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FORMULATION, OPTIMIZATION AND EVALUATION STUDY FOR AN ALOE EMODIN SYNTHETIC DERIVATIVE 4,5–DIHYDROXY-9,10-DIOXO-9,10-DIHYDROANTHRACENE-2 CARBOXYLIC ACID IN GEL DOSAGE FORM

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ABSTRACT: Dermatologists should be aware of cosmeceutical products and have access to accurate scientific theory with validated data-if any exists-to support cosmeceutical claims. The question remains whether it is possible to deliver adequate doses to the skin *in-vivo* and to produce clinical or histological effects. It is important to evaluate these new products with a critical and careful methodology, considering intended product use and the design of available studies supporting product use. The system was optimized with a primary aim to prepare this formulation is to reduce down the concentration of ethanol in formulation by addition of PEG-600 as a co-solvent. All formulations were observed for appearance of color and transparency of gel. The pH of gels was adjusted in the range 6.8 to 7.4. The formulation designed including the ingredients of carbopol 934, PEG (600), ethanol, and water. Evaluation carried out for the formulation with rheological studies. *In-vitro* diffusion study through flow through diffusion cell method. Drug content uniformity was determined spectrophotometrically. Spreadability indicates that the gel is easily spreadable by small amount of shear. Viscosity (cp) measured by a Brookfield programmable viscometer (RVD-II+) along with helipath assembly. The skin irritation assay was performed following the organization for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals, Section 4, No. 404 - Acute Dermal Irritation / Corrosion study, the formulations were found to be non-irritating and free of skin allergy. The gel formulation showed a significant consistency and passes all the relevant stability testing. The formulation further proceeds to pharmacological evaluation study.

Keywords: Aloe emodin, PEG-600, *In-vitro* diffusion study, Optimization, Spreadability, Skin irritation assay

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INTRODUCTION: Aging is a "biological reality, which has its own dynamic, beyond human control [1]. The aging process causes fundamental changes in the skin, soft tissue, and skeletal support structures of the human face [2].

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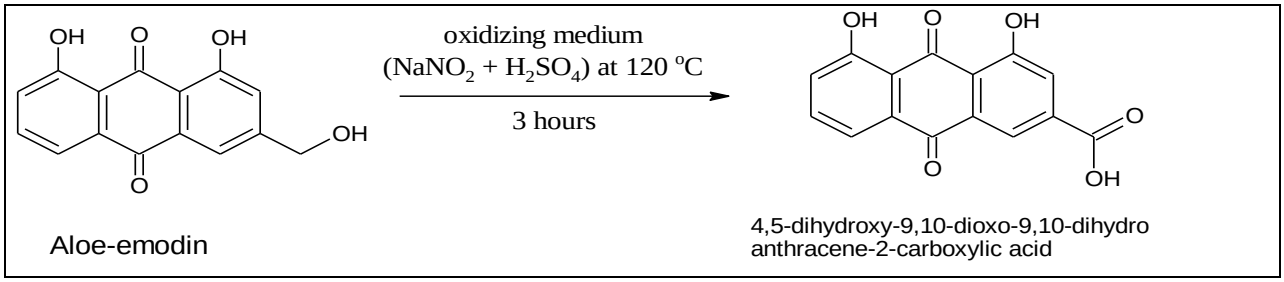
Ageing, a basic biological process seen in all living creatures, is not preventable [3]. Ageing are skin depressions that draw lines on ageing skin. As age increases, depressions become deeper, or fine and reducible lines evolve to form coarse and permanent lines. The most obvious examples of this are so-called expressional ageing like frown lines that can transiently appear on a child's face and turn into permanent lines in adults.

Although ageing can be found almost everywhere in aged skin, they develop preferentially on face, neck, and hands, which are photo exposed and

largely visible areas. Ageing are indeed one of the earliest and most visible signs of skin ageing. Preventing and treating them is thus of major interest for dermo-cosmetic companies. The use of sunscreen is a most common practice now a days that provides protection against the adverse effects of UV radiation [4]. Natural substances extracted from herbs acts as the potential photoprotective resources owing to their UV absorbing property in the UV region [5]. In addition, they exhibit antioxidant property flavonoids as potential protective agents against photo-oxidative skin damage [6]. For these reasons, dermatologists should be aware of cosmeceutical products and have access to accurate scientific theory with validated data—if any exists—to support cosmeceutical claims. For some products, in vitro evidence shows that these ingredients do have antiaging activity. The Question remains whether it is possible to deliver adequate doses to the skin *in-vivo* and to produce clinical or histological effects. It is important to evaluate these new products with a critical and careful methodology, considering intended product use and the design of available studies supporting product use. Then, one can decide if the product is useful as a main or adjuvant treatment for aging skin [7].

Docking Study: By use of computer-aided technique, the aloe emodin and its carbonyl derivative had docked on NEP protein. The NEP protein obtained from protein data bank. The results showed positive effect on binding the AE and AEC on protein receptor site. Thus, the study is further proceeding on formulation of aloe emodin and its derivative. In order to study the compatibility and stability of the synthesized compound [8].

Synthesis of 4,5–dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2 carboxylic acid from Aloe emodin Reaction [9]:



Formulation and Optimization of Topical Preparations:

Formulation of Based: Carbopol 934 grade is used as gelling reagent. Polyethylene glycol (PEG 600) is utilized as plasticizer. Triethanol Amine used as buffer to maintained the basic pH.

Optimization of Base Formula:

Optimization: The system was optimized with a primary aim to prepare this formulation is to reduce down the concentration of ethanol in formulation by addition of PEG-600 as a co-solvent and increase penetration as well to optimize concentration PEG-600 in the formulation to obtain a transdermal gel of good consistency.

Preparation: For the preparation of the gel first concentration of derivative of aloe emodin we have mentioned 0.25-0.50% in table 9 was dissolved in varying concentrations of ethanol and PEG 600. In a separate beaker varying concentration of Carbopol 934 (0.5% w/w) was dissolved in water with continuous stirring on an industrial stirrer, to that ethanolic solution of derivative with PEG600 was added gradually with continuous stirring. At the end triethanol amine was added drop wise to bring the pH to neutral. The details of the formulations are given in **Table 1**.

TABLE 1: BASE FORMULA OF TOPICAL GEL

S. no.	Ingredients	Percentage
1	Carbopol 934 w/v	0.5%
2	PEG 600 v/v	10 %
3	Ethanol (99.99%) v/v	50.00%
4	Triethanol Amine	02.00%
5	Active Ingredient	0.25-0.50%
6	Perfume	01.00%
7	Preservative	00.50%
8	Distilled water	Qs 100%

Appearance: All formulations were observed for appearance of color and transparency of gel.

pH Measurements: [10] The pH of each gel was measured using a pH meter. The pH meter was calibrated before each use with standard pH 4, 7 and 10 buffer solutions the calibration can be done at only 4 and 7 or 7 and 10. The pH of gels was adjusted in the range 6.8 to 7.4.

Drug Content: [11] The quantity of formulation equivalent to 10 mg of drug was dissolved in 100 ml methanol. From this solution, 1 ml samples were withdrawn and diluted to 10 ml with phosphate buffer pH 7.4. The samples were analyzed spectrophotometrically at a wavelength of 264.0 nm. The concentration of drug in each sample was determined from a previously prepared standard curve.

Formulation of Herbal Gel:

TABLE 2: COMPOSITION OF GEL FORMULATION

Formulation No.	Weight of Drug w/v	Weight of Carbopol 934 w/v	PEG(600) v/v	Ethanol v/v	Water v/v
GF_B	---	0.5%	10 %	50%	q.s
GF AE 25	0.25%	0.5%	10 %	40%	q.s
GF AE 50	0.50%	0.5%	10 %	35%	q.s
GF AEC 25	0.25%	0.5%	10 %	40%	q.s
GF AEC 50	0.50%	0.5%	10 %	35%	q.s

Where: GF_B- Gel Base Formulation; GF_{AE} = Gel Formulation of Aloe emodin; GF_{AEC} = Gel Formulation of Aloe emodin carbonyl.

Evaluation of Gel Formulation:

Rheological Study:

***In-vitro* Diffusion Study:** Flow through diffusion cell was used in the diffusion studies. This type of cell enhances the receiver compartment volume and maintains proper sink conditions by continuous circulation of receptor phase through the cell. The cell was fabricated without the outer glass jacket and has one inlet and an outlet connected to reservoir compartment. The cell has a diameter of 2.1 cm, effective diffusion area of 3.63 cm^2 and a receiver compartment volume (cell plus reservoir) of 500 ml. The receptor phase (phosphate buffer of pH 7.4) was kept in a reservoir, which was continuously circulated into the diffusion cell with the help of circulating pump at a uniform speed of 2 ml/ min to simulate with circulating blood in capillaries.

The temperature of the reservoir was maintained 37 ± 1 °C throughout the experiment. Synthetic membrane (cellophane) was used as diffusion barriers I guess when it is synthetic membrane it is drug release and diffusion needs animal skin. 1 gm of gel was placed in the donor compartment. At appropriate time intervals, 10ml of the sample was withdrawn from the reservoir and the same amount of fresh buffer solution was added to maintain the sink condition. The samples were analyzed spectrophotometrically to determine the concentration of drug in each sample.

Drug Content Uniformity: 1 gm of gel was weighed accurately and dissolved in 100 ml of ethanol. Suitable dilutions were made with ethanol to bring the concentration of drug in Beer-Lamberts range. The concentration was determined spectrophotometrically.

Spreadability: [11] The spreadability of the gel was determined using an apparatus described in the literature. The apparatus was fabricated in our laboratory. The apparatus consists of two glass slides (7.5 × 2.5cm), one of which being fixed onto the wooden board and the other is movable, tied to a thread which passes over a pulley, carrying a weight. Firstly, 1 gm of gel was placed between the two glass slides. 100 gm weight was allowed to rest on the upper slide for 1 to 2 min to expel the entrapped air between the slides and to provide a uniform film of the gel. The weight was subjected to a pull of 5 gm. The time necessary for top slide to travel pre marked 6.5 cm distance was noted.

Viscosity: [12] A Brookfield programmable viscometer (RVD-II+) along with helipath assembly was used to determine viscosity (cp). The spindle was T-bar spindle no. 1 (S91).

Gel was placed in a beaker, the spindle was dipped into it and the helipath was adjusted in such a way that neither it was touching the bottom of container nor did it come out of the sample during the movement of the helipath. The spindle was rotated

in the gels at a speed of 5rom. The corresponding viscosity (in cp) and torque (in %) of each formulation was noted.

Oil in water (O/W) emulsion-based gel [semisolid formulation] was formulated. The emulsifier (stearic acid) and other soluble component (cetyl alcohol, Mineral oil) were dissolved in the oil phase (PART: A) and heated to 75^0 C. The preservative and other water soluble component (Methyl paraben, triethanolamine, Propylene glycol, AE and AEC) were dissolved in aqueous phase (PART: B). Various possible combination of formulation containing dried derivative were tried to choose the most suitable combination having better consistency along with, triethanol amine, glycerin, AE and AEC, perfume, preservative, and distilled water, to choose out the best concentration ratio.

The gel thus obtained was evaluated for its organeoleptic properties like color, odour, and physical properties like specific gravity, viscosity and pH. The pH of the gel was found to be in range of 5.6 to 6.8. The viscosity rage of 28001-27025 All formulations produce uniform distribution of fractions in gel. This was confirmed by visual appearance and by touch.

RESULTS:

TABLE 3: PHYSICAL PROPERTY AE AND AEC BASED GEL FORMULATION

Formulation no.	Appearance	Color	Transparency	Spreadability (Time in Sec)
GF_B	Consistent	Greenish	Semitransparent	**5**
AE-25	Consistent	Greenish	Semitransparent	**8**
AE-50	Consistent	Greenish	Semitransparent	**9**
AEC-25	Consistent	Greenish	Semitransparent	**9**
AEC-50	Consistent	Greenish	Semitransparent	**9**

Where: GF_B- Gel Base Formulation; $GF_{AE25\text{-}AE50}$ = Gel Formulation of Aloe emodin; $GF_{AEC25\text{-}AEC50}$ = Gel Formulation of Aloe emodin carbonyl.

TABLE 4: EVALUATION OF AE AND AEC BASED GEL FORMULATION FOR PHYSICOCHEMICAL PARAMETERS

Formulation No.	pH	Percentage Drug content	Viscosity	*In-vitro* Diffusion (Drug content uniformity)
GF_B	7.4±0.1	-	954±0.46	75±0.98
AE-25	7.3±0.45	94±0.24	664±0.24	78±0.15
AE-50	7.2±0.11	98±0.12	687±0.14	64±0.18
AEC-25	7.4±0.21	98±0.43	652±0.17	55±0.74
AEC-50	7.6±0.01	98±0.29	625±0.34	67±0.19

Where: Each value is expressed as Mean ± SD of triplicate determinations. GF_B- Gel Base Formulation; $GF_{AE25\text{-}AE50}$ = Gel Formulation of Aloe emodin; $GF_{AEC25\text{-}AEC50}$ = Gel Formulation of Aloe emodin carbonyl.

Skin Irritation Assay: [13] The study was performed following the Organization for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals, Section 4, No. 404 - Acute Dermal Irritation / Corrosion study, adopted 24th April 2013.

Male rabbits (White, 1.2-2.5kg) shaved prior to the experiment. The back of the rabbit was divided into six mark areas for the topical application of the fraction. The 0.5g of each test product placed on each area (25x25mm) for 30min and another group for 6hours and then washed off by water. Scoring of the erythema and edema performed at 24 and 72 hours and both the treatment and control sites were covered and wrapped by cotton bandage. Reaction on skin measured after 24 h and 72 h in form of erythema and edema. The Score of Primary irritation (SPI) calculate for each rabbit as the following. Scores for erythema and edema at 24 h and 72 h carrying out as reported earlier [8, 4].

Based upon *in-vitro* antioxidant and enzyme inhibition assay as described earlier the formulation F_{AE} to F_{AEC} were subjected to skin irritation as described above. The formulations were found to be non-irritating and free of skin allergy.

In the present work, aloe emodin and aloe emodin carbonyl (AE and AEC respectively) did not show any type of skin irritation in rabbit.

TABLE 5: SCORE GRADE FOR SKIN IRRITATION

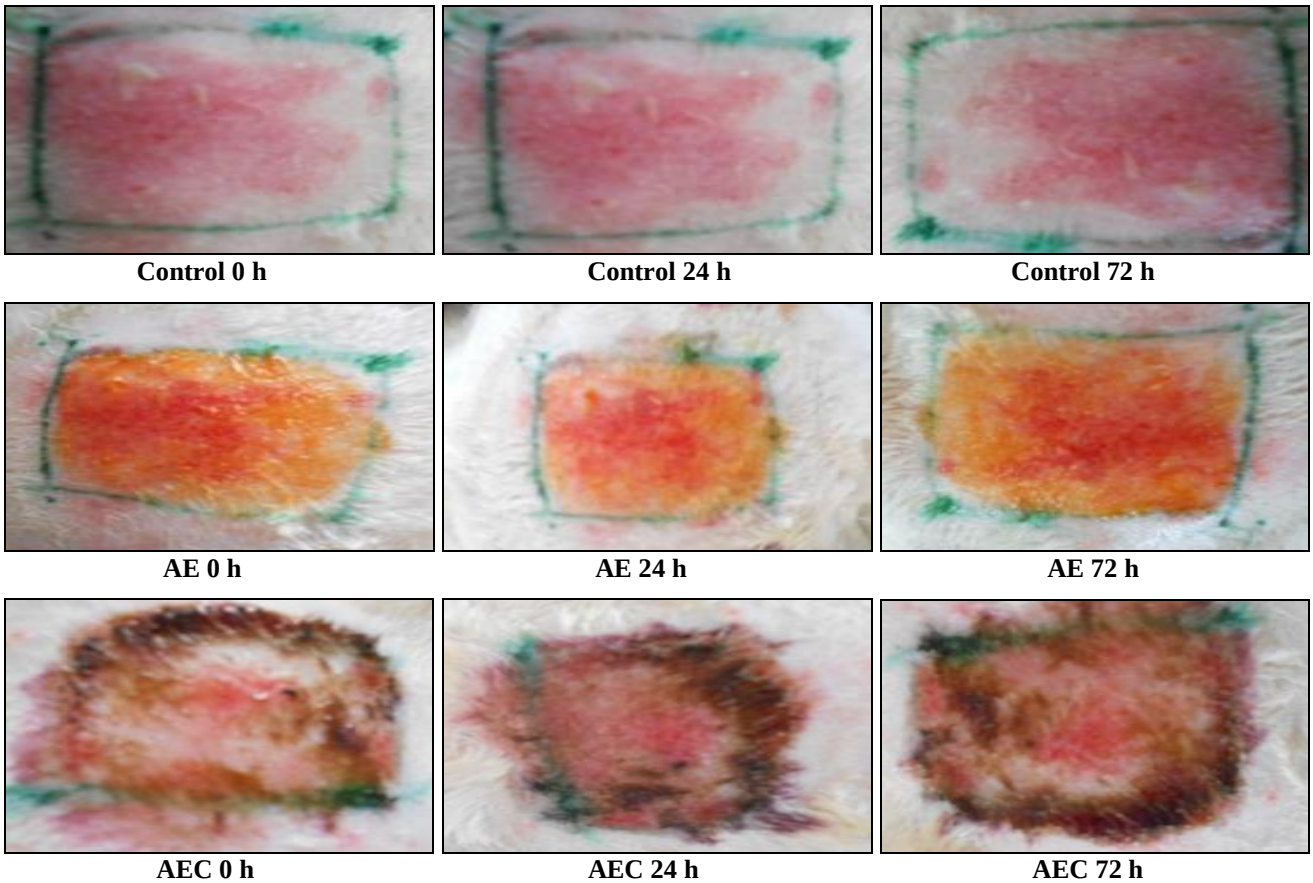
Reaction	Score
Erythema	
No Erythema	1
Very Slight Erythema (barely perceptible)	2
Well-defined erythema	3
Moderate to severe erythema	4
Severe erythema (beet redness) to eschar formation	
Edema	
No Edema	1
Very Slight Edema (barely perceptible)	2
Well-defined Edema	3
Moderate Edema (raising approx 1 mm)	4
Severe Edema (raised more than 1 mm and extending area of exposure)	

TABLE 6: EVALUATION OF THE AE AND AEC BASED GEL FORMULATION FOR SKIN IRRITATION ASSAY

S. no.	Reaction	Score Skin Reaction							
		Base Formulation							
		GF AE 25		GF AE 50		GF AEC 25		GF AEC 50	
		24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
1	Erythema	1	1	2	1	1	1	1	2
	Edema	1	2	1	2	2	2	1	2
2	Erythema	1	2	1	2	2	1	1	1
	Edema	2	1	2	1	1	2	1	2

Where: GF $_{AE25-AE50}$ = Gel Formulation of Aloe emodin; GF $_{AEC25-AEC50}$ = Gel Formulation of Aloe emodin carbonyl

DISCUSSION: The proto type of gel and gel formulations were developed to evaluate the base formulation. The different batches of varying quantity of surfactant, emulsifying agent were tried and all the formulations were found to be oil-in-water type emulsions.



Control 0 h **Control 24 h** **Control 72 h**

AE 0 h **AE 24 h** **AE 72 h**

AEC 0 h **AEC 24 h** **AEC 72 h**

FIG. 1: RABBIT SKIN IRRITATION TEST

The gel was formulated using carbopol 934 in different concentrations (0.25 %, and 0.50 %) and the formulation has shown the uniform distribution of drug throughout the batch. The developed herbal gel was greenish in color, translucent in appearance and showed good homogeneity with absence of lumps.

The appearance for all the formulation was found to be smooth and transparent. The consistent nature obtained for all the gel formulation. The greenish color obtained for the formulations giving good relevant compliance. The transparency studied showed semitransparent nature of the formulations. The rheological properties studied for the different formulations. It gave an idea of relative spreadability of the different gels. The spreadability measured in terms of time (sec) AEC 25 and AEC 50 showed considerable spreadability compared to the GB and AE formulations. The values of spreadability indicate that the gel is easily spreadable by small amount of shear.

The viscosities for formulation were found to be less as compared to the control formulation. During the accelerated stability studies the appearance was clear and no significant variation in pH was observed and spreadability. The topical gel thus formulated was non-irritant upon application on to the skin. The studies revealed that the developed herbal formulation consisting of derivative is comparatively better than later other formulations.

CONCLUSION: The gel formulation showed a significant consistency and passes all the relevant stability testing. The formulation further proceeds to pharmacological evaluation study.

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CONFLICT OF INTEREST: Nil

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