



# Formulation And Evaluation Of Herbal Sunscreen

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## 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.

## 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 suppression.<sup>1</sup> 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.<sup>2</sup> The goal of sunscreen formulation is to block UV rays and increase the level of protection from the UV-rays.

absorption but maximum formulations are of high cost and Incorporated synthetic molecules are with potential Toxicity and even carcinogenesis.<sup>4</sup> 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 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.<sup>5-10</sup> 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 antiinflammatory, 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.<sup>5</sup> 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. 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.

## **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 spectroscopic.

chromatographic studies. 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.

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

The aliquot prepared were scanned between 290-320 nm and the Obtained absorbance values were multiplied with the respective EE ( $\lambda$ ) And I ( $\lambda$ ) 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;

### Formula.

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

## 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 80oC. Afterwards, the aqueous phase was gradually added into the Oil phase with constantly stirring until the mixture was congealed at the Room temperature.<sup>16</sup> 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.

## Evaluation of Formulation

Physical Parameters Appearance, color and homogeneity are determined.

Subjective Properties Consistency, feel on application and irritation parameters. 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:  $S = M \cdot L / T$  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.

### **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:

### **Thermal stability**

In this test the oil separation from the cream were tested at 60-70% RH And  $37 \pm 1^\circ\text{C}$  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 6070% RH and temperature  $37 \pm 1^\circ\text{C}$ . To pass the test there Should not be any oil separation in the cream.

### **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.

## 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

## 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.

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

Photo stability determination 2 mg/cm<sup>2</sup> 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/m<sup>2</sup> UVA and 12 kJ/m<sup>2</sup> of UV radiation (UVB included).

## 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. Formulation.1950 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/cm<sup>2</sup> Reference control was actually a beaker covered with filter paper without sample application. Store the samples at 32°C 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.<sup>19</sup> 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.<sup>19</sup> 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 phosphomolybdate and phosphotungstate for the colorimetric assay Of phenolic and polyphenolic

### **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 .

### **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 cm<sup>2</sup> 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 cm<sup>2</sup> 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 350013,500 rpm at interval of 500 rpm for 10 min.22 The formulations were Observed for the phase separation.

### **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  $40 \pm 2^{\circ}\text{C}$  and relative humidity at  $75 \pm 5\%$  using stability .



## 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 Using silica gel G as stationary phase and toluene: ethyl acetate 20 Showed presence of curcumin at 8.2, quercetin at 8.2 and resveratrol at 8.0 and safranal at 7.2 RF value. All phyto chemicals were confirmed by UV-visible and FTIR Spectroscopic studies.<sup>11</sup> 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.

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 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. 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

**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.

## 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.



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