters and *in-vitro* SPF determination. The Boots Star Rating and Critical wavelength are associated with the claim for UV-A protection, as formulation show the UVA/UVB ratio 0.825; hence, there four Boot Star Rating. Critical Wavelength was found 388.00 showing that the formulation can provide excellent UVA/UVB Protection.[21] The result of Boots Star Rating of Formulation F-V is mentioned in Table 9.

Skin Irritation Study

The experimental protocol for this study was approved by the Institutional Animal Ethics Committee (IAEC) and the care of animals was taken according to the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India**.

- **Experimental Animals:** 3 Sprague Dawley Male Rats of 8 weeks age, weighing approximately 250-300 gm to test for the skin irritation.
- **Preparation of Animals prior to testing:** The back skin of area 6 cm² of each rat was shaved prior to the experiment and the animals were divided as
- **Control Animal:** No formulation was applied
- **Test Animals (Cream formulation Animal):** Formulation containing active ingredients was applied.
- **Base Formulation Animal:** Formulation containing only excipients (No active ingredients) cream base was applied.

No formulation was applied to group of control rats for whole period of experiment. 0.5 gm of herbal cream formulation was used as the test substance and applied to an area of approximately 6 cm² of skin and covered with a gauze patch. The patch was loosely held in contact with the skin by means of semi occlusive dressing for the duration of 1 hour and gauze was removed. At the end of exposure period (1 hour) residual test substance was removed without altering the existing response or the integrity of the epidermis. Observations were recorded after removal of the patch. 0.5 gm of base formulation i.e. cream formulated using all ingredients except the active drug materials, was applied to the animals and observations were made as similar to the test animals.[21]

Stability studies
Stability by Centrifugation

During the centrifugation studies, sunscreens were centrifuged at 3500-13,500 rpm at interval of 500 rpm for 10 min.[22] The formulations were observed for the phase separation. The results are shown in the Table 7

Stability studies as per ICH guidelines

For assessing the stability of formulated creams, the following parameters were taken into consideration like color, liquefaction, phase separation, viscosity; extrude ability, Spread ability, pH and SPF of formulation. These studies are essential to ensure that the product is stable throughout its designated shelf life.[22] The stability was carried out for thirty days at temperatures 40 ± 2°C and relative humidity at 75 ± 5% using stability chamber.

Table 2: Results of evaluation by physical parameters

Drug	Colour	Odour	Solubility	Melting point	UVmax wavelength (nm)
Curcumin	Bright yellow to orange	Characteristic	Methanol	181-183°C	420
Quercetin	Pale yellow	Odorless	Alkaline solution	314-316 °C	375
Resveratrol	White with yellow tinge	Characteristic	Ethanol	261-263°C	310
Safranal	Colorless	Aromatic	Ethanol	68°C	308

RESULTS AND DISCUSSION

Physical parameters

Physical evaluation like color, odor, solubility and melting point of purchased drugs were performed to confirm identity. All phyto chemicals were separated and confirmed by TLC. Chromatographic analysis[20] using silica gel G as stationary phase and toluene: ethyl acetate (7:3) showed presence of curcumin at 8.2, quercetin at 8.2 and resveratrol at 8.0 and safranal at 7.2 RF value. Results are shown in Figure 1. All phyto chemicals were identified by HPLC. Chromatographic conditions were used are as follows and graphs are from Figure 2 (a) to 2 (d).

All phyto chemicals were confirmed by UV-visible and FTIR Spectroscopic studies.[11] Spectrums are shown in Figure 3 to 4. Determination of UVmax of pure purchased phyto chemicals was done. Curcumin, quercetin, resveratrol and safranal showed 420, 375, 310 and 308 nm UVmax respectively. Drug excipients interaction study is very significant in relation to know compatibility of selected excipients with active drugs. Incompatibility is actually inactivation of active drug due to decomposition or alteration to a less effective physical or chemical form. When mixture of 2 or more active drugs and excipients are mixed together then chances of interaction with respect to change in appearance, elegance and most important chemistry of each other. To know chemical changes or interactions, generally chromatographic, spectroscopic and thermal analyses are preferred methods. Here TLC and FTIR studies are done for individual active drugs and final optimized formulation.
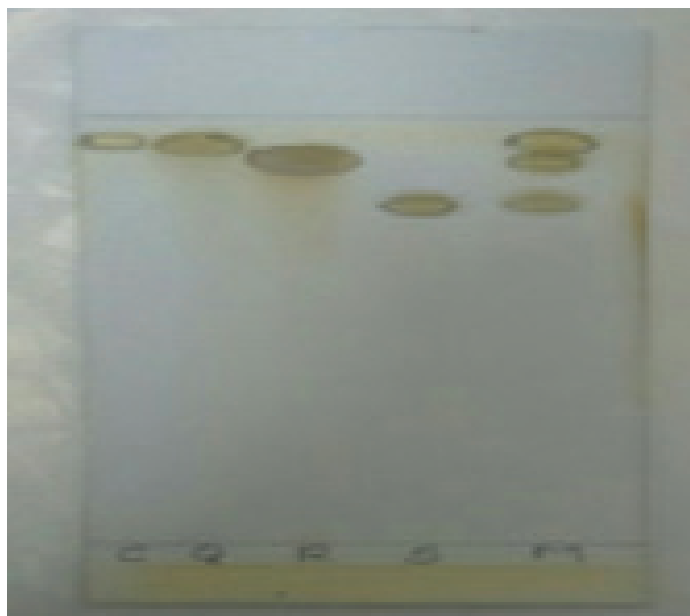

Figure 1: TLC of phytochemicals. *Stationary Phase:* Silica Gel F[254] *Mobile phase:* Toluene: Ethyl acetate (7:3), *Detection:* Iodine chamber Where C: Curcumin, Q: Quercetin, R: Resveratrol, S: Safranal and M: Mixture of all four constituents.

In-vitro SPF determination of active phytochemicals

All preliminary parameters and spectroscopic studies complied with previous literature standards and thus selected phytochemicals further processed to know SPF values. Values for curcumin, quercetin, resveratrol and safranal are shown in Table 3. SPF of curcumin, quercetin, resveratrol and safranal were found to 11.58, 14.81, 21.53 and 10 respectively measured by UV-Visible spectroscopic method. Resveratrol showed highest SPF value while safranal showed lowest SPF value.

In vitro antioxidant activity of active phytochemicals

Percentage antiradical activity calculated by DPPH method. DPPH (1, 1 diphenyl-2-picryl-hydrazyl) is stable free radical means which after reaction with antioxidant compound do not become unstable. Methanolic solution of it is used to evaluate the antioxidant activity of several natural compounds. 82.2, 81.04, 63.46, 72.14 and 51.15 % antioxidant activity exhibited by standard ascorbic acid, curcumin, quercetin, resveratrol and safranal respectively as shown in Table 4. All four selected polyphenolic phyto chemicals showed significant antiradical percentage at very low concentration and thus decided to be incorporated in formulation development.

Development of Formulation

As compared to lotion or any other dosage form, creams are more efficient due to good stability, spread ability, occlusivity, penetration power and cost effectiveness. Long contact time and hydrophobic active drug solubility in oil phase keeps cream dosage forms always a choice of manufacturers. Cream formulations of varying phyto chemicals composition were developed. All studied concentrations were in the legislated range.[5-10] Compositions are shown in Table 1.

Step I: Aqueous Phase Preparation: Disodium EDTA, Sodium Methyl Paraben and Triethanolamine weighed accurately and dissolved in De ionized Water; meanwhile, Carbopol was added to swell using a homogenizer and heated up to 80°C.

Step II: Oil Phase Preparation: Sodium propyl paraben, Stearic acid, Cetyl alcohol, Polyethylene glycol, Cetostearyl alcohol and respective quantities of essential active drugs curcumin, quercetin ,rutin and quercetin derivatives weighed accurately and mixed and heated at 80°C.

Step III: Mixing Phase: Oil phase was added to aqueous phase at 80°C with continuous stirring for 20-25 min and then it was homogenized till uniform emulsion formed. It was then poured into the wide mouth container and stored at temperature not exceeding 37°C.

Pharmaceutical Evaluation of Formulation

Physical Parameters

Appearance, color and homogeneity are determined. Results are shown in the Table 12. All formulations found to be uniform and homogenous (Figure 5).

Subjective Properties

Consistency, feel on application and irritation parameters are determined and results are shown in the Table 12. A result shows that all
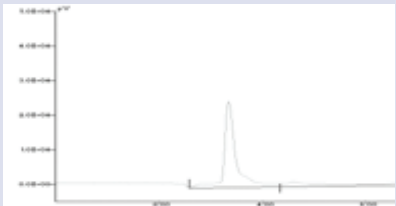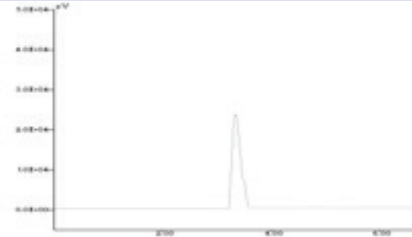
Parameters	Curcumin
Flow rate	0.80 ml/min
Stationary phase/column	C_{18}
Mobile Phase	Acetonitrile : water : 100 mM formic acid (70:10:20)
Methods	Isocratic
Detection	Photodiode array detector 200-500 nm
Retention time	1.1
	Quercetin
Flow rate	1 ml/min
Stationary phase/column	C_{18}
Mobile Phase	Methanol: 0.1% ortho phosphoric acid (65:35)
Methods	Isocratic
Detection	369 nm
Retention time	8.4
	Resveratrol
Flow rate	1ml/min
Stationary phase/column	C_{18}
Mobile Phase	methanol: 10 mM potassium dihydrogen phosphate buffer (pH 6.8): acetonitrile (63 : 30 : 7, v/v/v)
Methods	Isocratic
Detection	306 nm
Retention time	3.2
	Safranal
Flow rate	1ml/min
Stationary phase/column	C_{18}
Mobile Phase	Acetonitrile
Methods	Isocratic
Detection	Photodiode array detector 200-350 nm
Retention time	3.8

Figure 2 (a): HPLC chromatograph of Curcumin.

Figure 2 (b) : HPLC chromatograph of Quercetin.

Figure 2 (c): HPLC chromatograph of Resveratrol.

Figure 2 (d): HPLC chromatograph of Safranal.

formulations have good subjective properties such as consistency, texture and irritation on skin when it applied on the skin.

Spread ability

The spread ability of formulated cream was determined which indicates good spreading of cream when applied to the skin. Results are summarized in Table 12.

Extrude ability

This test is the measure of the force required to extrude the material from a collapsible tube when the certain amount of force has been applied on it in the form of weight. In the present study the quantity in percentage of cream extruded from the tube on application of certain load.[17] Results show that the all formulations have good extrude ability. The good extrude ability and Spread ability are required for the therapeutic efficiency of the formulation.

Thermal stability

In this test the oil separation from the cream were tested at 60-70% RH and 37 ± 1°C in humidity chamber. All these five formulations when placed in humidity chamber at 60-70% RH and 37 ± 1°C temperature there is no phase separation. This shows that formulations are thermally stable.

pH Determination

The pH of creams was determined to examine the possible side effects due to acidic or alkaline pH, which can leads to irritation of skin. Acidic or alkaline pH may cause irritation to the skin and influence the rate of hydration of polymer. The cream in general has a pH 6-9. Here all these five formulation have pH within these range to match skin pH (Table 12).
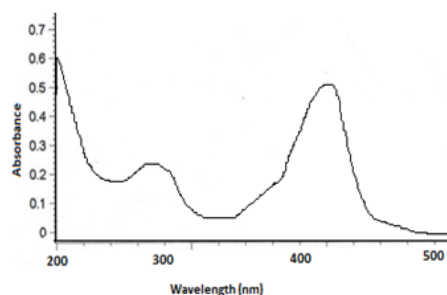
(a) Curcumin (420nm) in methanol
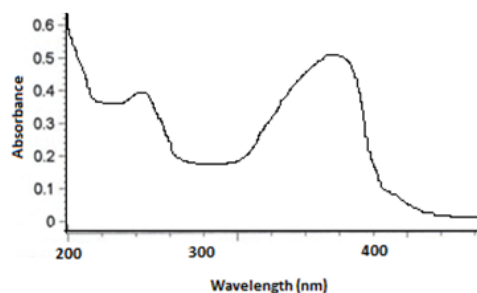
(b) Quercetin (375 nm) in ethanol
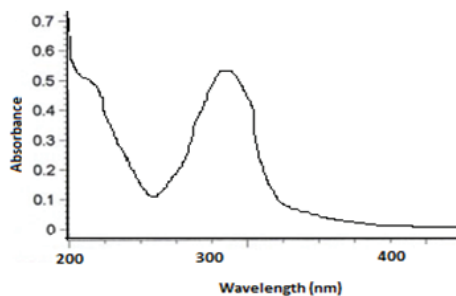
(c) Resveratrol (310 nm) in ethanol
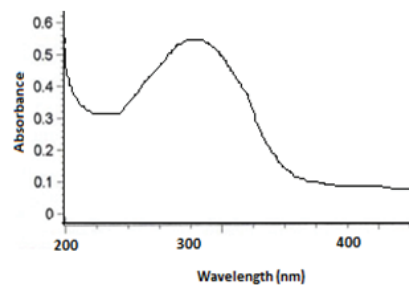
(d) Safranal (308 nm) in ethanol

Figure 3: UV spectrum of phytochemicals.
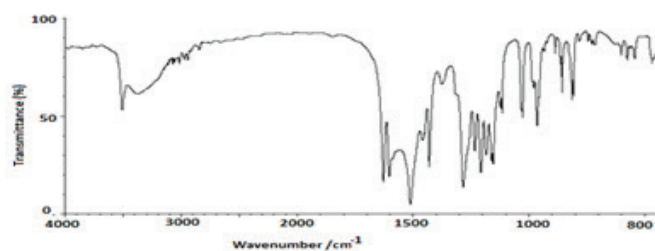
(a) FTIR spectrum of Curcumin

Wave number (cm⁻¹)	Details
3608 cm⁻¹	Streching vibrations of the free hydroxyl-group of phenol (Ar-OH)
2992, 2954 cm⁻¹	C-H Streching
1745 cm⁻¹	vibration of the carbonyl bond (C=O)
1568 cm⁻¹	C=C symetric aromatic ring streching
1463 and 1378 cm⁻¹	corresponding to the vibrational mode of C−O elongation of the alcohol and phenol groups
719, 815 and 962 cm⁻¹	Bending vibrations of the C-H bond of alkene groups (RCH=CH₂).
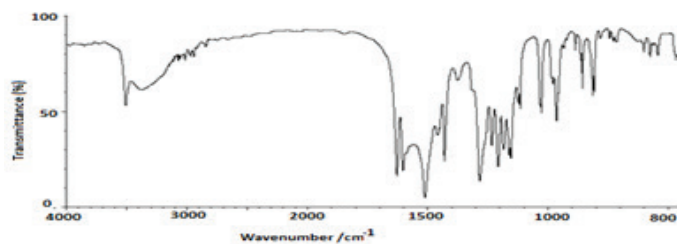
(b) FTIR spectrum of Resveratrol

Wave number (cm⁻¹)	Details
3300 cm⁻¹	Broad OH peak
3100 and 2900 cm⁻¹	Aromatic and aliphatic C-H bonds
1750 cm⁻¹	An ester Peak
1600 cm⁻¹	C=C bond
1200 cm⁻¹	Ester C-O-C peak
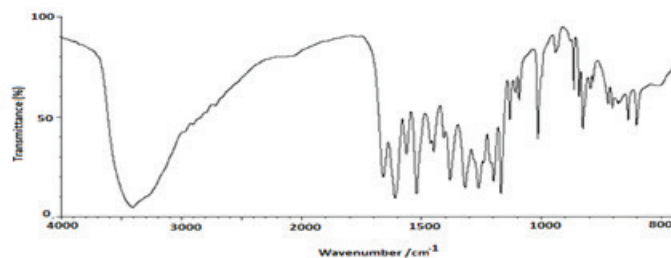
(c) FTIR spectrum of Safaranal

Wave number (cm⁻¹)	Details
3302 cm⁻¹	Broad OH peak
2925 cm⁻¹	C-H Streching
2034 cm⁻¹	-CO streching
1699 and 1637 cm⁻¹	-C=C streching
1462 cm⁻¹	corresponding to the vibrational mode of C−O elongation of the alcohol and phenol groups
1250 cm⁻¹	-CH wagging
1068 cm⁻¹	-CH streching
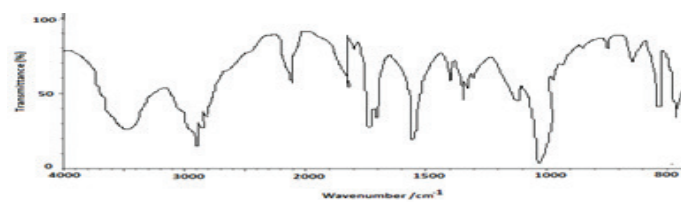
(d) FTIR spectrum of Quercetin

	Wave number (cm⁻¹)
3411 cm⁻¹	O-H strech vibration of phenol
1663 cm⁻¹	C=O aryl KetonicStrech
1608 cm⁻¹	C-C aromatic ring strech
1383 cm⁻¹	O-H bending of phenol
1318 cm⁻¹	C-H bond in aromatic hydrocarbon
1265 cm⁻¹	C-O strech of aryl ether
1203 cm⁻¹	C-O strech of phenol
1167	C-CO-C streching and bending in ketone
940,821,677 and 602	C-H bending of aromatic hydrocarbon

Figure 4: FTIR studies of phytochemicals.

Rancidity determination

This test is performed by using the Phloroglucinol solution. The rancidity is due to the oxidation of the fats and oils; during oxidation free fatty acids are liberated. These free fatty acids react with the Phloroglucinol solution and gives pink color. This indicates the rancidity of the product. All these five-formulation show no pink color (Table 12) when shaken with Phloroglucinol and HCl solution, so these five formulations shows no rancidity, all oils of formulation were free from oxidation.

Viscosity determination

Viscosity is the most important parameter in the evaluation of the cream. Viscosity governs the many properties of the product such as spread ability, pour ability of the product from the container etc. The viscosity of formulations was determined using Brookfield Viscometer and Viscosity

was found to be in range of 28000-32000 cp. (Table 12) The viscosity is determined by following formula:

Viscosity = Dial Reading × Factor. For LV-4 at 6 RPM Factor is 1M (1000)

Photo stability determination

The photo stability of the trial batches of sunscreen showed considerable stability of ingredients. Samples on plate showed similar spectrum before and after heating for 20 min at 50°C. No change in viscosity of cream observed in before and after scanning. (Table 5) The photo unstable sunscreens begin to degrade relatively rapidly when exposed to the sun.[18] After 120 min of UV nat, AUCI found to be <0.80 for sunscreens I to IV which indicates that these are unstable. Formulated sunscreens creams are containing organic chemical which are potent antioxidant and hence can be more. Sunscreen creams F-I, F-II, F-III and F-IV are found less photo stable than F-V after UV nat. AUCI was found between 0.66 and 0.73 for UVA and between 0.63 and 0.88 in the UVB range after 120 min. While exposure F-V showed, shift in wavelength to shorter range and found stable by both UV art and UV nat. Thus it can be said that F-V can give consumer satisfaction for photo-stable sunscreen product. All creams showed relatively good photo stability and hence it can be considered that antioxidant nature i.e. free radical scavenging efficacy can be reason behind it. Collective antioxidant capacity of all active phyto chemicals in F-V formulation is showing promising improved stability as compared to other batches. For the consumer it is very difficult to know what product to choose, since the photo stability varies between different brands and the photo stability is not marked on the bottle. To know which photoactive compound the sunscreen contains is not good enough. The stability also depends on factors like preservatives, oxygen radical scavengers, and base formulation.

In vitro occlusion studies

Occlusion factor of trial batches of sunscreen creams and reference control without cream were determined by *in-vitro* filter paper covered water-filled beaker method. The occlusive factor of all F-I to F-V was found to be greater than 85 and found to be directly depends on size of sample application. (Table 12) Occlusion is significant to moisturize and improve partitioning of skin which directly depends on thickness of applied sample layer.[19]

In-vitro skin permeation studies

The results of skin permeation studies are summarized in Table 12. The cumulative amount of each active drug from F-I to F-V is found between 75 to 82 % which indicates a good penetration of formulated cream. Dif-

Table 3: SPF Determination of SPF of pure phytochemicals

Wavelength (nm)	Curcumin			Quercetin		Resveratrol		Safranal	
	EE (λ) × I (λ)	Absorbance (λ)	EE (λ) × I (λ) × Abs (λ)	Absorbance (λ)	EE (λ) × I (λ) × Abs (λ)	Absorbance (λ)	EE (λ) × I (λ) × Abs (λ)	Absorbance (λ)	EE (λ) × I (λ) × Abs (λ)
290	0.015	1.124	0.01686	2.422	0.03633	1.452	0.02178	1.389	0.02084
295	0.0817	1.273	0.104	3.6121	0.29511	1.633	0.13342	1.472	0.12026
300	0.2874	1.363	0.39173	0.9423	0.27082	1.921	0.5521	0.962	0.27648
305	0.3278	1.182	0.38746	1.0421	0.3416	2.285	0.74902	0.774	0.25372
310	0.1864	0.885	0.16496	1.7546	0.32706	2.381	0.44382	1.295	0.24139
315	0.0837	0.915	0.07659	1.8416	0.15414	2.469	0.20666	0.849	0.07123
320	0.018	0.934	0.01681	3.144	0.05659	2.576	0.04637	0.942	0.01696
	Total = 1			Total = 1.1584		Total = 1.4817		Total= 2.1532		Total = 1.001
			SPF= 11.58		SPF= 14.81		SPF= 21.53		SPF= 10

Table 4: Antioxidant Activity determination by DPPH method

Concentration	Absorbance	Control Absorbance	% Antioxidant Activity
Ascorbic acid			
100 µg/ml	1.127	1.82	38.08
250 µg/ml	0.633	1.82	65.22
500 µg/ml	0.324	1.82	82.2
1000 µg/ml	0.105	1.82	94.23
Curcumin			
100 µg/ml	1.254	1.82	31.1
250 µg/ml	0.895	1.82	50.82
500 µg/ml	0.345	1.82	81.04
1000 µg/ml	0.278	1.82	84.73
Quercetin			
100 µg/ml	1.0915	1.82	40.03
250 µg/ml	0.8295	1.82	54.42
500 µg/ml	0.665	1.82	63.46
1000 µg/ml	0.395	1.82	78.3
Resveratrol			
100 µg/ml	1.072	1.82	41.1
250 µg/ml	0.745	1.82	59.07
500 µg/ml	0.507	1.82	72.14
1000 µg/ml	0.221	1.82	87.86
Safranal			
100 µg/ml	1.407	1.82	22.69
250 µg/ml	1.022	1.82	43.85
500 µg/ml	0.889	1.82	51.15
1000 µg/ml	0.424	1.82	76.7

ferent ingredients in cream base together are generally responsible for significant permeation.[20] And hence from results of *in-vitro* skin permeation studies it is confirmed that F-V due to efficacy of collective phyto chemicals role shows highest permeation across the skin.

In-vitro Antioxidant Activity

Endogenous production of radicals from cellular metabolism and exogenous sources from ultraviolet radiation and pollution can damage the skin on the cellular and tissue levels. Although the body possesses an elegant defense system to prevent radical damage, this innate system can be overwhelmed and lead to a state of oxidative stress or immune suppression, and can even trigger carcinogenesis. Topical supplementation of antioxidants can provide additional protection to neutralize reactive oxygen species from both endogenous and exogenous sources. 1, 1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl; DPPH) is stable free radical which able to delocalise free radical to generate violet color change in ethanol. But after reacting with antioxidant molecules, reduction takes place and loss of violet color intensity can be measured at 517nm. Percent inhibition of DPPH radical = absorbance before reaction- absorbance after reaction/ absorbance before reaction *100. Mix 0.2 ml of methanolic extract in 2 ml of 0.5 milimolar solution of DPPH. Measure absorbance of samples and standards at 517 nm after 30 min. the anti-radical activity was evaluated by DPPH method for equally diluted samples and activity found in concentration dependant manner. 75.87, 70.90, 77.45, 69.33, 50.63 and 89.83 % antioxidant activity shown by ascorbic acid, FI, FII, FIII, FIV and FV respectively. (Table 6) But as compare to antioxidant activity of standard ascorbic acid, resveratrol containing formulation possesses highest antioxidant activity while safranal incorporated formulation shows lowest activity. Combined polyphenolic phyto chemicals incorporation in formulation F-V shows synergistic antioxidant activity. As the optimized cream F-V shows excellent antioxidant activity thus it can be efficient as photo protective.

Table 5: Results of photo stability evaluation of sunscreen cream batches

| Formulation batches	Exposure time (min)	UVA radiation (kJ/m²)	UVA	UVB	UVA	UVB
			After UV natural exposure		After UV artificial exposure	
F-1	30	55	0.65	0.68	0.72	0.72
	90	165	0.68	0.635	0.65	0.69
	120	235	0.59	0.61	0.71	0.77
F-2	30	58	0.45	0.59	0.65	0.73
	90	160	0.85	0.88	0.78	0.81
	120	230	0.72	0.79	0.82	0.87
F-3	30	60	0.75	0.73	0.72	0.81
	90	159	0.65	0.67	0.54	0.77
	120	274	0.75	0.72	0.69	0.65
F-4	30	58	0.77	0.73	0.70	0.80
	90	170	0.64	0.76	0.66	0.81
	120	284	0.60	0.70	0.74	0.77
F-5	30	62	0.75	0.70	0.60	0.81
	90	155	0.79	0.72	0.65	0.80
	120	242	0.84	0.87	0.90	0.82
Marketed avobenzene containing formulation	30	62	0.72	0.74	0.77	0.70
	90	155	0.76	0.78	0.72	0.79
	120	242	0.78	0.77	0.73	0.75

F-I F-II F-III F-IV F-V
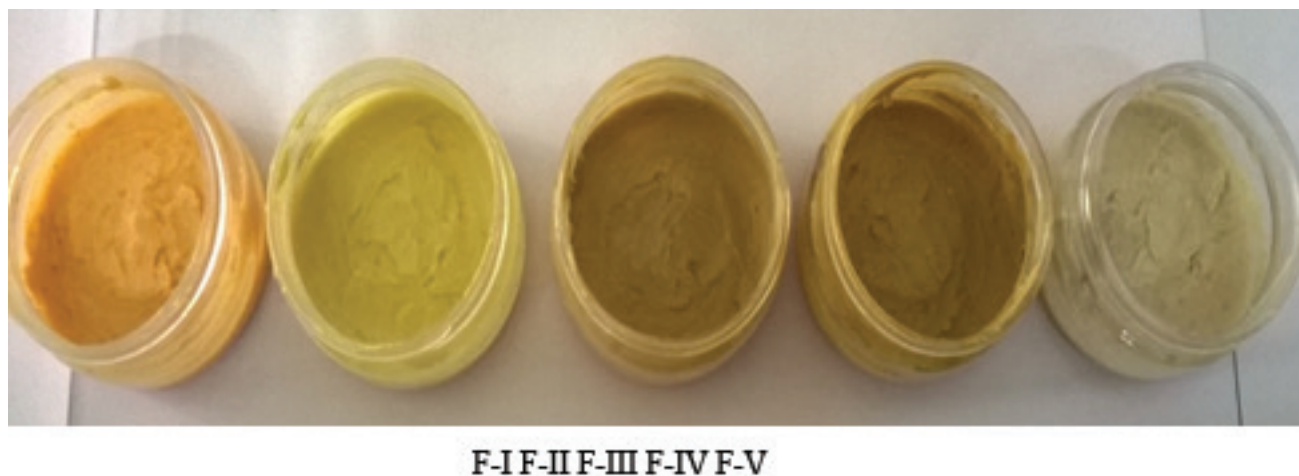
Figure 5: Photo protective cream formulations.

Table 6: Results of *in-vitro* antioxidant activity determination of trial batches of cream FI-to F-V

Concentration	Absorbance	Control Absorbance	% Antioxidant Activity
Ascorbic acid			
100 µg/ml	1.815	1.712	65.184
250 µg/ml	1.711	1.712	71.258
500 µg/ml	1.632	1.712	75.873
1000 µg/ml	1.558	1.712	80.195
F-I			
100 µg/ml	1.804	1.712	65.826
250 µg/ml	1.734	1.712	69.915
500 µg/ml	1.717	1.712	70.908
1000 µg/ml	1.642	1.712	75.289
F-II			
100 µg/ml	1.937	1.712	58.057
250 µg/ml	1.753	1.712	68.805
500 µg/ml	1.605	1.712	77.45
1000 µg/ml	1.575	1.712	79.202
F-III			
100 µg/ml	2.253	1.712	39.6
250 µg/ml	1.945	1.712	57.59
500 µg/ml	1.744	1.712	69.331
1000 µg/ml	1.789	1.712	66.702
F-IV			
100 µg/ml	2.255	1.712	39.483
250 µg/ml	2.184	1.712	43.63
500 µg/ml	2.064	1.712	50.639
1000 µg/ml	1.939	1.712	57.941
F-V			
100 µg/ml	1.712	1.712	71.2
250 µg/ml	1.587	1.712	78.501
500 µg/ml	1.393	1.712	89.833
1000 µg/ml	1.815	1.712	93.805

Stability studies

During centrifugation studies, all five formulations were centrifuged at 3500-13,500 rpm at interval of 10 minutes and it was observed that there was no phase separation, confirming that there was no effect of accelerated speed on formulation. Therefore, these formulations are stable. Stability studies are essential to ensure that the product is stable throughout its designated shelf life. The stability was carried out for thirty days at temperatures 40 ± 2°C and relative humidity at 75 ± 5% using stability chamber. Results are shown in Table 7. All formulations had increasing viscosity values after storage in freeze-thaw condition. All samples were oil-in-water creams; hence, their water content might lose at fluctuated temperatures. Therefore, the suggested storage condition for these products should be at constant temperature. Furthermore, it was obviously found that the viscosity values of the sunscreen creams were directly related to the obtained SPF values. The formulations with suitable viscosity could provide more adhesiveness and spreading efficacy. No phase separation and changing in color as well as odor were observed in all samples after stability test; however, they seemed to be more viscous. (Figure 6 and 7) From the results it is observed the given formulations are relatively stable at accelerated temperature and humidity.

Skin Irritation Study

By stepwise order skin irritation treating should be performed which is: a weight of evidence analysis, pH considerations, use of validated and accepted *in vitro* tests, and finally refinement of the animal testing. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the care of animals was taken according to the guidelines of CPCSEA, Ministry of Forests and Environment, Govern-

Figure 6: Freshly prepared F-V formulation.

Figure 7: Formulation F-V after 3 months storage.

Table 7: Results of Stability Study of Photo-protective Formulation

Formulation	Temperature	At 40 ± 2°C ; RH 70 ± 5	Stored in freeze
Color			
I	No change in Colour	Slight Change in colour	No change in Colour
II	No change in Colour	Slight Change in colour	No change in Colour
III	No change in Colour	Slight change in colour	No change in Colour
IV	No change in Colour	Slight Change in colour	No change in Colour
V	No change in Colour	Slight Change in colour	No change in Colour
Phase separation			
I	No Phase Separation	No Phase Separation	No Phase Separation
II	No Phase Separation	No Phase Separation	No Phase Separation
III	No Phase Separation	No Phase Separation	No Phase Separation
IV	No Phase Separation	No Phase Separation	No Phase Separation
V	No Phase Separation	No Phase Separation	No Phase Separation
Liquefaction			
I	No Liquefaction	Slight Liquefaction	No Liquefaction
II	No Liquefaction	Slight Liquefaction	No Liquefaction
III	No Liquefaction	Slight Liquefaction	No Liquefaction
IV	No Liquefaction	Slight Liquefaction	No Liquefaction
V	No Liquefaction	Slight Liquefaction	No Liquefaction
PH			
I	6.9	6.8	6.85
II	6.5	6.45	6.5
III	6.8	6.7	6.7
IV	6.7	6.6	6.7
V	7.2	7.0	7.2
Viscosity			
I	28450	28500	29954
II	30620	30255	31075
III	28500	28250	29000
IV	30005	29875	30296
V	32565	32158	33082
Spread ability			
I	20.55	20.08	17.09
II	21.75	14.58	17.85
III	19.49	19.05	17.04
IV	20.05	19.25	17.35
V	22.32	21.65	18.52
SPF			
I	12.25	12.34	10.84
II	16.63	15.87	14.78
III	24.05	22.62	20.57
IV	12.84	10.35	10.46
V	34.27	33.45	32.82
Extrudability			
I	Fair	Fair	Fair
II	Good	Good	Good
III	Good	Good	Good
IV	Excellent	Excellent	Good
V	Excellent	Excellent	Excellent

Centrifugation test for stability					
Formulation	I	II	II	IV	V
Observation	No Phase Separation	No Phase Separation	No Phase Separation	No Phase Separation	No Phase Separation
Inference	Stable	Stable	Stable	Stable	Stable

ment of India. The cream was applied to the skin for 7 days and observed for any sensitivity[19] and the reaction if any was graded as shown in Table 6.

Table 6: Grade of Sensitivity Reaction

Sensitivity Reaction	Grade
No reaction	A
Slight Patchy Erythema	B
Slight but confluent or moderate but patchy erythema	C
Severe Erythema with or without edema	D

The SPF of Formulation F-V found highest hence its skin irritation study was carried out. After 7 Days it was observed that, there was no any type of sensitivity reaction or irritation with erythema or edema. (Figure 8) The result is graded as - A (No Reaction).

Total poly phenolic content determination

Total polyphenolic compounds estimation in developed formulation can give idea of compatibility as well as total antioxidant potential responsible of each formulation.[20] Folin-Ciocalteu method is most simple and accurate method in determination of total polyphenolic compounds. Estimation studies shown from standard calibration curve of gallic acid (Table 7) that highest phenolic content of 40.74% in formulation-F-V which can be expected due to combination of all four active in gredients of polyphenolic class. 22.07, 21.89, 25.67 and 27.42% of total polyphenolic content of formulation F-I, F-II, F-III and F-IV are measured respectively.

Table 8: Results of standard calibration curve of gallic acid

Standard	Absorbance (765 nm)	Concentration (mg/ml)
1	0.875	0.2
2	1.698	0.4
3	2.315	0.6
4	2.667	0.8
5	2.995	1.0

DETERMINATION SUN PROTECTION FACTOR (SPF)

In-vitro Determination Sun Protection Factor (SPF) by UV-spectrophotometer

Sunlight despite of source of life and energy creating major health challenges like sunburn, pigmentation, wrinkles, dermatitis, urticaria, ageing, immune-suppression and number of skin cancers too. Sun protective clothes and or sunglasses provide insufficient and less convenient approach to get rid of all these health hazards. So sunscreen protection is popular mean among various regions of world. Efficacy of sunscreens is expressed in the form of Sunburn Protection Factor (SPF). The absolute protection performance of a sun care product against erythermal-effective UV radiation, calculated from the measured *in vitro* transmittance and weighted with the erythema action spectrum and with the "standard" output spectrum of a UV solar simulator used for SPF testing. SPF of active drugs, formulated creams, marketed sunscreen products and synthetic sunscreen agents were calculated by the application of equa-

Control   Cream base applied   F-V applied

Figure 8: Skin irritation study by using rats.

tion. The aliquot prepared were scanned between 290-320 nm and the obtained absorbance values were multiplied with the respective EE (λ) and I (λ) values. Then, their summation was taken and multiplied with the correction factor i.e. 10. All formulations i. e. FI (11.89), FII (16.11), FIII (23.10), FIV (12.35) and F-V (34.58) shows the significant SPF. (Table 9) SPF of synthetic sunscreen agents was found 22.40 for oxyben zone and 18 for avoben zone. The marketed formulations found with SPF 18.29 and 16.06 having lower SPF than label claims. The comparison with standard synthetic compounds and marketed herbal formulation shows promising results of developed formulation with higher SPF values. Combination of selected poly phenols in formulation F-V exhibited highest SPF value due to synergistic action. Resveratrol found to be more photo protective as compared to others. Efficacy of photo protection found: resveratrol › quercetin › safranal › curcumin. SPF of formulated phytochemicals found to be higher, which indicates synergism and compatibility of excipients too. These results reveal that the prepared formulations have good SPF and good sun protection activity.

In-vitro Determination of SPF by UV 2000S Ultraviolet Transmittance Analyzer (Lab sphere)

The evaluation parameter of cream complies with the acceptance criteria and SPF of this cream found to be 22.60 indicates that the formulated cream can be considered as an efficient topical product. Statistical parameters are expressed in Table 10. The SPF graph report of topical herbal photo protective formulation is shown in Figure 9. Thus SPF of Formulation-V found highest when determined by UV-Spectrophotometer hence its SPF determined by UV- 2000S Ultraviolet Transmittance Analyzer.[13,16] (KET's Scientific Research Center, Mumbai)

The above readings are averages of 3 replicate each consisting of 6 scans.

The Boots Guidelines for Star Rating: The Boots star rating is based on the average UVA/UVB ratio. A different rating is given to a range of values as shown below in Table 11. Rating levels differ for different wavelength sampling interval.

CONCLUSION

In present research work wound-healing curcumin, strong antioxidant quercetin, photo protective resveratrol and moisturizing as well as cooling safranal are incorporated together to develop efficient all-in-one sunscreen product. It is recommended that in detail studies of the safety, efficacy and toxicity of selected photo protectors are essential to establish product in market without any evidence of interactions.

ACKNOWLEDGEMENT

Table 9: SPF Determination of trial batches of sunscreen creams, marketed formulations and synthetic sunscreen chemicals

Wavelength (nm)	F-I EE (λ) ×I (λ)	F-I Abs (λ)	F-I EE (λ) × I (λ) × Abs (λ)	F-II Abs (λ)	F-II EE (λ) × I (λ) × Abs (λ)	F-III Abs (λ)	F-III EE (λ) ×I (λ) × Abs (λ)	F-IV Abs (λ)	F-IV EE (λ) ×I (λ) × Abs (λ)	F-V Abs (λ)	F-V EE (λ) ×I (λ) × Abs (λ)	Marketed formulation I Abs (λ)	Marketed formulation I EE (λ) ×I (λ) × Abs (λ)	Marketed formulation II Abs (λ)	Marketed formulation II EE (λ) ×I (λ) × Abs (λ)	Oxybenzone Abs (λ)	Oxybenzone EE (λ) ×I (λ) × Abs (λ)	Avobenzone Abs (λ)	Avobenzone EE (λ) ×I (λ) × Abs (λ)
290	0.015	1.943	0.0291	1.844	0.0277	2.995	0.0449	0.805	0.0121	3.204	0.0481	3.324	0.05136	3.2733	0.049099	3.522	0.05276	3.522	0.05283
295	0.0817	1.448	0.1183	1.632	0.1333	2.672	0.2183	1.739	0.1421	4.739	0.3872	3.675	0.30024	3.4743	0.283850	3.553	0.30738	3.553	0.29028
300	0.2874	0.937	0.2693	2.735	0.7877	1.735	0.4997	0.76	0.2184	3.495	1.0045	1.0346	0.29734	0.9246	0.266099	0.942	0.27159	0.942	0.27073
305	0.3278	1.423	0.4665	0.529	0.1734	2.964	0.9716	1.502	0.4924	2.903	0.9516	1.1393	0.37346	1.0413	0.341338	1.056	0.34616	1.056	0.34567
310	0.1864	0.972	0.1812	1.804	0.3363	1.925	0.3588	1.432	0.2669	4.067	0.7581	2.8140	0.52452	3.1486	0.586899	3.116	0.58082	3.116	0.59536
315	0.0837	1.205	0.1009	1.54	0.1289	1.975	0.1653	0.905	0.0757	3.105	0.2599	2.8033	0.23519	2.8856	0.023961	3.219	0.27477	3.219	0.27007
320	0.018	1.294	0.0233	1.325	0.0239	2.839	0.0511	1.53	0.0275	2.732	0.0492	2.6106	0.04699	3.0563	0.054946	3.009	0.06170	3.009	0.05416
			Total= 1.1885		Total= 1.6111		Total= 2.3097		Total = 1.2351		Total = 3.4585		Total= 1.829		Total = 1.606		Total= 2.24		Total= 1.88
			SPF= 11.89		SPF= 16.11		SPF= 23.10		SPF= 12.35		SPF= 34.58		SPF= 18.2		SPF = 16.06		SPF= 22.40		SPF= 18.80

SPF Mean	22.62
SPF STD	0.12
SPF COV	4.45%
UVA/UVB Ratio Mean	0.826
UVA/UVB Ratio STD	0.006
UVA/UVB Ratio COV	0.68%
Ratio Calculation	Labsphere Method
Lambda Critical Mean	388.00
Lambda Critical STD	0.00
Lambda Critical COV	0.00%

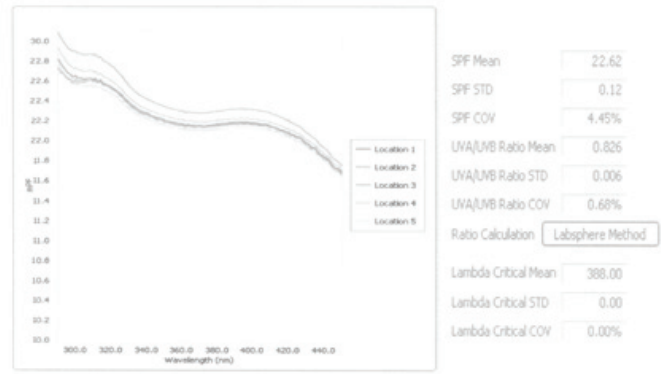(Legend: Location 1, Location 2, Location 3, Location 4, Location 5)

Figure 9: SPF Graph Report of Formulation F-V determined by UV- 2000S Ultraviolet Transmittance Analyzer.

Table 10: Sun Protection Factor of Formulation-V by UV- 2000S Ultraviolet Transmittance Analyzer

No	Test Sample	Scans Parameters	1	2	3	Average Values
1		SPF	21.56	22.63	22.62	22.60
2		Standard Deviation	0.09	0.12	0.12	0.11
3	Photo Protective Formulation	UVA/UVB Ratio	0.825	0.823	0.826	0.825
4		Critical Wavelength	388.00	388.00	388.00	388.00
5		Boots Star Rating	***	***	***	***

Table 11: The Boots Guidelines for Star Rating

Mean UVA/UVB ratio	Star Rating Category
0.0 to 0.59	No Rating
0.6 to 0.79	* * *
0. 8 to 0.9	* * * *
0.9 and over	* * * * *

The study yielded a 22.60 Sun Protection Factor (SPF) and ****Boots Star Rating as presented below following the application of the test samples on the transpore tape.

CONFLICT OF INTEREST

There is no conflict of interest.

Table 12: Summary of various pharmaceutical evaluation parameters

Parameters	Formula F-V	F-I	F-II	F-III	F-IV
Appearance	Cream like	Cream like	Cream like	Cream like	Cream like
Color	Reddish brown	Reddish brown	Reddish brown	Reddish brown	Reddish brown
Homogeneity	Uniform and homogeneous	Uniform and homogeneous	Uniform and homogeneous	Uniform and homogeneous	Uniform and homogeneous
Consistency	Good	Good	Good	Good	Good
Texture	Smooth	Smooth	Smooth	Smooth	Smooth
Irritation	No	No	No	No	No
Spread ability	22.73	20.41	21.88	21.36	19.58
Extrudation	94.29 %	78.57	82.52	81.44	93.94
Thermal Stability	No Phase Separation (Thermally Stable)	No Phase Separation (Thermally Stable)	No Phase Separation (Thermally Stable)	No Phase Separation (Thermally Stable)	No Phase Separation (Thermally Stable)
pH	7.2	6.9	6.5	6.8	7.1
Rancidity	No Pink Color, No Rancidity	No Pink Color, No Rancidity	No Pink Color, No Rancidity	No Pink Color, No Rancidity	No Pink Color, No Rancidity
Viscosity	32780	28400	30200	28540	30500
Centrifugation test	No Phase Separation (Stable)	No Phase Separation (Stable)	No Phase Separation (Stable)	No Phase Separation (Stable)	No Phase Separation (Stable)
photo stability	Good	Good	Good	Good	Good
Occlusion Factor (F)	95	90	88	90	93
Cumulative amount permeated (ug/cm2)	92.45	72.81	80.23	86.34	90.11
Total phenolic content	27.42 %	ND	ND	ND	ND
SPF by UV-spectrophotometer	34.58	ND	ND	ND	ND
SPF by UV 2000S Ultraviolet Transmittance Analyzer (Lab sphere)	22.62	ND	ND	ND	ND
Boots Star Rating	****	ND	ND	ND	ND
In-vitro antioxidant activity by DPPH method	93.805 (1000 µg/ml)	ND	ND	ND	ND

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