Phytoconstituents are gaining popularity as ingredients in cosmetic formulations as they can protect the skin against exogenous and endogenous harmful agents and can help remedy many skin conditions. Exposure of skin to sunlight and other atmospheric conditions causes the production of reactive oxygen species, which can react with DNA, proteins, and fatty acids, causing oxidative damage and impairment of antioxidant system. Such injuries damage regulation pathways of skin and lead to photoaging and skin cancer development. The effects of aging include wrinkles, roughness, appearance of fine lines, lack of elasticity, and de- or hyperpigmentation marks. Herbal extracts act on these areas and produce healing, softening, rejuvenating, and sunscreen effects. We have selected a few photoprotective phytoconstituents, such as curcumin, resveratrol, tea polyphenols, silymarin, quercetin and ascorbic acid, and have discussed the considerations to be undertaken for the development of herbal cosmetic formulations that could reduce the occurrence of skin cancer and delay the process of photoaging. This article is aimed at providing specific and compiled knowledge for the successful preparation of photoprotective herbal cosmetic formulations.

**Key words:** Antiaging phytoconstituents, botanical antioxidants, photoprotective herbs, ultraviolet radiations

**ABSTRACT**

Phytoconstituents are gaining popularity as ingredients in cosmetic formulations as they can protect the skin against exogenous and endogenous harmful agents and can help remedy many skin conditions. Exposure of skin to sunlight and other atmospheric conditions causes the production of reactive oxygen species, which can react with DNA, proteins, and fatty acids, causing oxidative damage and impairment of antioxidant system. Such injuries damage regulation pathways of skin and lead to photoaging and skin cancer development. The effects of aging include wrinkles, roughness, appearance of fine lines, lack of elasticity, and de- or hyperpigmentation marks. Herbal extracts act on these areas and produce healing, softening, rejuvenating, and sunscreen effects. We have selected a few photoprotective phytoconstituents, such as curcumin, resveratrol, tea polyphenols, silymarin, quercetin and ascorbic acid, and have discussed the considerations to be undertaken for the development of herbal cosmetic formulations that could reduce the occurrence of skin cancer and delay the process of photoaging. This article is aimed at providing specific and compiled knowledge for the successful preparation of photoprotective herbal cosmetic formulations.

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lines, lack of elasticity, and de- or hyperpigmentation marks. Herbal extracts act on these areas and produce healing, softening, rejuvenating, and sunscreen effects.[7]

Various synthetic agents are used as photoprotectives, but they have limited use because of their potential toxicity in humans and their ability to interfere in certain selected pathways of multistage process of carcinogenesis. Several botanical compounds have been shown to be antimutagenic, anticarcinogenic, and nontoxic and have the ability to exert striking inhibitory effects on a plethora of cellular events at various stages of carcinogenesis. Because multiple pathways are involved in photocarcinogenesis, a mixture of several botanical antioxidants working through various mechanisms, in conjunction with the use of sunscreen, could also be an effective approach for reducing UV-generated ROS-mediated photodamage, immunosuppression, and skin cancer in humans. Few examples include tea polyphenols, curcumin, silymarin, garlic compounds, apigenin, resveratrol, ginkgo biloba, beta-carotene, and ascorbic acid.[8] There is a need to develop herbal formulations that could combat the harmful effects of both UV-A and UV-B radiations. The present researches are aimed to develop novel strategies to reduce the occurrence of skin cancer and delay the process of photosaging.

PREFORMULATION STEPS FOR THE DEVELOPMENT OF HERBAL COSMETIC FORMULATIONS

Selection of herbal extracts

Herbal extracts produce healing, softening, rejuvenating, and sunscreen effects. Botanical extracts are multifunctional in nature because they possess various properties, such as photoprotection, antiaging, moisturizing, antioxidant, astringent, antiirritant, and antimicrobial, which are correlated with each other.[9] Natural compounds belong to chemical classes, such as polyphenols, monoterpenes, flavonoids, organosulfides, and indoles.[8] After a detailed literature survey of the herbs acting as photoprotectives, we could select a few herbal constituents according to their nature, availability, estimation methods, stability, and utility of the developed formulation as well as on the basis of previous research.

Nature of phytoconstituents

Solubility is an important criterion for the development of novel formulations. According to the nature of the phytoconstituent, that is, hydrophilic or lipophilic, best-suited formulation was selected. In Table 1, we have discussed the chemical profile and solubility of important photoprotective phytoconstituents in various solvents.

Selection criteria for suitable type of formulation

Novel technology has shown great potential for improving the effectiveness and efficiency of the delivery of nutraceuticals and bioactive compounds. Recent advances in nanotechnology show their promise as potential cosmetics for poorly soluble, poorly absorbed, and labile herbal extracts and phytochemicals. An innovative approach can improve both the esthetics and performance of a cosmetic product. The application of novel approaches can also improve its efficacy regarding continuous action of herbs on the human body.[7] The formulation and selection of the approach to be used for herbal cosmetics will depend on the purpose of preparation (ie, for topical or systemic effect; inherent properties of drug or herb extract, such as

Figure 1: Mechanism of photoreactions
Table 1: Profile of photoprotective phytoconstituents

<table>
<thead>
<tr>
<th>Name of phytoconstituents</th>
<th>Chemical structure</th>
<th>Nomenclature</th>
<th>Chemical data</th>
<th>Solubility</th>
</tr>
</thead>
</table>
| Curcumin                  | ![Curcumin structure](image) | 1,7-Bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione. | Mol. Formula: C_{17}H_{16}O_{5}  
Mol. mass: 368.38 g/mol | Acetone (20 mg/mL)  
ethanol, DMSO, dimethyl formamide (1 mg/mL), 0.1 M NaOH  
(3mg/mL) soluble in alkali.  
Insoluble in methylene chloride, water at acidic and neutral pH |
| Resveratrol               | ![Resveratrol structure](image) | (trans-3,5,4’-Trihydroxystilbene) | Mol. formula: C_{14}H_{12}O_{3}  
Mol. mass: 228 g/mol | Ether, chloroform, ethanol, acetic acid, acetone.  
Insoluble in water |
| Quercetin                 | ![Quercetin structure](image) | 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one | Mol. formula: C_{15}H_{10}O_{7}  
Mol. mass: 302.24 g/mol | Order of solubility Isopropyl myristate < oleyl alcohol < propylene glycol monolaurate < oleyl macrogol-6 glycerides < linoleyl macrogol-6 glycerides < propylene glycol laurate (POL) < propylene glycol monacaprylate (PGMC) < polyethylene glycol-8 glyceryl linoleate < aplytocaproyl macrogol-6 glycerides < diethylene glycol mono ethyl ether (DGME).  
Poorly soluble in water. |
| Genistein                 | ![Genistein structure](image) | 5,7-Dihydroxy-3-(4-hydroxyphenyl) chromen-4-one. OR 4',5,7-trihydroxyisoflavone | Mol. formula: C_{15}H_{10}O_{5}  
Mol. mass: 270.24 g/mol | Soluble in ethanol, DMSO, dimethyl formamide. (30 mg/mL).  
Poorly soluble in water |
| Silibinin                 | ![Silibinin structure](image) | (2R,3R)-3,5,7-Trihydroxy-2-[(2R,3R)-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-2,3-dihydrobenzo| Mol. formula: C_{21}H_{18}O_{10}  
Mol. mass 482.44 g/mol | Simulated gastric fluid (pH 1.2)  
(0.209 ± 0.65 mg/mL), Simulated Intestinal Fluid (pH 6.8) 0.148 ± 0.88 mg/mL, Dioxane.  
Poorly soluble in water. |
| Ascorbic acid             | ![Ascorbic acid structure](image) | (5R)-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxypurran-2(5H)-one | Molecular formula C_{6}H_{8}O_{6}  
Molar mass 176.12 g/mol | Solubility in water 33 g/100 mL  
Solubility in ethanol 2 g/100 mL  
Solubility in glycerol 1 g/100 mL  
Solubility in propylene glycol 5 g/100 mL.  
Insoluble in diethyl ether, chloroform, benzene, petroleum ether, oils, fats, fat solvents |

hydrophilic or hydrophobic; surface characteristics of a system, such as permeability and charges; degree of biodegradability, biocompatibility, and toxicity; release profile and size of the product required; and antigenicity of the final product. [10] Table 2 discusses the applications of various novel formulations.

IMPORTANT PHOTOPROTECTIVE PHYTOCONSTITUENTS

The use of active photoprotectives is very beneficial in combating the deleterious effects of UV rays. Important categories of beneficial phytoconstituents include phenolic acids, flavonoids, and high molecular weight polyphenols. [21,22] Naturally occurring phenolic acids include hydroxycinnamic acid and hydroxybenzoic acid. High molecular weight polyphenols, also known as tannins, include condensed polymers of catechins or epicatechins and hydrolyzable polymers of gallic or ellagic acids. [21] Many flavonoids, such as quercetin, luteolin, and catechins, are found to be better antioxidants than ascorbic acid, alpha-tocopherol, and beta-carotene. [23] Other photoprotective phytoconstituents include curcumin, garlic compounds caffeic and ferulic acid, apigenin, genistein, resveratrol, nordihydroguaiaretic acid, carnosic acid, silimarins, tea polyphenols, Capparis spinosa extract, Calendula officinalis H.B.K. leaf extract, French maritime pine bark extract, Ginkgo biloba extract, Grape seed extract, K. triandra root extract, Prunus persica flower extract, S. officinalis root extract, and Sedum telephium leaf extract. Mechanism of photoreactions and stages for their prevention could be understood with the help of Figure 1. We have selected few phytoconstituents and given their detailed profile. Mechanisms of action of photoprotective phytoconstituents on topical application have been depicted in Table 3.
Curcumin

Curcumin, (diferuloylmethane), a yellow colored polyphenol is one of the 3 curcuminoids of Curcuma longa (turmeric). The other 2 curcuminoids are demethoxycurcumin and bisdemethoxycurcumin. Out of which curcumin was found to be more potent showing antiinflammatory and antiproliferative properties.\[36\] Antioxidant and antiinflammatory properties of curcumin in mouse models are well documented.\[24,37,38\] Curcumin is obtained by solvent extraction from dried turmeric roots. A simple, selective, precise, and stability-indicating high-performance thin-layer chromatography (HPTLC) method of analysis of curcumin both as a bulk drug and in formulations was developed and validated. The method used TLC aluminum plates precoated with silica gel 60 F254 as the stationary phase. The solvent system consisted of chloroform:methanol (9.25:0.75 v/v). This system was found to give compact spots for curcumin (Rf value of 0.48 ± 0.02). Densitometric analysis of curcumin was carried out in the absorbance mode at 430 nm.\[36\]

Silymarin

Silymarin, also known as Silibinin, is a polyphenolic flavonoid derived from milk thistle. Silymarin consists of 3 phytochemicals: silybin, silydianin, and silychristin. Silybin is the most active phytochemical and is largely responsible for the claimed benefits
of silymarin. The different analytical methods that have been reported for its determination include high-performance liquid chromatography (HPLC), TLC, HPTLC, potentiometric titration, diffuse reflectance fourier transform infrared spectroscopy, and UV spectrophotometry. Few spectrophotometric methods have been reported for the assay of silymarin, based on the formation of colored complex of the drug with 2,4-dinitrophenyl hydrazine in the presence of tetramethyl amine hydroxide; reaction with diazotized sulfanilic acid in alkaline medium forms an orange-red colored chromogen. Oxidation by Fe(III) and reduced Fe(II) was estimated with 1,10-phenanthroline at 510 nm and reaction with Folin-Ciocalteu reagent to form a blue colored complex in the presence of NaOH, and subsequent determination was done at 740 nm. It has also been determined in drug formulations based on its oxidation with potassium permanganate at a neutral pH by measuring a decrease in the absorbance at 530 nm.[39]

The method is based on the oxidation of drug and 3-methyl-2-benzothiazolinone hydrazone hydrochloride with potassium persulfate in alkaline medium and subsequent coupling to form an intensely colored product. The reaction is followed spectrophotometrically by measuring the increase in absorbance with time (for 15 min) at 430 nm. The 2 calibration procedures, namely, initial rate and fixed time methods (at 12 min) are utilized for the assay of drug in the concentration range of 16.0-192.0 μg/mL.[39]

**Quercetin**

Quercetin belongs to the flavonoids family and consists of 3 rings and 5 hydroxyl groups. Quercetin is also a building block for other flavonoids. Quercetin occurs in food as an aglycone (attached to a sugar molecule). Only a small percentage of the ingested quercetin will get absorbed in the blood. Quercetin is found in many common foods, including apple, tea, onion, nuts, berries, cauliflower, and cabbage. Direct spectrophotometric method for the determination of quercetin in the presence of ascorbic acid was established. The influences of medium, wavelength, pH, temperature, and the ionic strengths on quercetin determination were investigated. The best conditions for calibration curve are 50% ethanol, λ = 370 nm, pH = 4.2, T = 34 °C, and I = 7.5 × 10⁻³ M. Beer’s law is obeyed in the concentration range 1.0-12.0 μg/mL for quercetin. The corresponding detection limit is 0.76 μg/mL.[40] The colored complex formed on reaction of quercetin with vanadyl sulfate is used as the basis for a sensitive and reproducible spectrophotometric method for the estimation of quercetin. The 1:1 complex exhibits maximum absorption at 425 nm in aqueous ethanol (80% v/v) adjusted to pH 3.3. Beer’s law is followed over the quercetin concentration range of 0.3-20 μg/mL. The molar absorptivity is 1.4 × 10⁴ L/mole/cm. This method can be used for the determination of quercetin isolated from biological sources. In the metal complex, the 4-carbonyl and 3-hydroxy groups of the quercetin are involved.[41]

**Ascorbic acid**

Ascorbic acid is the main water soluble vitamin in nature. It is present in fruits and vegetables, mainly citrus plants and their juices. It possesses antioxidant activity owing to its ability to scavenge ROS. Titration with iodine has proved to be inadequate for the determination of ascorbic acid in natural products, because they contain other reducing substances and also because the color of such products interfere with the determination of the endpoint of titration. For estimation of ascorbic acid in pure solution to 5 mL of freshly prepared standard ethanolic solution of ascorbic acid (11 mg/100 mL ethanol), 1 mL of freshly crystallized perinaphthoidehydrate in ethanol (2 mg/mL) is added, mixed thoroughly, and stoppered. The color gradually develops and reaches its maximum after 10 min. The stability of the color permits its measurement with ease any time from 10 min to 24 h after the reagent has been added. The percentage transmission of the sample is recorded with reference to a blank tube of ethanol set at 100% transmission. The amount of dihydroxy perinaphthoide is formed is first estimated photoelectrically and then spectrophotometrically using a 5-mm cell, and the ascorbic acid present calculated from this spectrophotometric method.[42]

**Resveratrol**

Resveratrol belongs to a class of polyphenolic compounds called stilbenes, found largely in the skins of red grapes and root of Polygonum cuspidatum Sieb. et Zucc (Japanese knotweed). Resveratrol is a fat soluble compound that occurs in a trans and a cis configuration. Resveratrol, a naturally occurring polyphenolic phytoalexin, is present in many plants and fruits, including red grapes, eucalyptus, spruce, blueberries, mulberries, peanuts, and giant knotweed. Also, red wine contains a lot of it. The longer the grape juice is fermented with the grape skins, the higher the resveratrol content will be. Resveratrol is an effective antioxidant with strong antiinflammatory and antiproliferative properties.[43]

**Genistein**

Genistein is the aglycone (without sugar component) of the glycoside genistin. Before genistein can act, it needs to be released from genistin. The main sources of genistein are soya beans, peas, and other legumes. Other legumes, such as chickpeas, contain small amounts of genistein. Genistein is an isoflavone belonging to the group of flavonoids. Because of the similarity in the structure of genistein to that of estrogen, genistein is also a phytoestrogen. It acts as a phytoestrogen and as an antioxidant. Topical application of genistein and its gastrointestinal metabolites, such as eqol, isoeqol, and dehydroeqol, to hairless mice skin substantially inhibited the UV-B-induced hydrogen peroxide (H₂O₂) production, contact hypersensitivity, and reduced the inflammatory edema reaction.[43] Genistein was analyzed by reverse phase HPLC with X Terra C18 column (4.6 × 250 mm, 5 μm) at 40°C and isocratic elution with the mobile phase of a mixture of acetonitrile:50 mM ammonium formate buffer (5:5, v/v) delivered at a flow rate of 1.0 mL/min. Samples (30 μL) were injected onto the HPLC system. The eluent was monitored at 260 nm with a UV-vis detector.[44]

**Green tea extract**

Green tea is obtained from the plant Camellia sinensis of the
Theaceae family. The water-extractable fraction of green tea contains several polyphenolic compounds known as catechins. Generally, a typical cup of green tea contains 100-150 mg catechins, including 50% of (-)-epigallocatechin-3-gallate (EGCG), 15% of (-)-epigallocatechin (EGC), 15% of (-)-epicatechin-3-gallate, and 8% of (-)-epicatechin (1). All these polyphenols act as potent antioxidants and can scavenge ROS, such as lipid-free radicals, superoxide radicals, hydroxyl radicals, H$_2$O$_2$, and singlet oxygen. Afq et al. have reported in their publications that topical application or oral feeding of a polyphenolic fraction prepared from green tea prevents photocarcinogenesis.[1] Epicatechin is also known as epicatechol. Pure epicatechin is an odorless white powder. Epicatechin is a flavonol belonging to the group of flavonoids. High quantities of it can be found in cocoa, tea, and grapes.

**EVALUATION PARAMETERS OF PHOTOPROTECTIVE FORMULATIONS**

**Morphological studies**
Penetration of the skin barrier is size dependent, and nano-sized particles are more likely to enter more deeply into the skin than larger ones. Hence, particle or vesicular size determination is an important aspect in designing the formulation for topical application. The $\bar{z}$-average diameters of vesicles or particles of formulations are determined by dynamic light scattering using a Malvern Zetasizer. The samples are diluted to avoid multiple scattering. As a measure of the particle size distribution, the polydispersity index is calculated, which ranges from 0.0 (monodisperse) to 1.0 (very much heterogenous). Polydispersity signifies the uniformity of droplet size within the formulation. If the polydispersity value of the formulation is very low, it indicates uniformity of the droplet size within the formulation. The scanning electron microscope produces images by probing the specimen with a focused electron beam that is scanned across a rectangular area of the specimen; can produce images that are good representations of the 3-dimensional shape of the sample. The ability to determine the positions of atoms within materials has made the transmission electron microscope (TEM), an important tool in the research and development of nanotechnologies. Formulations are visualized by TEM at ×80,000 and ×1,20,000 magnifications. Differential scanning calorimetry is an important means of detecting the incompatibility between active constituents and excipients used in the formulation.

**Entrapment efficiency determination**
Entrapment efficiency is the percentage of the initial drug or active constituent incorporated into vesicular systems. Entrapment efficiency is estimated by centrifugation method. The prepared formulations are placed in centrifugation tube and centrifuged at 14,000 rpm for 30 min. The supernatant (1 mL) is withdrawn and diluted with phosphate-buffered solution (PBS) (pH 7.4) or distilled water. The entrapped phytoconstituent (drug) is determined by UV spectrophotometer at a wavelength at which the maximum peak ($\lambda$ max) is obtained for that constituent. The samples from the supernatant are diluted 100 times before taking the absorbance measurement. The free drug in the supernatant gives us the total amount of unentrapped drug. Entrapment efficiency is also determined by a Sephadex G-50 minicolumn centrifugation technique.[15] Encapsulation efficiency is expressed as the percent of drug trapped. After fusing the vesicles with Triton X-100, the amount of trapped drug was estimated by UV spectrophotometer with suitable dilution by pH 7.4 PBS. The vesicles are washed first with PBS pH 7.4 and then 3-4 times with distilled water and suspended in distilled water.[48]

In vitro drug release
Drug release is observed using the dialysis method at room temperature. After reconstituting the freeze-dried formulation in distilled water/PBS, an aliquot of each formulation (0.1 mL) is placed in a dialysis tube (molecular weight cutoff dialysis membrane: 12,000-14,000 Mw), which is tightly sealed. The tube is immersed in 200 mL release medium, PBS (pH 7.4), to maintain sink condition and stirred at 300 rpm on a magnetic stirrer. Samples (0.5 mL) are taken at predetermined time intervals for 24 h, and replenished with an equal volume of fresh medium. The concentration of drug is determined by HPLC or UV after appropriate dilution with acetonitrile without further treatment.[48]

In vitro skin permeation studies
In vitro skin penetration studies are performed with human cadaver skin, using Keshary-Chien cells or Franz diffusion cells. Human cadaver skin from the abdominal region, after removing hair and subcutaneous fat tissue, is mounted on the diffusion cell. PBS serves as a receptor fluid. A small quantity (0.5 g) of the gel/formulation is applied to the skin surface. At the end of 24 h, the amount of drug in the receptor compartment, the drug remaining on the skin, and the drug concentration in the skin is determined by extraction into a suitable solvent followed by spectrophotometric analysis using UV-vis spectrophotometer.[49]

In dermatologic treatment, improving the efficacy demands high drug levels in the skin. In an experiment with nanoparticle dispersion, it was found that a greater quantity of drug remained localized in the skin, with lesser amounts penetrating into the receptor compartment as compared with conventional gels. Thus, drug localizing effect in the skin seems possible with novel colloidal particulate drug carriers, such as solid lipid nanoparticle. This colloidal carrier, being submicron in size, enhances the drug penetration into the skin, and because of its lipoidal nature, the penetrated drug concentrates in the skin and remains localized for a longer period of time, thus enabling drug targeting to the skin.[49]
In vitro sun protection factor determination by UV spectrophotometer

Ratio of UV doses protected to unprotected gives the sun protection factor (SPF). The in vitro method measures the reduction of the irradiation by measuring the transmittance after passing through a film of product. The most common in vitro technique involves measuring the spectral transmittance at UV wavelengths from 280 to 400 nm. The observed absorbance values at 5 nm intervals are calculated using the following formula:

$$\text{SPF}_{\text{spectrophotometric}} = \frac{\sum_{\lambda} EE(\lambda) \times I(\lambda) \times \text{Abs}(\lambda)}{290}$$

Where $CF =$ correction factor (10), $EE(\lambda) =$ erythmogenic effect of radiation with wavelength $\lambda$, $\text{Abs}(\lambda) =$ spectrophotometric absorbance values at wavelength $\lambda$. The values of EE $\times I$ are constants. They were determined by Sayre et al. The aliquots prepared were scanned between 290 and 320 nm and the obtained absorbance values were multiplied with the respective EE $\times I$ values. Then their summation was taken and multiplied with the correction factor (10).

In vitro occlusion studies

The occlusivity of formulations is determined by occlusion factor. First investigations were performed by de Vringer. The in vitro model consists of a beaker of water covered by a filter paper, 200 mg of the formulation is spread on a filter surface of 18.8 cm²; a reference control is maintained, which is a beaker filled with nitrogen and stored at 4 and 25°C for different periods of time (10, 20, and 30 days). The stability of the formulations is assessed by monitoring the residual drug content, size, and morphology of the vesicles, with respect to time.

Bioengineering methods

Viscoelasticity determination

The viscoelastic properties are measured using cutometer. The measuring principle is suction/elongation. An optical system detects the decrease of infrared light intensity depending on the distance the skin is being sucked into the probe. In this study, the strain time mode has been applied. A probe with a 2 mm opening was used and a pressure of 500 m bar was applied in order to suck the skin into the probe. Each measurement consists of 5 suction cycles (3 s of suction followed by 3 s of relaxation and was performed in triplicate on volar fore arm). The following parameters (absolute and relative) were analyzed: $U_e$, elastic deformation; $U_v$, viscoelasticity; $U_0$, total deformation; $U_r$, retraction; $R_2$ or $U_a$, overall elasticity of the skin; $R_5$, or $U_r/ U_e$, pure elasticity of the skin without viscous deformation; $R_7$ or $U_r/ U_v$, biological elasticity, that is, the ratio of retraction to extension; $R_6$ or $U_v/ U_e$, the ratio of viscoelasticity to elastic deformation; and $R_8$ or $(U_a)$, pliability, that is, the ability of the skin to return into its original state.

Hydration measurement by corneometer

In photoaging, the epidermis becomes pale, thin, and dry. So hydration is also responsible for the increased drug penetration into the skin.

Primary skin irritation studies

Primary skin irritation studies of the selected formulation is performed using albino rabbits in accordance with the guidelines of the Consumer Product Safety Commission. The study has to be approved by the Institutional Ethics Committee. The scores for erythema and edema are totaled for intact and abraded skin for all rabbits at 24 and 72 h. The primary irritation index (PII) is calculated based on the sum of the scored reactions divided by 24 (2 scoring intervals multiplied by 2 test parameters multiplied by 6 rabbits). If the developed formulation showed no erythema or edema on the intact and abraded rabbit skin, the PII of the formulation is calculated to be 0.0. Thus, the formulation can be classified as a nonirritant to the rabbit skin. In addition, a subjective evaluation of product safety is conducted by a dermatologist on a predetermined scale, that is, $0 =$ no sign of irritation, $1 =$ mild irritation, $2 =$ moderate irritation, and $3 =$ strong irritation, and a self-report diary.

Stability studies

The formulations are sealed in vials (10 mL capacity) after flushing with nitrogen and stored at 4 and 25°C for different periods of time (10, 20, and 30 days). The stability of the formulations is assessed by monitoring the residual drug content, size, and morphology of the vesicles, with respect to time.

In vivo skin hydration studies

The skin hygrating effect of the selected formulation is investigated in vivo and compared with the conventional gel. The topical formulations were applied to the shaved skin of female albino rats. After 24 h, the animals were humanely killed and the skin was isolated, vertically sliced using microtome, and stained with hematoxylin and eosin. The slides were observed under an optical microscope and the thickness of stratum corneum was detected and evaluated.
**Erythema determination by mexameter**

Skin erythema is measured using mexameter, before and after a single and 1-, 6-, and 12-week period of daily application. Erythema can be determined by investigating the histologic, ultrastructural, biochemical, and immunologic effects of UV-R on skin and its relationship to photodamage and skin cancer.

**Method:** Hairless mouse model could be used for photoprotection studies. Before experimentation, 4 hairless mice are anesthetized (using ketamine) and a rectangular area approximately 2.5 × 4 cm is marked off on the dorsal area of each animal. Then sunscreen formulations are applied and one mouse is left as control, which is untreated. After 15 min drying period, UV-R are produced by a planar array of 2 UV-A 340 fluorescent lamps, which stimulate UV-R present in the sunlight from wavelength 295 to 365 nm. Irradiance could be measured using erythema UV-A and UV-B. Intensity meter and minimal erythemal dose (MED) is calculated. One MED is defined as the amount of UV-R necessary to cause a slight reddening of the skin 24 h after exposure. For hairless mouse, 1 MED is approximately 140 mJ/cm².

**Lipid concentration determination**

The lipid concentration was measured using Sebumeter.® The test product is applied twice daily to the face for a period of 12 weeks. A clinical assessment and instrumental measurements are done before and after the treatment period. Casual sebum level on the forehead and both cheeks is determined with a photometric device (Sebumeter).

**Biological studies**

**Lipid damage determination**

UV-R induces the formation of ROS resulting in the damage to various components of the skin—lipid damage, which is oxidative degradation of unsaturated free fatty acids and cholesterol. It is observed that UV exposure decreases lipid melting temperature of the mouse skin and that application of sunscreens prior to UV-R would reduce this epidermal damage. The detail method as described by Felton has been already discussed in our earlier work.

**Quantification of UV-induced DNA damage**

To estimate whether the application of the test formulation exhibits a protective effect on DNA lesions, the test formulations were applied to volunteers twice daily. To assess DNA damage by single-cell gel electrophoresis (Comet assay), epidermal keratinocytes were isolated from suction blister epidermis and embedded in low-gelling agarose gels.[53]

**Histologic studies**

These studies include epidermal cell turnover determination, sunburn cell count, edema determination and wrinkle determination. The decrease in corneocyte size is correlated with accelerated epidermal turnover. The determination is carried out by image analysis of D-Squame® sheets.[61] Inflammation is an acute biological response to UV-R. The vasodilatation of cutaneous blood vessels results in erythema (reddening) and edema (swelling). The treatment reduces this edema and thus, it is also an evaluation parameter for photoprotectives. Edema is calculated by the difference in skinfold thickness between the baseline and post-UV exposure data. Facial wrinkles in the crow’s feet area are evaluated by means of in vivo topometry, using phase shift rapid in vivo measurement of human skin phase induction PRIMOS. The PRIMOS system represents an established and widely used method to quantify the effects on skin wrinkles.[54,60]

**HERBAL PHOTOPROTECTIVE FORMULATIONS**

Ashawat et al.[58] prepared and characterized herbal creams for the improvement of skin viscoelasticity and hydration.[81] They prepared and characterized herbal cosmetic cream comprising extracts of *Glycyrrhiza glabra*, *Curcuma longa* (roots), seeds of *Porroba orifilfa*, *Cassia tora*, *Areca catechn*, *Panica granatum*, fruits of *Embelia officinalis*, leaves of *Centella asiatica*, dried bark of *Cinnamon zeylanium* and fresh gel of *Aloe vera* for the protection of skin against UV-induced aging. Ashawat et al.[56] evaluated UV absorption ability of *Boerhavia diffusa* and expressed in terms of SPF values.[51]

The study was done to develop quercetin-loaded nanoparticles (QUEN) by a nanoprecipitation technique with Eudragit®E (EE) and polyvinyl alcohol as carriers, and to evaluate the antioxidant effects of quercetin and its nanoparticles. The release of the drug from the QUEN was 74-fold higher compared with the pure drug. In addition, the antioxidant activity of the QUEN was more effective than pure quercetin on DPPH (2,2-Diphenyl-1-Picrylhydrazyl) scavenging, antisuperoxide formation, superoxide anion scavenging, and antilipid peroxidation.[62]

Plianbanchang et al., studied the efficacy and safety of curcuminoids-loaded solid lipid nanoparticles facial cream as an antiaging agent.[64] Curcuminoids are easily degraded by acid and alkal hydrolisis, oxygenation, and photodegradation, whereas solid lipid nanoparticles (SLN) promote its stability, prolong the release, enhance the penetration of active substances through the stratum corneum by increasing its hydration and forming an intact film while drying.[64] Wissing and Muller (2003) confirmed the higher effectiveness of creams containing SLN on skin hydration and viscoelasticity as compared with conventional creams.[60]

The quercetin self-emulsified formulation was optimized based on the quercetin solubility in different oils, and the self-microemulsified efficiency of various combinations of emulsifiers and coemulsifiers was evaluated using the pseudoternary phase diagram.[69] The solubility of quercetin is significantly increased in the self-emulsified system and the formulation is stable and easy to prepare. Casagrande et al. took quercetin-loaded formulations of non-ionic emulsion with high lipid content and anionic emulsion with low lipid content and found that quercetin remains functionally stable in formulations, and measuring the antioxidant activity is an efficient approach to evaluate quercetin.
skin retention with minimal interference of the tissue products. Furthermore, these data suggest that formulations containing quercetin may be used as topical active products to control UV-B-mediated oxidative damage of the skin.[67] Martelli et al in their invention used curcumin at concentrations between 0.0005 and 10% of the total composition weight for cosmetic or pharmaceutic-dermatologic use and found it suitable for maintaining skin cells and enabling them to effect regeneration of the skin.[68]

Resveratrol, the main active polyphenol in red wine, was incorporated into various combinations of emulsions and liposomes to examine its physicochemical characteristics and cardiovascular protection. They concluded that encapsulation by the emulsion-liposome blends is a potent way to enhance the preventative and therapeutic benefits of resveratrol.[69] Sreekumar et al in their patent took a cosmetic skin care composition comprising resveratrol in an amount from 0.00001 to 10 wt%, a retinoid selected from the group consisting of retinoic acid, retinol, retinyl acetate, and retinyl linoleate, and a cosmetically acceptable vehicle. It was a cosmetic method of improving the appearance of wrinkled, lined, dry, flaky, aged, or photodamaged skin and improving skin thickness, elasticity, flexibility, and plumpness—the method comprising applying to the skin.[70]

The possibility of improving the efficacy of resveratrol, a polyphenol with strong antioxidant and free-radical scavenging properties, on cell proliferation and photoprotection by liposomal incorporation was investigated. Interestingly, liposomes prevented the cytotoxicity of resveratrol at high concentrations, even at 100 μM, avoiding its immediate and massive intracellular distribution, and increased the ability of resveratrol to stimulate the proliferation of the cells and their ability to survive under stress conditions caused by UV-B light.[71]

Zhang et al reported that green tea extract and EGCG exhibit antiangiogenic activities in various experimental tumor models.[72] Huang et al reported that EGCG is a potent agent against UV-B-induced damage in HaCaT keratinocytes.[73] Camouse et al have experimentally determined that topical application of green tea and white tea extracts prevent simulated solar radiation-induced oxidative damages to DNA and Langerhans cells that may lead to immune suppression and carcinogenesis.[74]

CONCLUSION

There are sufficient number of photoprotective phytoconstituents, which could be an important part of photoprotective formulations, but very less work has been done thus far and there is need for more research taking these phytoconstituents, establishing more effective formulations as several botanical compounds have been shown to be antimutagenic, anticarcinogenic, and nontoxic and have the ability to exert striking inhibitory effects on a plethora of cellular events at various stages of carcinogenesis. Because multiple pathways are involved in photocarcinogenesis, a mixture of several botanical antioxidants working through various mechanisms, in conjunction with the use of sunscreens could also be an effective approach for reducing UV-generated ROS-mediated photodamage, immunosuppression, and skin cancer in humans. Further development of novel delivery systems along with the use of botanical extracts will be a good approach for fighting against photocarcinogenesis. This article would be helpful for the development of stable herbal photoprotective formulations with least side effects, more efficiency, and longer duration of action.

ACKNOWLEDGEMENTS

Authors are thankful to UGC [major project, F.No 32-133/2006(SR)], New Delhi for financial support and Head, Cosmetic laboratory, University Institute of Pharmacy, Raipur CG for providing instrumental and other facilities. We also pay gratitude to National Medical Library, New Delhi for library facility and NISCAIR, New Delhi for online journals facility.

REFERENCES


Conflict of Interest: None declared.

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