FORMULATION AND EVALUATION OF PHYTOSOMAL CREAM FORMULATION FOR PHOTO PROTECTIVE EFFECT

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ABSTRACT

Skin play significant role in the management of harmful effect of sunlight, due to the presence of endogenous antioxidant system beneath the skin. UV radiation induced free radicals destroy the antioxidant system of skin and causes harmful effect on skin. Sunscreen is use to protect the skin from the harmful effect UVR. The aim of the present study was to prepare and evaluate the novel phytosomal cream formulation for photoprotective effect. Combination of phenolic fraction was used to prepare the phyto-phospholipid complex. Total five batches of cream formulations loaded with phytophospholipid complex were prepared by taking different concentration of phytophospholipid complex, and evaluated for pharmaceutical parameters and therapeutic evaluation. Result of pharmaceutical evaluation showed that all prepared batches of phytosomal cream formulation showed pharmaceutical parameter in the required range. The result of photoprotective evaluation showed that optimized batch of phytosomal cream formulation (PCF5) showed optimum photoprotective effect.

.**Keywords:** UV radiation, harmful effect, antioxidant system, Photoprotectvie, Phenolic constituents, phytophopsholipid complex.

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INTRODUCTION

Plant phenolic biomolecules play crucial role in the management of diseases. Polyphenolic constituents are the well-known plant biomolecules present in most of the plant. Plant phenolic compounds have potent therapeutic potential due to their free radical scavenging activity use for the treatment of heart diseases, cancer, diabetes, CNS disorders and skin disorders.[1,2]Phenolics became important part of drug discovery and formulation development since from last three to four decades. Low solubility, poor bioavailability, instability, and poor dermal penetration limits their therapeutic potential [3,4].To overcome these challenges, novel approach was adopted in this study to improve the therapeutic potential of phenolic compound. Emphasis is given to improve the photoprotective efficacy of phenolic biomolecules, previous study showed that synthetic photoprotective agent induces allergic reaction, acne and erythema. Development of natural photoprotective

formulation faces the challenges of instability, poor absorption and dermal penetration. Nano encapsulation of phenolic biomolecules involves the entrapment of phenolic bioactive in small capsules which improved the solubility, stability, bioavailability, dermal penetration and control release of bioactive[5]Previous research proved that phytophospholipd complex show better therapeutic efficacy than the conventional formulation or extracts[6].In this study novel potential cream formulation were prepared by incorporating the phytophospholipid complex of combination of phenolic bioactives with phospholipid in different molar ratio.

Materials and Methods

All the selected plants were purchase from the local crude drug shop and the taxonomic identification was confirmed by the department of pharmacognosy, PWCOP Yavatmal.

Chemicals- Soya lecithin, tetrahydrofuran, ethanol, methanol, ethyl acetate, 1,1-diphenyl-2-picrylhydrazyl potassium ferricyanide. All chemicals of AR analytical grade were purchased.

Preparation of extract

200 gm powder of each crude drug (*C. sinensis, G. glabra, M. ferrea, and N. nucifera.)* Was extracted by soxhlet apparatus using ethanol for 10 hrs. and filtered. The filtrate was evaporated to dryness under pressure. Dissolve dried extract in ethyl acetate and filter. Process was repeated for three times. The filtrate was evaporated under reduced pressure.

Preparation of Phytophospholipid complex

Phytophospholipid complex was prepared by solvent evaporation method. Different molar concentration of combination of extract and soya lecithin was taken. Dissolved the required amount of mixture of extract and soya lecithin in tetrahydrofuran followed by stirring (RPM was adjusted to 400 rpm by using rotary evaporator) two hours at a temperature not exceeding 40^0C. Thin film of sample obtained to which n-hexane was added and continuously stirred until monolayered of phospholipid form, precipitate was obtained, filtered, collected, and stored in amber color bottle at room temperature.[7-9].

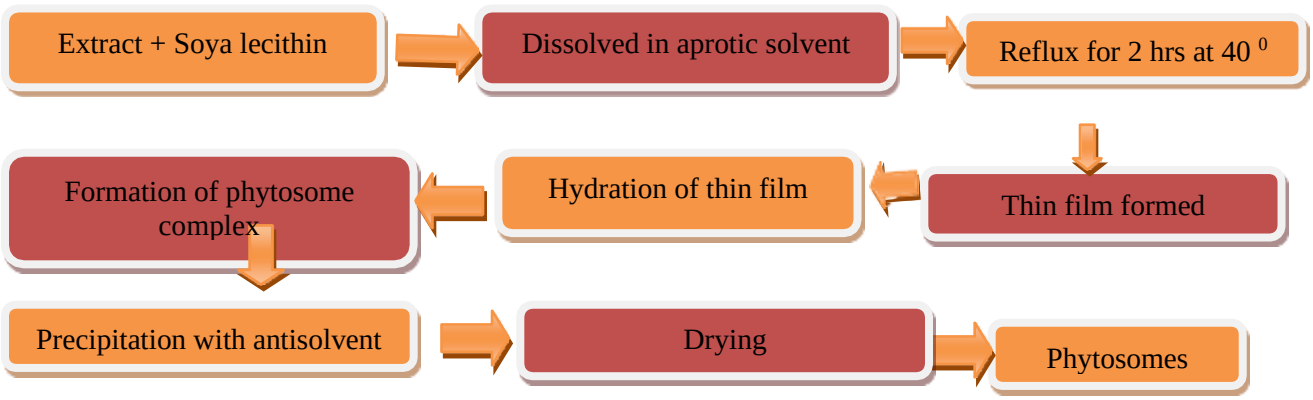

Fig1: graphical presentation of preparation of phytosomes.

Preparation of sunscreen formulation

Total six batches of cream formulation loaded with phytosomes containing combination of extract were prepared. The sunscreen cream formulation was prepared by heating both oil phase and water phase in separate beakers at 75°C on water bath. Aqueous phase was added

drop wise to the oil phase with constant stirring for 15 – 20 minutes until it becomes a homogeneous mixture. Glycerin was added while stirring as a moisturizing agent. To this base cream specified quantity of combination of extract and phytosomes loaded with combination of extract was added at different concentration [10-11]Stirred the cream formulations at 3000 rpm using high speed stirrer to form homogenous cream. The cream was stored in tightly closed containers.

Evaluation of sunscreen formulation

1. **Organoleptic evaluation**

   All the trial sunscreen cream formulations were evaluated with visual observation for the color, odor, texture and appearance. The outcomes of the test are given table 2.

2. **PH Test**

   PH of all cream formulation was measured with the help of previously calibrated digital PH meter.

3. **Spreadability**

   All the prepared cream formulation were evaluated for spreadability. It wasdetermined in terms of time (sec) taken by two glass slides to slip off the cream formulation placed in between the glass slides of uniform thickness under the direction of the heavy weight kept over the slides. The spreadability was calculated by using the formula:

$$S = \frac{M \times L}{T}$$

4. **Viscosity**

   Viscosity of sunscreen cream formulations was determined by using Brookfield viscometer at 25°C temperature.

5. **Phase Separation Test**

   Cream formulations were transferred to container and closed with tight leads. Containers containing respective cream formulations were maintained at 25 – 100 °C temperature in dark condition. Phase separation was monitored for 24 hours. Formulations were kept for 30 days to observe any phase separation during one month period.

6. **Drug content**

   Drug content of all formulations were analyzed by the supernatant solution diluted with mobile phase and analyzed by UV spectroscopy 730nm.

7. **Stability study**

   According to ICH guideline stability study was performed. Optimized batch PCF5 and MCF5 was kept at different temperature 25^0c ± 2 ^{0}c/65% RH ± 5% RH, 40^0c ± 2 ^{0}c/75% RH± 5% RHand 4^0c ± 2 ^{0}c for three month. After three month physicochemical and therapeutic parameter was evaluated.

Therapeutic Evaluation

In-Vitro SPF Determination by UV spectrophotometer

Photoprotective effect of optimized phytosomal cream formulation was determined using Shimadzu UV-Spectrophotometer. Stock solution was prepared by taking 1% w/v of optimized sunscreen cream formulation containing phytophospholipid complex (PCF5), and extract cream formulation(ECF) in ethanol. Then dilution was made, 0.1% stock solution was prepared, and absorbance of each aliquot prepared were determined from 290-320 nm, at 5 nm intervals, taking ethanol as blank, determinations were made in triplicate at each point. SPF of sunscreen formulation was calculated by the following equation:

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

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).

Skin Irritation Study

Skin irritation study of optimized phytosomal cream formulation (PCF5),extract cream formulation (ECF) and base cream was conducted on Albino wistar rats. As per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) regulations the animals were maintained and study was performed after due permission by IAEC (Institutional Animal Ethics Committee) permission letter no.650/PO/RE/S-2002/2022/CPCSEA/34.Primary skin irritation study result was compared with control. Cream formulation was applied for 7 days and daily observed for any sign of irritation, inflammation, redness or edema.

Result and discussion

Table:1 Preparation of phytosomal and extract cream formulation

Sr. No	Ingredients	PCF1 (1%)	PCF2 (2%)	PCF3 (3%)	PCF4 (4%)	PCF5 (5%)	ECF
1	Castor oil (Oil Phase)	2 %	2 %	2 %	2%	2%	2%
2	Combination of extract	-	-	-	-	-	5%
3	Phytosomes	1 %	2 %	3 %	4 %	5 %	-
4	Stearic acid	25 %	25 %	25 %	25 %	25 %	25 %
5	Cetyl alcohol	15 %	15 %	15 %	15 %	15 %	15 %
6	Glycerine	10 %	10 %	10 %	10 %	10 %	10 %
7	Methyl paraben	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
8	Distilled water	25%	25%	25%	25%	25%	25%
9	Rose oil	0.5 %	0.5 %	0.5 %	0.5 %	0.5 %	0.5 %
10	Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s

Table:2 Result of pharmaceutical evaluation.

Parameters	**PF1**	**PF2**	**PF3**	**PF4**	**PF5**	**ECF**
Color	Faint brownish	Faint brownish	Brownish	Brownish	Brownish	Faint creamish
Odor	Pleasant	Pleasant	pleasant	pleasant	Pleasant	Pleasant
Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Appearance	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid
P^H	5.9	6.2	6.7	6.6	6.5	6.4
Spreadability **(g. cm/sec)**	23.11	21.09	12.35	23.33	23.10	22.78
Viscosity	22344	22032	21870	21510	22612	22877
Phase separation	No phase separation	No phase separation	No phase separation	No phase separation	No phase separation	No phase separation
Skin irritancy	Nil	Nil	Nil	Nil	Nil	Nil
Drug content	91.27	92.10	92.22	93.30	94.27	93.89
Stability	Stable	Stable	Stable	Stable	Stable	Stable

Table:3 Skin irritation study of sunscreen cream formulation.

Formulation	Erythema	Edema	Primary irritation index
Control	0.00±0.00	0.00±0.00	0.00±0.00
PCF5	0.00±0.00	0.00±0.00	0.00±0.00
ECF	0.00±0.00	0.00±0.00	0.00±0.00
Base	0.00±0.00	0.00±0.00	0.00±0.00

All the values are expressed as mean ±SD, n=6

SPF determination by UV spectrophotometer

Table: SPF value of optimized cream formulation PCF5 &ECF.

	PCF5			ECF		
Wavelength (nm)	EE (λ) × I (λ)	Abs (λ)	EE (λ) × I (λ)× Abs (λ)	EE (λ) × I (λ)	Abs (λ)	EE (λ) × I (λ)× Abs (λ)
290	0.015	1.257	0.018855	0.015	1.599	0.023985
295	0.0817	4.000	0.3268	0.0817	1.897	0.154985
300	0.2874	4.000	1.1496	0.2874	1.945	0.558993
305	0.3278	3.409	1.1174702	0.3278	1.889	0.619214
310	0.1864	2.991	0.5575224	0.1864	2.695	0.502348
315	0.0837	3.304	0.2765448	0.0837	2.468	0.206572
320	0.018	2.724	0.049032	0.018	2.74	0.04932
	Total = 1		Total = 3.495			Total= 2.115
			SPF= 3.495 × correction factor (10) =34.95			SPF=2.11 × correction factor (10)= 21.15

Conclusion

Optimized batch of phytophospholipid complex was incorporated in sunscreen cream formulation in different concentration. Pharamaceutical evaluation result of all the prepared phytosomal cream formulation (PCF1, PCF2, PCF3, PCF4, PCF5 and ECF) showed that pharmaceutical parameters of all the prepared cream formulation was found within the required range. Drug content of PCF1, PCF2, PCF3, PCF4, PCF5 & ECF was found 91.27%,92.10%,92.22%, 93.30%,94.27% and 93.89% respectively. Based on drug content, cream formulation batch PCF5 was selected as optimized batch and use for further therapeutic evaluation. Skin irritation study showed that optimized cream formulation was safe and effective. Invitro SPF result showed that novel phytosomal sunscreen formulation (PCF5) showed excellent photoprotective effect.

Conflict of Interest: No conflict of interest.

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