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DEVELOPING AND ASSESSING A TOPICAL DRUG DELIVERY SYSTEM INCORPORATING PHYTOCONSTITUENT FOR THE TREATMENT OF CONTACT DERMATITIS

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ABSTRACT

Objective: The objective of this study is to optimize a cream base and design a topical dosage form containing phytoconstituent for treating contact dermatitis, specifically focusing on its potential as an alternative for managing contact dermatitis. **Method:** A base cream was formulated by fusion method with varying concentration of cetyl alcohol, stearic acid, propylene glycol, triethanolamine and glycerine. Organoleptic and physicochemical properties were evaluated. The selected cream (CB1) was used for loading resveratrol and evaluated for physical characteristics, stability, drug content, ex-vivo diffusion study, skin retention, and microbial examination. **Result:** Controlled release was observed over 24 hours, with less than 1% release in buffer after 360 minutes. High drug retention in skin was noted for all formulations. Cream formulations remained stable after one month of storage. **Conclusion:** Resveratrol-loaded creams show promise as topical drug delivery system for treating contact dermatitis. Further in vivo studies are warranted to validate these findings.

KEYWORDS: Contact Dermatitis (CD), Creams, Optimization, Resveratrol, Topical delivery.

1. INTRODUCTION

Contact Dermatitis is delayed hypersensitivity reaction causing inflammation, erythema dryness, and vasiculation. It's localized to skin area in contact with allergens, spreading from hands to distant sites. Over 4000 chemical substances can trigger CD, resulting in Type I and

Type IV reactions.[1,2] Contact urticaria and dermatitis are common manifestations, with some cases being photoallergic.[3] Contact dermatitis involves two phases: sensitization and elicitation. During sensitization, an allergen penetrates the skin, forming a complex with endogenous proteins, activating the innate immune system, and priming T cells, primarily TH1 type. In the elicitation phase, keratinocytes release cytokines, leading to activation of the innate immune system. Antigen-specific T cells proliferate, enter the systemic circulation, and induce inflammation upon exposure to allergens.[4,5] Primary management of CD involves allergen education and avoidance. Topical treatments include Polidocanol, but can cause itching and burning.[6] Other options include skin protectants and chelator creams for specific allergens. Class II and III topical corticosteroids are recommended for inflammation, but long-term use may cause skin atrophy.[7,8] Phytoconstituents from Artemisia *vestita*, *Panax ginseng, Scutellaria baicalensis* and *Sophora flavescens* are used in traditional system of medicines for CD treatment.[9] Resveratrol, found in grapes, peanuts, and mulberries, is a polyphenol with anti-inflammatory properties.[10] It inhibits cytokine release, halting T cell activation and inflammation, making it a potential treatment for CD.[11] Topical drug delivery for CD offers benefits like localized treatment, enhanced bioavailability, immediate cessation of treatment for adverse effects, and improved patient compliance.[12] In the proposed research, a cream containing resveratrol will be formulated and investigated for its potential in managing contact dermatitis.

2. MATERIALS AND METHOD

All the material used in this study were of analytical grade and procured from following source as shown in tables below.

Table 1: List of materials.

S. No	Name of chemical	Manufacturer/ supplier
1.	Resveratrol	Yucca Enterprises Mumbai, India
2.	Cetyl alcohol	CDH Fine Chemical, New Delhi.
3.	Stearic acid	CDH Fine Chemical, New Delhi.
4.	Triethanolamine	CDH Fine Chemical, New Delhi.
5.	Methyl paraben	CDH Fine Chemical, New Delhi.
6.	Isopropyl alcohol	Qualikems Fine Chemical Ltd, New Delhi
7.	n-octanol	Qualikems Fine Chemical Ltd, New Delhi
8.	Cyclohexane	Qualikems Fine Chemical Ltd, New Delhi
9.	Propylene glycol	Qualikems Fine Chemical Ltd, New Delhi
10.	Glycerin	TITAN Biotech Ltd. Rajasthan
11.	Phenolphthalein	CDH Fine Chemical, New Delhi

Table 2: List of Equipments.

S. No	Equipment	Manufacturer
1.	Weighing balance	Wensar, Magnetic Analytical Balance
2.	Melting point apparatus	Digital melting point apparatus, Rolex, Ambala, India
3.	UV-Visible Spectrophotometer	LABINDIA UV 3000^{+}, Mumbai, India
4.	FTIR photometer	IR-Affinity-I, SHIMADZU, Japan
5.	Hot air oven	Universal Hot Air Oven, Rolex, India
6.	Heating mantle	ROLEX
7.	IR Presss	(Model M 15), Technosearch Instruments
8.	Brookfield Viscometer	R/S Plus
9.	Ultraviolet cabinet	Rolex
10.	Digital pH meter	Deluxe pH meter, Rolex, Ambala, India
11.	Digital water bath shaker	Rolex, Ambala, India
12.	Stability chamber	Thermolab Scientific Equipment Pvt. Ltd, Mumbai, India.

2.1 Preformulation studies

2.1.1 Organoleptic properties: The resveratrol was characterized for organoleptic properties i.e color and odor and compared with monograph in official book**.**

2.1.2 Determination of melting point: Melting point of resveratrol was determined by using digital melting point apparatus**.**

2.1.3 Determination of absorption maxima (λ_{max}) and preparation of calibration curve

The absorption maxima (λ_{max}) of Resveratrol in ethanol and pH 6.8 Phosphate buffer were determined at concentrations 0.5 µg/ml and 1 µg/ml, respectively, using a UV-Visible spectrophotometer (LABINDIA 3000^{+})**.** A standard curve ranging from 1µg/ml to 5µg/ml was prepared in ethanol, and absorbance was measured at 307 nm.[13,14]

2.1.4 Determination of Partition coefficient: The partition coefficient of resveratrol was determined using shake-flask method with n-octanol and pH 6.8 phosphate buffer. Equal volumes (10 ml each) were mixed in a separating funnel and shaken for one hour to reach equilibrium. Resveratrol (10 mg) was added and the mixture shaken for four hours, with intervals of 15 minutes. Then layers were separated, and the concentration in aqueous layer was measured using UV-visible spectrophotometer after filtration and appropriate dilutions. The organic layer (2 ml) was evaporated; the residue dissolved in 10 ml of pH 6.8 phosphate buffer, filtered and measured similarly.[15,16]

$$\text{Partition coefficient (P)} = \frac{\text{Concentration of drug in organic layer}}{\text{Concentration of drug in aqueous layer}}$$

2.1.5 Determination of drug solubility: The solubility of resveratrol was determined in water, methanol, ethanol and pH 6.8 Phosphate buffer. Incremental amounts of the drug were added to 10 ml of each solvent until saturation was reached. The solutions were equilibrated at 32 ± 0.5ºC for 72 hours, filtered using whatmann filter paper, and the absorbance of the filtrate was measured at 307 nm using a UV-Visible spectrophotometer.[17]

2.1.6 Fourier transformed infrared (FTIR) studies FTIR spectrum of pure drug was recorded by potassium bromide (KBr) press pellet method. Pure drug resveratrol was uniformly mixed with dry powdered KBr in the ratio of 1:100 and mixture was compressed into transparent disc under high pressure using special dies. The disc was placed in FTIR photometer (Shimadzu IR Affinity-1) and spectrum was recorded.[18,19]

2.1.7 Drug-excipients compatibility Study: Compatibility screening between resveratrol and excipients (cetyl alcohol, Stearic acid, methyl paraben, propyl paraben, glycerin, triethanolamine and propylene glycol) was assessed by comparing their FTIR spectra with that of pure resveratrol.

2.1.8 Thin layer chromatography (TLC): In thin layer chromatography (TLC), resveratrol was dissolved in methanol and spotted on a TLC plate with a mobile phase of ethyl acetate, cyclohexane, and n-butanol (9+9+2% v/v). The plate was developed until the solvent reached 3/4th height, then removed, and observed under UV light for detection. Rf value was calculated using formula.[20]

Rf = Distance travel by solute/ Distance travel by solvent.

2.2 Formulation and evaluation of cream base

2.2.1 Preliminary formulation of cream base: The base cream (25 g) was formulated by fusion followed by trituration method. The preliminary compositions of the cream base are shown in Table No. 3 and Table No. 4. The oil phase was prepared by melting the emulsifying agent and stiffening agent i.e., cetyl alcohol and stearic acid, respectively at 65°C in a china dish kept on heating mantle. The aqueous phase containing stabilizing agent, humectant, pH stabilizer i.e., propylene glycol, glycerin and triethanolamine, respectively was also maintained at 65°C. Methyl paraben and propyl paraben in the ratio of 1:0.5 was added into aqueous phase as preservative. The oil phase was mixed with aqueous phase with constant stirring followed by the trituration of mass in a mortar pestle to get a smooth cream. In the first stage, formulations C1 to C14 were formulated, to check the effect of variation in

the concentration of cetyl alcohol and stearic acid, respectively by evaluating organoleptic and physicochemical properties of creams. In the second stage, formulations F1 to F4 were formulated, to check the variation in the concentration of stabilizer and humectant i.e., propylene glycol and glycerin respectively by evaluating organoleptic and physicochemical properties of creams. In the third stage, formulations F5 to F7 were formulated, to check the variation in the concentration of triethanolamine as pH stabilizer by evaluating organoleptic and physicochemical properties of creams.[21]

Table 3: Formula for preliminary optimization of concentration of cetyl alcohol and stearic acid in cream base.

S. No	Ingredients (%w/w)	Formulations													
		C 1	**C 2**	**C 3**	**C 4**	**C 5**	**C 6**	**C 7**	**C 8**	**C 9**	**C 10**	**C 11**	**C 12**	**C 13**	**C 14**
1	**Cetyl alcohol**	1	1	1	1	2	2	2	2	3	3	3	3	4	5
2	**Stearic acid**	2.5	5.0	7.5	10	2.5	5.0	7.5	10	2.5	5.0	7.5	10	10	10
3	**Propylene glycol**	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4	**Glycerin**	5	5	5	5	5	5	5	5	5	5	5	5	5	5
5	**Methyl paraben**	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
6	**Propyl paraben**	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
7	**Triethanolamine**	2	2	2	2	2	2	2	2	2	2	2	2	2	2
8	**Double distilled water**	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100	Upto 100

2.2.2 Preliminary evaluation of cream base: The preliminary evaluation of cream base included assessing physical appearance, grittiness, thermal stability, and pH. Physical appearance was examined for color, homogeneity, smoothness and consistency. Grittiness was checked by spreading a small amount of cream between glass slides. pH of the cream was determined by dissolving a 5g±0.1g in 45ml distilled water at 45°C, stirring for 15 minutes, filtering, and measuring pH at 27°C.

2.2.2.1 Spreadability: Spreadability was assessed using a concocted instrument with glass plates. A weight of 50g was applied to the upper plate, and time taken for the plates to slide a designated distance of 7cm was recorded. Spreadibility was calculated using formula

$$S = M \times L/T$$

Where, S is the spreadability, M is the weight (g), L is the length (cm) and T is the time taken (s) for the plates to slide the entire length**.**

2.2.2.2 Thermal Stability: The cream formulations were spread on the internal wall of a 100 ml beaker and placed in stability chamber shown in Fig. **1** at 60-70 % RH and 45 ± 1°C for 48 hours. After this period, formulations were inspected for any separation of the oil phase**.**

Fig. 1: Samples in Stability chamber for thermal stability.

Table 4: Formula for optimization of concentration of propylene glycol, glycerin and triethanolamine in cream base.

S.no	Ingredients (%w/w)	Formulations						
		F1	F2	F3	F4	F5	F6	F7
1	Cetyl alcohol	3	3	3	3	3	3	3
2	Stearic acid	10	10	10	10	10	10	10
3	Propylene glycol	-	2.5	7.5	10	5	5	5
4	Glycerin	10	7.5	2.5	-	5	5	5
5	Methyl paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1
6	Propyl paraben	0.05	0.05	0.05	0.05	0.05	0.05	0.05
7	Triethanolamine	2	2	2	2	1	1.5	2.5
8	Double distilled water	upto 100	upto 100	upto 100	upto 100	upto 100	upto 100	upto 100

2.2.2.3 Viscosity: Cream base formulation was evaluated for viscosity using Brookfield rheometer (R/S Plus) shown in Fig. **2** One gram sample was taken, placed on the plate at maintained temperature (25 ± 1° C) and the cone was rotated at 10 rpm.

Fig. 2: Brookfield rheometer.

2.3 Investigation for final optimization of cream base formula: In the investigation for final optimization of cream base formula, cream bases were formulated based on narrowed concentrations of cetyl alcohol and stearic acid shown in Table No. **5**. These cream bases were then evaluated for physical appearance, grittiness, thermal stability, pH, spreadibility, viscosity, acid value, Saponification value, total fatty content and for total residue content. Evaluation methods were as outlined under sub section 2.2.2.

2.3.1 Determination of total fatty content: A 2 gram sample was diluted with 25 ml of hydrochloric acid in a round bottom flask and refluxed until clear. The solution was transferred into a 300 ml separating funnel, rinsed with 50 ml of ethyl ether, shaken and left to separate into aqueous and non-aqueous layers. The aqueous layer was extracted twice more with ethyl ether. The combined layers were washed with water until acid-free (methyl orange indicator), filtered over dried sodium sulphate, and then ether layer was dried at 60°C ± 2°C to constant mass.

Fatty acid (%) = 100× M1/M2

Where, 'M1'is mass obtained from residue, 'M2'is total mass.

2.3.2 Determination of residue: A five-gram of formulation was accurately weighed and placed into a clean, dry squat form weighing bottle. It was dried to a constant mass at 105±1°C, then cooled in a desiccator and weighed again.

Residue (percent by mass) = $100(M_1/M_2)$

Where, **'**M_1' mass in gram of the residue, 'M_2' mass in gram of the material taken for test.

Table 5: Formula for optimization of cetyl alcohol and stearic acid in cream base.

S.no	Ingredients (%w/w)	Formulations								
		CB1	CB2	CB3	CB4	C12	CB5	CB6	CB7	CB8
1	Cetyl alcohol	2.5	3	3.5	2.5	3	3.5	2.5	3	3.5
2	Stearic acid	9	9	9	10	10	10	11	11	11
3	Propylene glycol	5	5	5	5	5	5	5	5	5
4	Glycerin	5	5	5	5	5	5	5	5	5
5	Methyl paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
6	Propyl paraben	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
7	Triethanolamine	2	2	2	2	2	2	2	2	2
8	Double distilled water	upto 100	upto 100	upto 100	upto 100	upto 100	upto 100	upto 100	upto 100	upto 100

2.3.3 Determination of acid value: A 50 ml mixture of equal volume of ether and alcohol was previously neutralized with 0.1N NaOH. Ten gram of formulation was accurately weighed and dissolved in mixture. One or two drops of phenolphthalein indicator were added, and the solution was titrated with 0.1N NaOH until a faint pink colour persisted for 30 seconds after shaking.

$$\text{Acid value} = n \times 5.61/w$$

Where, ‘n’ is number of ml of 0.1 N sodium hydroxide required, ‘w’ is Weight in gram of the formulation.

2.3.4 Determination of saponification value: Two gram formulation was placed in a 200 ml round bottom flask fitted with reflux condenser. Twenty-five ml of 0.5 N alcoholic KOH was added and the mixture was refluxed on a water bath for 30 minutes, with frequent rotation. After refluxing, the flask was cooled, and 1-2 drops of phenolphthalein indicator were added. The solution was titrated with 0.5 N HCl, and the volume of titrant used was noted as (a). A blank titration was performed with the sample, and the volume of titrant used was noted as (b).

$$\text{Saponification value} = (b-a) \times 28.5/w$$

Where, ‘w’ is weight in gram of the formulation.

2.4 Formulation and evaluation of resveratrol loaded cream

2.4.1 Formulation of resveratrol loaded creams: In the formulation of resveratrol-loaded creams, an optimized cream base was chosen as shown in Table No. **6**, and different doses of resveratrol were added to aqueous phase uniformly dispersed in propylene glycol. Creams (60 g) were formulated following method reported in section 2, under subsection 2.2.1.

Evaluation of resveratrol loaded creams included assessing physical appearance, grittiness, thermal stability, pH, spreadibility, viscosity, total residue content, total fatty content, type of cream, drug content, ex-vivo diffusion study, skin retention study, microbial examination and stability study. Evaluation methods were as outlined in section 2, under subsection 2.2.2.

2.4.1.1 Type of cream: Dye test was used to estimate the type of cream. A water soluble dye (amaranth) was mixed with a proportion of cream and visualize under the microscope.[22,23]

2.4.1.2 Drug content: For drug content analysis, a 1g sample was dissolved in ethanol in a 100 ml volumetric flask. The solution was filtered and absorbance was measured using a UV-Visible spectrophotometer at λ_{max} 307 nm against a similarly treated blank.

2.4.1.3 Ex - vivo diffusion study using goat skin: Ex-vivo permeation studies of formulation were conducted using Franz diffusion cell assemblies with goat skin. The shaven skin was washed with normal saline, mounted on the diffusion cell assembly, with the stratum corneum facing the donor compartment. Formulation equivalent to 1 mg of drug was placed on the skin in the donor compartment, while the receptor compartment contained 30ml of pH 6.8 phosphate buffer at 37°C. Samples were withdrawn at suitable time interval and replaced with fresh diffusion medium. Cumulative permeated resveratrol through goat skin was measured over time.

2.4.1.4 Skin retention study: At the end of the in ex-vivo diffusion study, the remaining formulation on the epidermal layer was scrapped off and dilute in 100 ml ethanol. Incisions were made on the remaining skin, which was then chopped and added to 100 ml ethanol. After 24 hrs, both solutions were filtered and analyzed for the amount of drug retained in skin and cream using UV spectrophotometer.[24,25]

Table 6: Formulation of resveratrol loaded cream.

S.no	Ingredients (%w/w)	RB1	RB2	RB3	RB4
1	**Resveratrol**	0.05	0.075	0.1	0.125
2	**Cetyl alcohol**	2.5	2.5	2.5	2.5
3	**Stearic acid**	9	9	9	9
4	**Glycerin**	5	5	5	5
5	**Propylene glycol**	5	5	5	5
6	**Triethanolamine**	2	2	2	2
7	**Methyl paraben**	0.1	0.1	0.1	0.1
8	**Propyl paraben**	0.05	0.05	0.05	0.05
9	**Double Distilled water**	upto 100	upto 100	upto 100	upto 100

2.4.1.5 Microbiological Evaluation: Microbial examination of prepared formulations was performed in accordance with Indian Standards methods specified in IS 11648; 1999. Total viable counts, including bacteria, yeast, and mould, were recorded using colony counter. Bacterial counts were assessed with soyabean casein digest agar, while mould and yeast counts were estimated using peptone agar medium as per Indian Pharmacopoeia. The formulations were diluted 1: 10 in sterile broth with appropriate neutralizers. Then, 1 ml of each diluted sample was plated onto soyabean casein digest agar for bacterial growth and onto sabouraude dextrose agar for fungal, yeast, and mold growth. The plates were incubated at 20-35 °C for 48 hours for 5 days for fungal, yeast, and mould growth.[26]

2.4.1.6 Stability study: The prepared cream formulation were filled in the collapsible tubes and stored at ambient temperature (30°C & 40°C). Cream formulations were evaluated for phase separation, physical appearance and drug stability after 1 month.[22]

3. RESULT AND DISCUSSION

3.1 Preformulation study

Resveratrol was identified as an off-white powder possessing a distinctive odor**.** Melting point was determined to be 253°C, matching the reported literature range of 253-255°C, which indicates the compound's purity. The absorption maximum of resveratrol was determined to be 307 nm in both ethanol and pH 6.8 phosphate buffer (Fig. **3**, **4**). The calibration curve data and plot for resveratrol in ethanol is presented in Table No. **7** and Fig. **5**, respectively.[13]

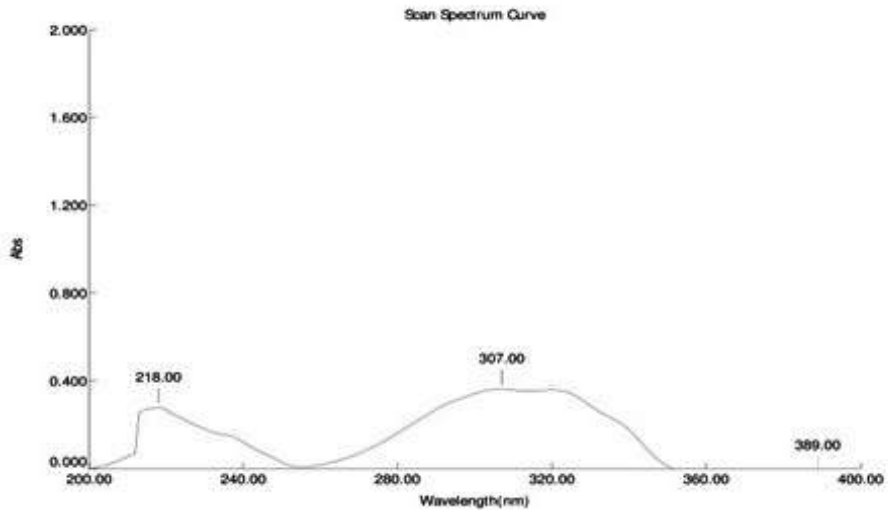


Fig. 3: UV spectra of Resveratrol in ethanol.

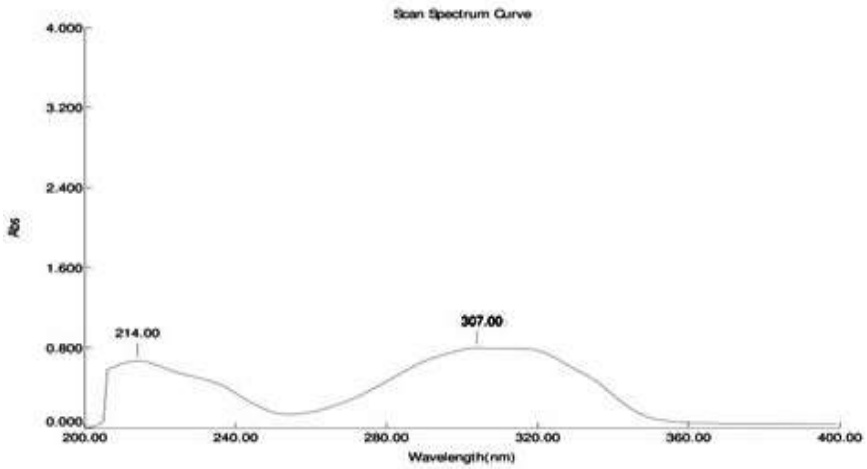


Fig. 4: UV spectra of resveratrol in pH 6.8 phosphate buffer.

Table 7: Calibration curve data of resveratrol in ethanol at 307 nm.

Sr. No.	Concentration (µg/ml)	Absorbance ± SD
1	0.5	0.131 ± 0.007
2	1.0	0.259 ± 0.001
3	1.5	0.324 ± 0.005
4	2.0	0.398 ± 0.007
5	2.5	0.491 ± 0.008
6	3.0	0.587 ± 0.005
7	3.5	0.663 ± 0.005
8	4.0	0.749 ± 0.006
9	4.5	0.826 ± 0.005
10	5.0	0.886 ± 0.007

Mean ± SD; (n=6).

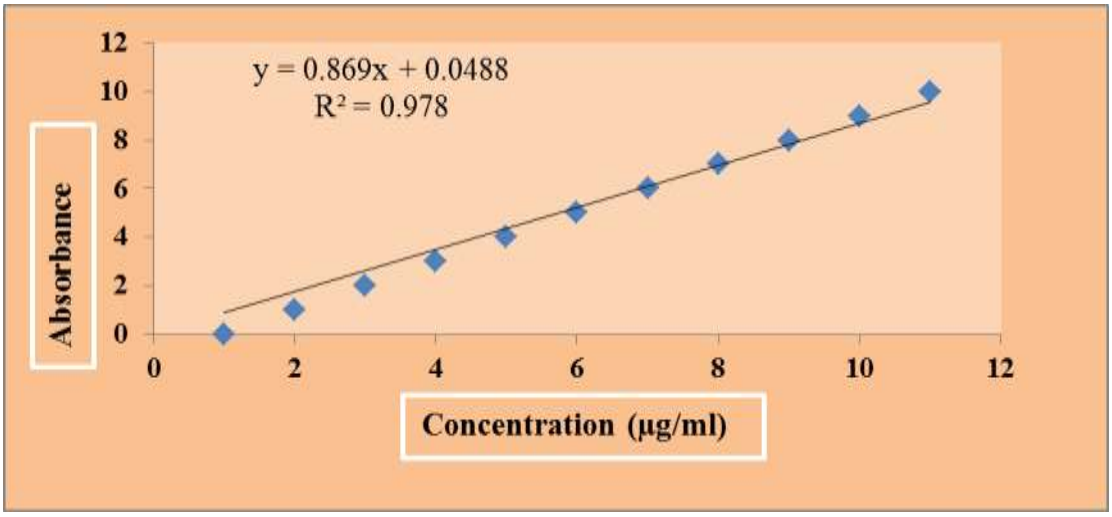


Fig. 5: Calibration curve of Resveratrol in ethanol at 307 nm.

Partition coefficient of resveratrol was found to be 3.01 ±0.512 indicating lipophilic nature of drug. The reported log P value of resveratrol was 3.06 [15, 16]. It was found to be freely soluble in ethanol and methanol but practically insoluble and only very slightly soluble in distilled water and pH 6.8 phosphate buffer are detailed in Table No. 8.[17]

Table 8: Solubility profile of resveratrol in different solvents.

Solvents	Conc. (mg/ml) ± SD	Solubility
Ethanol	85.92 ± 0.002	Soluble
Methanol	75.45 ± 0.005	Soluble
Distilled Water	0.05 ± 0.005	Practically insoluble
pH 6.8 phosphate buffer	0.31 ± 0.001	Very slightly soluble

Mean ± SD; (n=3)

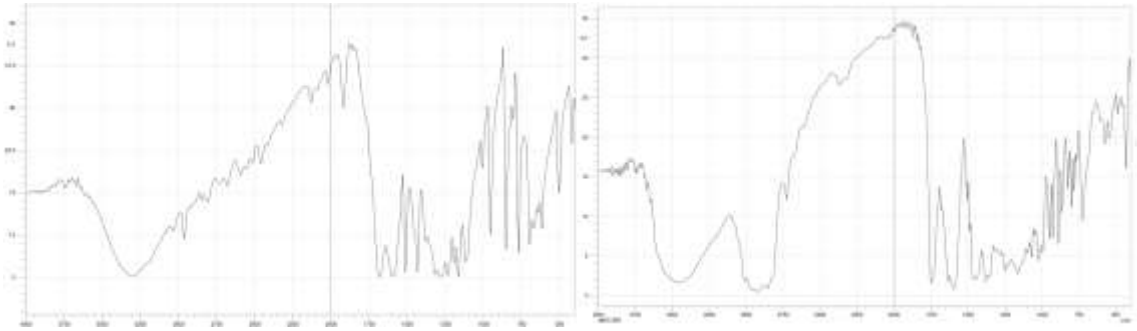
The FTIR spectrum of resveratrol is shown in Fig. **6a**. The peak positions for important functional groups are detailed in Table No. **9**. The detailed study of peak position for different functional groups in comparison with standard indicated the purity of the resveratrol. Compatibility study of resveratrol with the excipients used in the formulation of cream was studied by FTIR spectroscopy. All the major peaks of resveratrol were examined in FTIR spectrum with different excipients is shown in Fig. **6b**. There was no significant variation in peak positions of resveratrol was observed are detailed in Table No. **10**, indicating the compatibility of drugs with excipients used in the formulation of cream.[18,19]

Table 9: Peak position of resveratrol.

Functional groups	Standard values (cm^{-1})	Observed values (cm^{-1})
Phenolic -OH group stretching	3294.19	3286.4
Aromatic C-H group stretching	3016.46	3025.2
Aliphatic C=C	1589.2	1582.1
Aromatic C=C	1149.50	1134.5

Table 10: Peak positions of resveratrol in physical mixture.

Functional groups	Standard values (cm^{-1})	Observed values (cm^{-1})
Phenolic -OH group stretching	3294.19	3288.4
Aromatic C-H group stretching	3016.46	3024.6.
Aliphatic C=C	1589.2	1589.1
Aromatic C=C	1149.50	1142.5



(a) **(b)**

Fig. 6: FTIR spectra (a) Resveratrol (b) Physical mixture of Resveratrol and excipients.

The R_f value of the resveratrol was found to be 0.75 ± 0.00 is shown in Fig. **7**. The previously reported R_f value of resveratrol is 0.78. It indicated the purity of phytoconstituent.[20]

Fig. 7: TLC chromatogram Resveratrol.

Fig. 8: Cream base (C12).

3.2 Preliminary investigation for cream base

The results of preliminary investigation of cream bases are shown in Table No. **11**. The consistency of formulation C1 to C9 was not good. Formulations showed flowable consistency. The consistency of the formulations C10 to C12 was good. The color of C10 and C11 was dull white. The color of C12 was pearlescent white and was having more pleasant appearance as compared to C10 and C11 is shown in Fig. **8**. In the formulations C10, C11

and C12, no grittiness or presence of foreign particles was observed. The C10 was found to thermally unstable. The pH of formulation C12 was more appropriate for the treatment of skin in CD. In the first stage of preliminary evaluation, composition C12 (cetyl alcohol and stearic acid 3%w/w and 10%w/w, respectively) was considered optimum for cream base.

Table 11: Preliminary evaluation parameters of cream bases.

Formulations	Evaluation Parameters						
	Consistency	Appearance	Grittiness	Thermal stability	pH	Spreadibility (g.cm/sec)	Viscosity (cps)
C1	Not good	-	-	-	-	-	-
C2	Not good	-	-	-	-	-	-
C3	Not good	-	-	-	-	-	-
C4	Not good	-	-	-	-	-	-
C5	Not good	-	-	-	-	-	-
C6	Not good	-	-	-	-	-	-
C7	Not good	-	-	-	-	-	-
C8	Not good	-	-	-	-	-	-
C9	Not good	-	-	-	-	-	-
C10	Good	Dull White	-ve	Not Stable	-	-	-
C11	Good	Dull White	-ve	Stable	6.1 ± 0.384	10.38 ± 1.842	2783.7 ± 80
C12	Good	Pearlescent white	-ve	Stable	6.6 ± 0.153	11.79 ± 1.364	2938.2 ± 32
C13	Good	Pearlescent white	-ve	Stable	6.7 ± 0.294	20.37 ± 2.307	6170.5 ± 57
C14	Good	Pearlescent white	-ve	Stable	6.6 ± 0.517	25.03 ± 2.197	6481.3 ± 64
Marketed	-	-	-	-		10.96 ± 1.037	2834.4 ± 39

Mean ± SD (n=3), No grittiness (-ve).

The results of variation in the concentration of propylene glycol, glycerin and triethanolamine in comparison to C12 are shown in Table No. **12**. The creams were not formed when either glycerin or propylene glycol were used alone. The formulation F2 and F3 were thermally unstable. The results indicated that the physicochemical properties of base cream i.e color, consistency, pH and thermal stability were more acceptable in ACD, when propylene glycol and glycerin were used in equal concentration. The variation in the concentration of triethanolamine (F5-F7) in comparison to C12 indicated that physicochemical properties of base cream were more acceptable with 2%w/w concentration.

Table 12: Preliminary evaluation parameters of cream bases.

Formulations	Evaluation Parameters						
	Consistency	**Appearance**	**Grittiness**	**Thermal stability**	**pH**	**Spreadibility (g.cm/sec)**	**Viscosity (cps)**
F1	Not good	-	-	-	-	-	-
F2	Good	Pearlescent white	-ve	Unstable	6.6 ± 0.387	8.36 ± 1.394	2534.6 ± 76
F3	Good	Pearlescent white	-ve	Unstable	6.5 ± 0.126	8.57 ± 1.927	2597.1 ± 91
F4	Not good	-	-	-	-	-	-
F5	Not good	-	-	-	-	-	-
F6	Good	Pearlescent white	-ve	Unstable	-	-	-
F7	Good	Pearlescent white	-ve	Stable	7.3 ± 0.294	12.67± 1.964	3641.6 ± 67
Marketed	-	-	-	-	6.5 ± 0.314	10.96 ± 1.037	2834.4 ± 34

Mean ± SD (n=3), No grittiness (-ve)

3.3 Investigation for final optimization of cream base formula

The results of the final optimization and selection of cream base formula are shown in Table No. **13**. The color of all the formulations was pearlescent white, had the desired consistency for the creams, and was stable. Increasing the concentration of cetyl alcohol and stearic acid led to higher viscosity and spreadibility of the formulations. Key chemical parameters critical for skin creams, including included pH (4 -9), acid value (in the range of 3.5-6.5), saponification value, total fatty content (minimum 5% by mass of cream sample) and total residue content (minimum 10% by mass), were all within acceptable ranges across all formulations. The skin's natural barrier, known as the acid mantle, has an acidic pH. However, frequent washing and soap use can disrupt this acidity. Consequently, creams intended to normalize the skin should ideally have an acidic range pH range. The acid value, which indicates the free fatty acid content and potential skin irritation of a skin cream, was within the acceptable range of formulations CB1 to CB3. This suggests that these formulations may be non-irritating to the skin. The saponification values of the formulations reflect the presence of free ester, which can influence the stability and pH of the formula. In stable cream formulations, low saponification value is desirable.

All cream base formulation met the acceptable range for total fatty content and residue content as per the BIS 2004 guidelines for skin creams. After considering all physicochemical parameters, CB1 was chosen for further loading of resveratrol.

Table 13: Evaluation parameters for final optimization and selection of cream base formula.

Parameters	Formulation Code									
	CB1	CB2	CB3	CB4	C12	CB5	CB6	CB7	CB8	Marketed
Appearance	Pearlescent white	Pearlescent white	Pearlescent white	Pearlescent white	Pearlescent white	Pearlescent white	Pearlescent white	Pearlescent white	Pearlescent white	-
Consistency	Good	Good	Good	Good	Good	Good	Good	Good	Good	-
Grittiness	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-
Thermal stability	Stable	Stable	Stable	Stable	Stable	Stable	Stable	Stable	Stable	-
pH	5.9 ± 0.153	6.1 ± 0.153	6.4 ± 0.153	6.5 ± 0.153	6.6 ± 0.153	7.2 ± 0.153	7.4 ± 0.153	7.5 ± 0.153	7.7 ± 0.153	
Spreadibility (g.cm/sec)	9.12 ± 0.937	9.59 ± 1.071	10.63 ± 1.617	10.92 ± 1.037	11.79 ± 1.364	11.87 ± 1.914	12.91 ± 1.439	13.09 ± 0.541	15.37 ± 1.319	10.96 ± 1.037
Viscosity (cps)	2512.1 ± 30	2609.7 ± 29	2698.9 ± 30	2842.5 ± 24	2938.2 ± 32	3012.2 ± 28.6	3182.3 ± 31	3210.8 ± 33	3279.1 ± 29.1	2834.4 ± 39
Acid value	5.83 ± 0.128	5.90 ± 0.120	6.12 ± 0.131	6.51 ± 0.218	7.12 ± 0.194	8.19 ± 0.215	16.4 ± 0.202	18.3 ± 0.126	19.4 ± 0.214	-
Saponification value	26.2 ± 1.51	30.3 ± 1.97	35.7 ± 2.1	37.7 ± 1.02	39.3 ± 2.6	43.7 ± 2.13	47.4 ± 1.42	49.1 ± 2.49	53.7 ± 2.17	-
Total fatty content (%)	7.0 ± 3.5	7.9 ± 2.16	11.4 ± 3.01	15.5 ± 2.87	17.2 ± 1.27	20.9 ± 3.64	26.3 ± 2.07	29.4 ± 2.11	32.3 ± 3.23	-
Total residue content (%)	16.7 ± 0.51	17.9 ± 0.79	20.4 ± 0.65	21.3 ± 0.28	22.7 ± 0.45	24.0 ± 0.601	31.1 ± 0.46	33.7 ± 0.29	35.2 ± 0.73	-

Mean ± SD (n=3), No grittiness (-ve)

3.4 Formulation and evaluation of resveratrol loaded cream

The creams formulated were of slightly brown color is shown in Fig. **8**. In identification of type of emulsion formed by dye test, dispersion medium was found to be red in color is shown in Fig. **9**. Hence, it indicates that the cream was of O/W type cream.

The results for the evaluation of physicochemical parameters of resveratrol creams are shown in Table No. **14**. The consistency of all the formulation was good and acceptable. All the formulations were free from grittiness. The pH of the formulations was in the range of 5.8 ± 0.121 to 6.0 ± 0.129. The viscosities of the formulations were in the range of 2501.5 ± 20 to 2519.3 ± 18 cps. The acid value, saponification value, total fatty content and total residue content were in acceptable range for the skin creams. The values indicate that the topical creams might be non-irritant and stable. The drug content of the formulations was in the range of 95.3 ± 0.002 to 96.7 ± 0.001.[21, 22, 23]

Fig. 8: Resveratrol loaded cream.

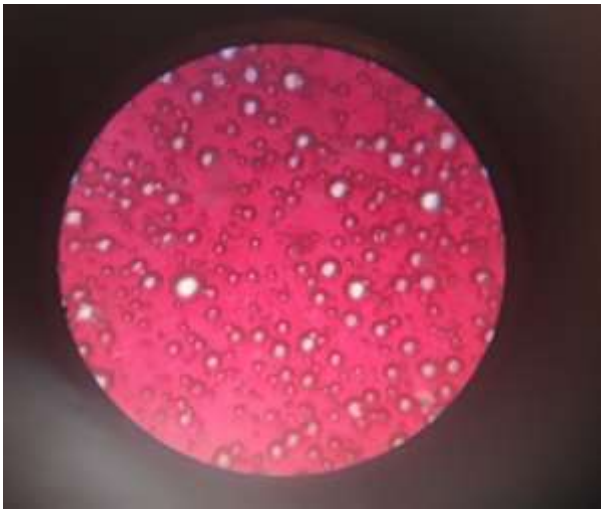


Fig. 9: Cream base under microscopic examination.

Table 14: Evaluation of Resveratrol loaded cream.

Parameters	Formulations			
	RB1	**RB2**	**RB3**	**RB4**
Appearance	Light brown	Light brown	Light brown	Light brown
Consistency	Good	Good	Good	Good
Grittiness	-ve	-ve	-ve	-ve
Thermal stability	Stable	Stable	Stable	Stable
pH	5.9 ± 0.113	5.8 ± 0.132	5.8 ± 0.121	6.0 ± 0.129
Spreadibility (g.cm/sec)	9.12 ± 0.826	9.12 ± 0.937	11.2 ± 0.912	11.2 ± 0.941
Viscosity (cps)	2501.5 ± 20	2519.3 ± 18	2511.2 ± 18.6	2511.7 ± 20
Acid value	5.83 ± 1.23	5.81 ± 2.01	5.84 ± 1.14	5.84 ± 1.06
Saponification value	26.2 ± 1.36	26.8 ± 0.09	26.1 ± 1.00	26.2 ± 1.24
Total fatty content (%)	7.12 ± 0.51	7.01 ± 0.214	7.03 ± 0.293	7.2 ± 0.19
Total residue content (%)	16.2 ± 1.37	16.7 ± 1.31	16.1 ± 1.42	16.5 ± 1.65
Drug content (%)	95.3 ± 0.102	95.9 ± 0.217	96.7 ± 0.111	95.4 ± 0.131

Mean ± SD (n=3)

Table 15: Cumulative percent release of resveratrol from creams.

S.no	Time (min)	%CPR			
		RB1	RB2	RB3	RB4
1	10	0.037 ± 0.002	0.050 ± 0.001	0.055 ± 0.001	0.054 ± 0.007
2	20	0.037 ± 0.005	0.141 ± 0.008	0.107 ± 0.007	0.119 ± 0.006
3	30	0.072 ± 0.002	0.223 ± 0.003	0.182 ± 0.004	0.167 ± 0.001
4	60	0.084 ± 0.001	0.236 ± 0.009	0.21 ± 0.001	0.220 ± 0.003
5	120	0.154 ± 0.005	0.255 ± 0.002	0.301 ± 0.005	0.303 ± 0.008
6	180	0.245 ± 0.001	0.331 ± 0.007	0.346 ± 0.003	0.335 ± 0.002
7	240	0.303 ± 0.007	0.354 ± 0.001	0.412 ± 0.003	0.398 ± 0.005
8	300	0.456 ± 0.002	0.396 ± 0.001	0.478 ± 0.005	0.489 ± 0.009
9	360	0.478 ± 0.008	0.479 ± 0.005	0.601 ± 0.002	0.566 ± 0.001

Mean ± SD (n=3)

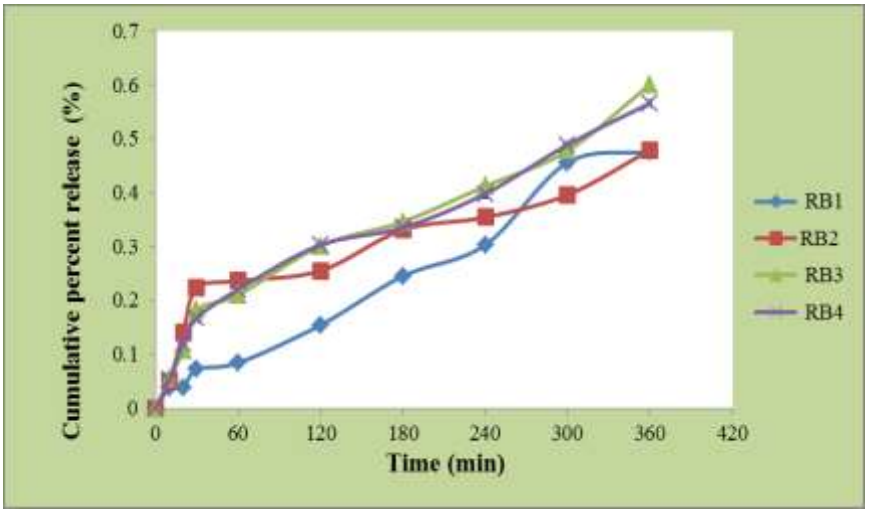


Fig. 10: Cumulative percent release of resveratrol from cream formulations.

3.4.1 Ex- vivo diffusion study using goat skin and skin retention: The drug release (%) from the formulations is detailed in Table No. **15 and** Fig **10)**. Notably, in buffer solution, all formulations exhibited less than 1% drug release after 360 minutes. Furthermore, the drug content (%) of resveratrol in both goat skin and cream within the donor compartment remained high across all the formulations, demonstrating desirable drug retention for topical formulations is shown in Table No. **16**.

Table 16: Skin retention (%) and unreleased drug (%) from cream.

Parameters	Formulations			
	RB1	RB2	RB3	RB4
Skin retention (%)	82.1 ± 0.061	87.3 ± 0.032	91.8 ± 0.046	90.3 ± 0.059
Unreleased drug from cream (%)	9.1 ± 0.028	8.3 ± 0.011	4.6 ± 0.026	5.23 ± 0.016

Mean ± SD (n=3).

3.4.2 Microbial examination: The microbial count in all the formulations was within the acceptable range; with count below 1000 cfu/g is detailed in Table No. 17.[26]

Table 17: Result of microbiological study of cream formulations.

Parameters	Formulations			
	RB1	RB2	RB3	RB4
Microbial count	34±5	42±7	39± 1	39± 3

Mean ± SD (n=3)

Table 18: Stability data of creams at zero day and after 1 month.

Parameters	Formulations							
	At 0 day				After 1 month			
	RB1	RB2	RB3	RB4	RB1	RB2	RB3	RB4
Appearance	Light brown	Light brown	Light brown	Light brown	Light brown	Light brown	Light brown	Light brown
Consistency	Good	Good	Good	Good	Good	Good	Good	Good
Thermal stability	Stable	Stable	Stable	Stable	Stable	Stable	Stable	Stable
pH	5.9 ± 0.113	5.8 ± 0.132	5.8 ± 0.121	6.0 ± 0.129	5.9 ± 0.123	5.86 ± 0.114	5.9 ± 0.106	6.0 ± 0.132
Viscosity (cps)	2501.5 ± 20	2519.3 ± 18	2511.2 ± 18.6	2511.7 ± 20	2500.5 ± 19	2521.3 ± 15	2501.2 ± 20	2510.7 ± 20
Drug content (%)	95.3 ± 0.002	95.9 ± 0.007	96.7 ± 0.001	95.4 ± 0.001	94.8 ± 0.005	95.3 ± 0.004	96.1 ± 0.001	95.0 ± 0.001

Mean ± SD (n=3)

3.4.3 Stability study: The stability study of creams was conducted for one month. Based on the basis of comparison of data at day 0 and after 1 month, it was concluded that there were no changes in colour is shown in Fig. **11**, pH or viscosity of creams during storage. All the cream formulations exhibited thermal stability. Assessment of drug content from creams after one month suggested negligible or minimal drug loss during storage is detailed in Table No. 18.[22]

(a) **(b)**

Fig. 11: Resveratrol loaded cream appearance (a) at zero day (b) after 1 month.

4. CONCLUSION

Preformulation study gives profile of drug and other excipients added to the formulation development and found to comply with standard specifications. Hence, these ingredients were used for further development of cream base.

In the preliminary investigation base cream was formulated by fusion method. In the first stage formulations C1 to C14 were formulated, to check the effect of variation in the concentration of cetyl alcohol and stearic acid. In the second stage, formulations F1 to F4 were formulated, to check the variation in the concentration of stabilizer and humectant i.e., propylene glycol and glycerin. In the third stage, formulations F5 to F7 were formulated, to check the variation in the concentration of triethanolamine as pH stabilizer. Formulated creams were evaluated for organoleptic and physicochemical properties of creams. From evaluation, composition C12 (cetyl alcohol and stearic acid 3%w/w and 10%w/w, respectively) was considered optimum for cream base. Based on the preliminary investigation, the concentration of cetyl alcohol and stearic acid was more narrowed to investigate the effect on physicochemical properties of cream base; and to obtain an optimized cream base for loading of resveratrol. Considering all the physicochemical parameters, CB1 was selected for further loading of resveratrol.

Different doses of resveratrol were selected for drug loading in cream base on the basis of literature survey. Formulated creams were evaluated for physical appearance, grittiness, thermal stability, pH, spreadibility ,viscosity, total residue content, total fatty content, type of cream, drug content, Ex-vivo diffusion study and tissue retention using goat skin. The drug

release in buffer was less than 1% from all the formulations after 360 minutes. The results of drug content (%) of resveratrol in goat skin and cream remained in donor compartment indicates that the drug retention in skin was high in all the formulations, which is desirable for the topical formulations. The stability study indicates that cream formulations remained stable after one month. The present study supports that resveratrol cream is an advantageous topical drug delivery system for the treatment of contact dermatitis. However, detailed in-vivo studies should be conducted in the future to confirm the in-vitro findings.

5. CONFLICTS OF INTEREST

It is hereby declared that this paper does not have any conflict of interest.

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