ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
Research Article
www.ijptonline.com

FORMULATION AND EVALUATION OF UV PROTECTIVE HERBAL SKIN CREAMS

[1]Sujith S Nair*, [2]Sreena K and [3]Molly Mathew
[1,2]Crescent College of Pharmaceutical Sciences, Kannur, Kerala.
[3]Malik Deenar College of Pharmacy, Kasargod, Kerala.
Email: tosujithsnair@gmail.com

Received on 12-10-2014 *Accepted on 02-11-2014*

Abstract

The sunscreen is excellent photo protecting agents and guards the body from UV attack. Further sunscreens are used to shoot up the body's natural defense mechanisms to protect against harmful UV radiation from the sun. The goal of this study is to develop cost effective, nontoxic and effective sun protection formulations. The four formulations prepared exhibited significant SPF value (Sun Protection Factor) and formulation F1 containing *P.emblica* displayed higher value compared to that of the other formulations which might be due to the presence of potent phytochemicals in the plant extract. When concentration of the extract in the formulation was increased, formulation F1 showed highest SPF value than the marketed one. The HPLC study revealed the occurrence of marker ingredient in the formulations and it was compared with the standard by HPLC method. The presently formulated products using herbal extracts have significant sun protection property against UV irradiation along with antioxidant activity which is a major factor looked for cosmetic industry.

Key words: Herbal creams, UV protection, SPF, HPLC quantification.

Introduction

Medicinal herbs are gaining much attention in mainstream healthcare as it provides safe remedies and approaches to healthcare. There is a global demand for herbal preparations, herbal health products, herbal pharmaceuticals, nutraceuticals, food supplements and herbal cosmetics as they are non-toxic with minimal adverse effects, compatible with the physiological flora and moreover highly economical [1, 2]. The main purpose of the cosmetic application is to intensify the general appearance of face and other body parts to minimize the skin defects to a considerable extent [3].

The penetration of UV radiation into the skin can trigger detrimental photochemical reactions and elicits acute inflammatory conditions such as erythema and chronic conditions such as photoageing, the main contributor to changes in skin's appearance with age and skin cancers such as cutaneous malignant melanoma, basal cell carcinoma and squamous cell carcinoma. The sunscreen is excellent photoprotecting agents and guards the body from UV attack [4, 5]. Further sunscreens are used to shoot up the body's natural defense mechanisms to protect against harmful UV radiation from the sun. Its function is based on its ability to absorb, reflect or scatter the sun's rays. Any substance that reduces oxidative damage such as that caused by free radicals is called as anti-oxidants. Free radicals are highly reactive chemicals that attack molecules by capturing electrons and thus modifying chemical structures [6]. The four plants parts were selected for prepairing the herbal formulations; fresh roots and rhizomes of *Glycyrrhiza glabra* and *Hemidesmus indicus, rhizomes of Curcuma longa and* fruits of *Phyllanthus emblica* [7, 8, 9, 10]. The goal of this study is to develop cost effective, nontoxic and effective sun protection formulations and to find out whether the prepared formulations are effective and comparable to the existing marketed one.

Materials and Methods

Preparation of extract:

The Selected plant parts were collected from Payangadi, Kannur and identified by Dr. Jayasmitha S J,Taxonomist, Parassinikadavu Ayurveda Medical College, Kannur and dried it for 2 days in sunlight. Then crushed it to fine powder and passed it from sieve # 60. The collected powder was subject to maceration with 70% ethanol in iodine flask for 7 days. The extract was filtered and decolorized with charcoal to get clear liquid. Evaporate this extract to get the semisolid mass [11].

Preliminary Phytochemicals screening (Qualitative Analysis):

The preliminary phytochemicals studies were performed for testing different chemical groups present in ethanolic extract [12, 13].

Preparation of Cream:

The four different formulations were prepared as O/W cream, F1 with extract *Phylanthus emblica*, F2 with extract *Glycyrrhiza glabra*, F3 with extract *Curcuma longa* and F4 with extract *Hemidesmus indicus.* The moisturizer conditioner was mixture of propylene gycol: glycerine: sorbitol (2:1:1). All the aqueous soluble ingredients were

dissolved in water and all oil soluble ingredients were mixed at 75^0c± 5^0c in separate beakers. The aqueous phase was then added to oil phase slowly with constant stirring and the mixture was homogenized for 30 min [14].

***In vitro* sun protection factor determination by UV spectrophotometer:**

The effectiveness of a sunscreen cream is expressed by the sun protection factor (SPF), which is defined as the UV energy required to produce a minimal erythemal dose (MED) in protected skin, divided by the UV energy required producing an MED in unprotected skin. The minimal erythemal dose (MED) is defined as the lowest time interval or dosage of UV light irradiation sufficient to produce a minimal, perceptible erythema on unprotected skin. The higher the SPF, the more effective is the product in preventing sunburn. 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 [15, 16]. The observed absorbance values at 5 nm intervals are calculated using the following formula,

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

Where CF = correction factor (10), EE (λ) = erythmogenic effect of radiation with wavelength λ, Abs (λ) = spectrophotometric absorbance values at wavelength λ. The values of EE × *I* was constant and 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 (λ) × *I* (λ) values. Then their summation was taken and multiplied with the correction factor (10).

Quantitative determination of marker ingredient by HPLC [17, 18, 19, 20].

The formulated creams were tested for the presence of the marker bio ingredients by HPLC.

HPLC Quantification of Lupeol

The Lupeol present in the formulation was analyzed by the following chromatographic conditions [17].

Mobile Phase: IPA: water (80:20,v/v). Column: ABZ plus column, having dimension 250×4.6 and particle size of 5µ. Detector: Photodiode-array detector (SPD-10A). Injection volume: 20µl. Wavelength for recording the chromatogram: 210 nm.

HPLC Quantification of Gallic acid

The Gallic acid present in the formulation was analyzed by the following chromatographic conditions [18].

Mobile Phase: Water: Acetonitrile: Acetic acid (90:10:0.2,v/v/v). Column: ODS (Octadecyl silane) C_{18}, 5m size, 250 x 4.6mm (Merck) RP-18, Lichrocart 250-4. Detector: SPD-M-10Anp- Photo Array Detector. Injection volume: 20 µl. Wavelength for recording the chromatogram: 254 nm.

HPLC Quantification of Glycyrrhetinic acid

The Glycyrrhetinic acid present in the formulation was analyzed by the following chromatographic conditions [19].

Mobile Phase: Acetonitrile / Phosphoric acid (3/1 and PH=2.5). Coloumn: C_{18} reversed phase column (VP-ODS, (250×4.6 mm, 5 mm)) and LC-10AD pump. Detector: Photodiode-array detector (SPD-10A). Injection volume: 20µl. Wavelength for recording the chromatogram**:** 254 nm.

HPLC determination of Curcumin

The Curcumin present in the formulation was analyzed by the following chromatographic conditions [19].,

Mobile Phase: acetonitrile-5 % acetic acid (75:25, v/v). Column: Diamonsil C_{18} analytical column having dimension of 4.6 x 100 mm,5 µ). Detector: Photodiode-array detector (SPD-10A). Injection volume: 20µl. Wavelength for recording the chromatogram: 420 nm.

After analysis, the HPLC chromatogram of extracts and formulations were compared with the pure standard. Then the quantity of marker ingredient present in the extract and in the formulation was determined by using the following formula.

$$\text{ASSAY (\%w/w):} \quad \frac{\text{Sample area} \times \text{standard weight} \times \text{purity}}{\text{Standard area} \times \text{sample weight}}$$

Statistical Analysis:

All data's were recorded in six trials (i.e., n=6). The probability (p) values were determined by paired t–test using Graphpad software and $p<0.01$ were considered as significant.

Results

Preliminary Phytochemical screening

Phytochemical screening of the extracts was done to reveal the major constituents present in the extracts and the results are summarized in the table 1. These constituents present in the extracts are responsible for the antioxidant activity and the antioxidant activity of *curcuma longa* is due to the presence of curcumin.

Table-1: Phytochemical screening of the prepared plant extracts.

S.No.	Type of Constituents	NAME OF THE PLANT EXTRACT			
		PE	GG	CL	HI
1	Sterols	+	+	-	+
2	Triterpenoids	-	+	-	+
3	Saponins	-	++	-	+
4	Alkaloids	-	-	-	+
5	Carbohydrates	+	+	-	+
6	Reducing sugars	+	-	-	-
7	Tannins	++	-	-	+
8	Phenolic compounds	+	+	-	+
9	Glycosides	+	+	-	+

(+) Present (-) Absent

PE – *Phylanthus emblica*, GG – *Glycyrrhiza glabra*, CL –*Curcuma longa*, HI – *Hemidesmus indicus*.

***In vitro* Sun Protection Factor study**

Sun Protection Factor of selected formulations, F1, F2, F3 & F4 are displayed in table 2 A. In this study, all the formulations exhibited significant sun protection, but F1 displayed the highest effect, which was evident from the SPF value (9.105) compared to that of the other formulations and when the concentration of extract in F1 increased from 10% w/w to 12% w/w then SPF value was increased to 11.96 and it was found to be more than that of marketed cream table 2 B.

Table-2: A. Sun Protection Factor study of prepared formulations.

Formulations	SPF
Blank	2.67 ± 0.039
F1	9.105 ± 0.16**
F2	7.650 ± 0.040**
F3	8.90 ± 0.059**
F4	8.35 ± 0.12**
Marketed cream	10.94 ± 0.054

Values are Mean±SEM, n=6, ** t-test Significant ($p < 0.01$).

F1 with *Phylanthus emblica*, F2 with *Glycyrrhiza glabra*, F3 with *Curcuma longa*, F4 with *Hemidesmus indicus.*

Table-2: B. SPF of F2 with increased concentration of the extract.

Formulation with increased concentration of extract (12% w/w)	SPF
F2	11.96±0.14**

Values are Mean±SEM, n=6, ** t-test Significant ($p < 0.01$).

HPLC Quantification of marker ingredient in the formulations

The Retention time of Gallic acid in standard and formulation F1 was 9.567 and 9.508 respectively. (Figure 1A and 1B). The Retention time of Glycyrrhetinic acid in standard and formulation F2 was found to be 19.346 and 19.358 (Figure 2A and 2B). The Retention time of Curcumin in standard and

formulation F3 were 10.922 and 10.762 respectively (Figure 3A and 3B) and the Retention time of lupeol in standard and formulation F4 was found to be 7.290 and 7.367 respectively (Figure 4A and 4B). Thus, it confirms the presence of the marker ingredient in the formulations.

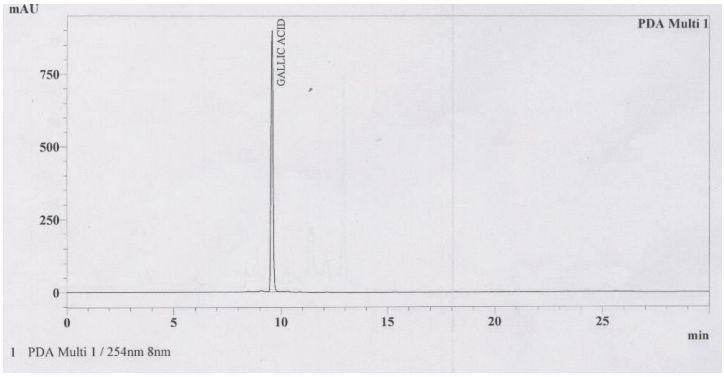

Figure 1 A. HPLC Chromatogram of standard gallic acid

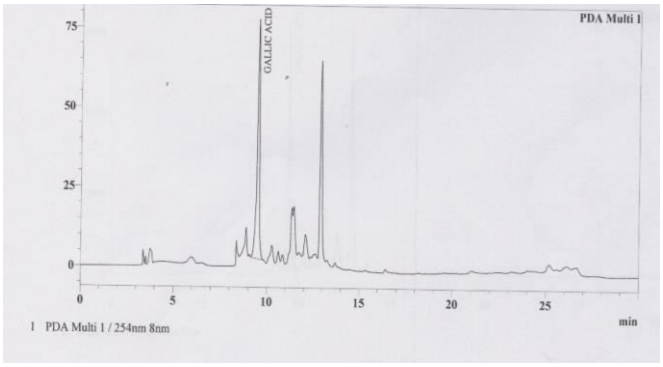

Figure 1 B. HPLC Chromatogram of F1

$$\% \text{ of Gallic acid present in F1} = \frac{3469074 \times 21.3 \times 25 \times 97}{5997440 \times 100 \times 258.5} = 1.15\% \text{w/w}$$

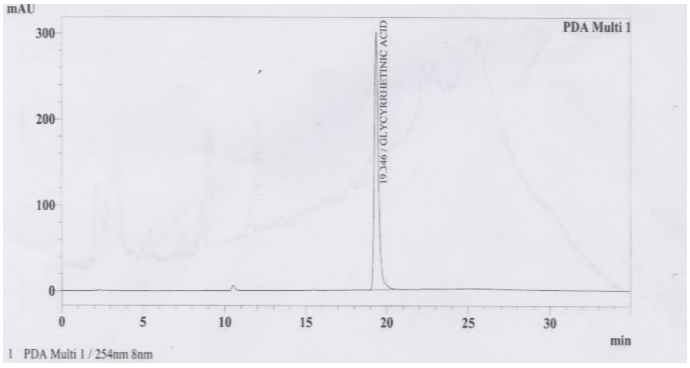

Figure 2 A. HPLC Chromatogram of standard Glycyrrhetinic acid

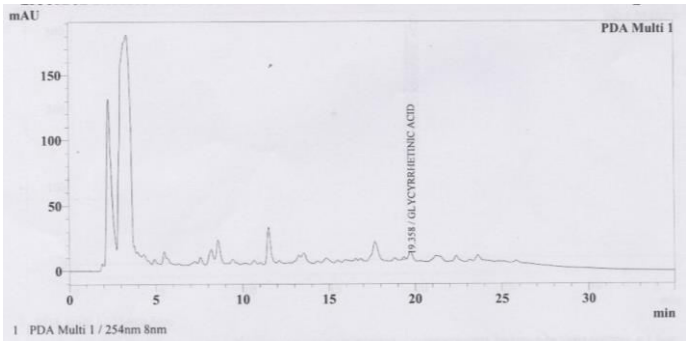

Figure 2 B. HPLC Chromatogram of F2

$$\% \text{ Glycyrrhetinic acid present in F2} = \frac{149120 \times 23.52 \times 97}{5397453 \times 205.03} = 0.31\% \text{w/w}$$

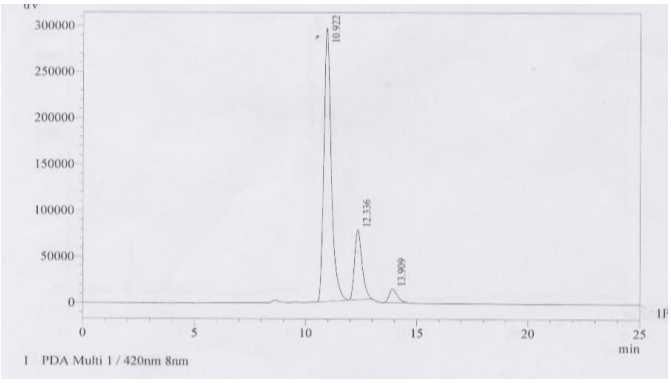

Figure 3 A. HPLC Chromatogram of standard curcumin

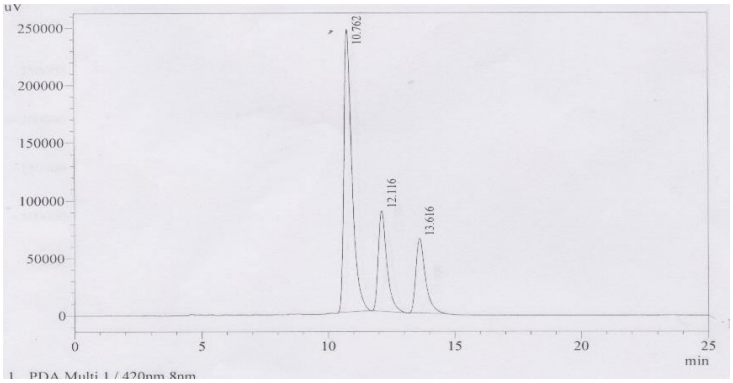

Figure 3 B. HPLC Chromatogram of F3

$$\% \text{ Curcumin present in F3} = \frac{595806 \times 56.25 \times 5 \times 50 \times 95.11}{6849814 \times 50 \times 405.72 \times 5} = 1.14\% \text{w/w.}$$

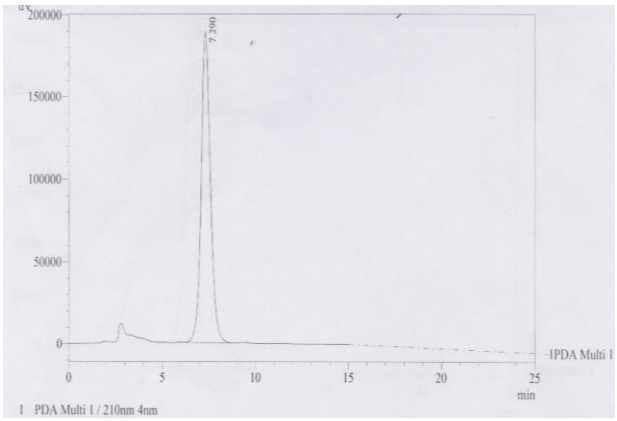

Figure 4 A. HPLC Cgromatogram of standard lupeol

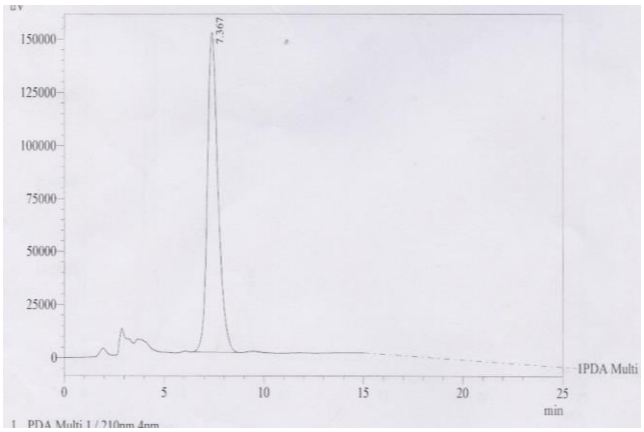

Figure 4 B. HPLC Chromatogram of F4

$$\% \text{ Lupeol present in F4} = \frac{584677 \times 25.95 \times 81.5}{6558743 \times 236.36} = 0.79\% \text{w/w.}$$

Discussion

The phytochemical study indicates the possibility of plant metabolites to be responsible for their potent antioxidant activity. The antioxidant properties of plant extracts which have free radical scavenging activity, receive considerable attention for possible use in protection of cells against oxidative damage. The four formulations exhibited significant SPF value and formulation F1 containing *P.emblica* displayed higher value compared to that of the other formulations which might be due to the presence of potent phytochemicals in the plant extract. When concentration of the extract in the formulation was increased, formulation F1 showed highest SPF value than the marketed one. The HPLC study revealed the occurrence of marker ingredient in the formulations and it was compared with the standard by HPLC method. So the presently formulated products using herbal extracts have significant sun protection property against UV irradiation.

Conclusions

In the present research work, the selected four formulations exhibited significant SPF value and formulation F1 containing *P.emblica* displayed higher value compared to that of the other formulations which might be due to the presence of potent phytochemicals in the plant extract. So from the present study it can be concluded that the presently formulated products using herbal extracts have significant sun protection property against UV irradiation along with antioxidant activity which is a major factor looked for cosmetic industry. The clinical studies in humans have to be conducted in future to prove its complete safety and efficacy to market these formulations for the benefit of the society.

Acknowledgement

The Authors are thankful to the authorities of Crescent College of Pharmaceutical Sciences, Kannur, India for providing necessary facilities for carrying out the study.

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Corresponding Author:

Sujith S Nair*,

Email: *tosujithsnair@gmail.com*